OXACILLIN RESISTANT STAPHYLOCOCCUS AUREUS AMONG HIV INFECTED AND NON-INFECTED PATIENTS IN KENYA

BY

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A DEGREE OF MASTER OF SCIENCE (INFECTIOUS DISEASES DIAGNOSIS) IN THE SCHOOL OF PURE AND APPLIED SCIENCES, KENYATTA UNIVERSITY.
DECLARATION

I declare that this thesis is my own original work and has not been presented for a degree in any other university or any other award.

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This thesis is dedicated to my mother Dorine Ngoche Ouko and my parents in law Mr. and Mrs Otieno and to the love of my family, Nickson, Franklin and Judy. Thus far has the Lord brought us.
I wish to acknowledge the support of my supervisors, Dr. J. N. Ngeranwa, Dr. G. O. Orinda and Dr. C. N. Wamae for their guidance, patience and technical expertise in the field of microbiology and making this thesis what it is. My appreciations also go to Japan International Cooperation Agency (JICA) for kindly availing supplies and necessary equipments that made the study possible.

This project was carried out at the Centre for Microbiology Research (Opportunistic Infection Laboratory) KEMRI. I would especially like to thank the Director KEMRI for offering an enabling environment during the study period.

My special and sincere thanks go to Dr. N. Wamae for her financial assistance which ensured easy running of the program. May the good Lord reward you abundantly. I thank my family and friends for offering humble time during the study, I will always cherish your great help and kindness.

Thanks are to God for giving me life, strength and talent.
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<tr>
<td>MRSA</td>
<td>Methicillin Resistant <em>Staphylococcus aureus</em></td>
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<td>MSSA</td>
<td>Methicillin Sensitive <em>Staphylococcus aureus</em></td>
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<tr>
<td>HA-MRSA</td>
<td>Hospital Acquired Methicillin Resistant <em>Staphylococcus aureus</em></td>
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<td>CA-MRSA</td>
<td>Community Acquired Methicillin Resistant <em>Staphylococcus aureus</em></td>
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<tr>
<td>ORSA</td>
<td>Oxacillin Resistant <em>Staphylococcus aureus</em></td>
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<tr>
<td>PBP</td>
<td>Penicillin Binding Protein</td>
</tr>
<tr>
<td>PBP 2' or PBP 2a</td>
<td>Penicillin Binding Protein Two Prime</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>SCCmec</td>
<td>Staphylococcus Cassette Chromosome</td>
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<tr>
<td>NNISS</td>
<td>National Nosocomial Infections Surveillance System</td>
</tr>
<tr>
<td>EARSS</td>
<td>European Antimicrobial Resistance Surveillance System</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>UNAIDS</td>
<td>United Nations AIDS</td>
</tr>
<tr>
<td>DHQPN</td>
<td>Division of Health Quality Promotion National Centre</td>
</tr>
<tr>
<td>VISA</td>
<td>Vancomycin Intermediate <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>VRSA</td>
<td>Vancomycin Resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>VRE</td>
<td>Vancomycin Resistant <em>Enterococci</em></td>
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<tr>
<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standards</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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ABSTRACT

The emerging and re-emerging multi-drug resistant bacterial isolates to the commonly used antibiotics has continued to increase and pose a global challenge in management and control of infectious diseases, especially with the advent of HIV and AIDS. Infections due to methicillin resistant *S. aureus* present a considerable dilemma to clinicians, since therapeutic options are limited and sub-optimal dosing contributes to heightened mortality and increased length of hospital stay particularly among the HIV infected patients. The objective of the study was to assess the prevalence and relative risk of MRSA infections in HIV infected patients with a view to improving on healthcare management strategies of HIV patients with staphylococcal infections.

This was an analytical, cross sectional study carried out on both HIV infected and non-infected patients suspected of staphylococcal infections from four health institutions in Nairobi and Busia Districts, Kenya. Microbiological cultures were performed on blood agar and manitol. Identification of *Staphylococcus aureus* isolates was done by coagulase and API and MRSA by both disk diffusion and MIC on oxacillin and cefotaxime. *MecA* gene and its coded PBP 2' was determined using PCR and monoclonal latex agglutination tests. The MIC of oxacillin, cefotaxime, vancomycin, amoxicillin clavulanic acid, sulphamethoxazole trimethoprim, erythromycin, chloramphenicol, tetracycline and gentamycin were performed as per the NCCLS 2003. ATCC *S. aureus* 25923, 29213 and 3359, *E. coli* 25922, *P. aeruginosa* 27853, *E. faecalis* 29212 were used as quality control strains. Analysis of variance was done using chi square. A total of 436 patients suspected of staphylococcal infections were recruited and sampled between 2006 and 2007 with 220 and 216 HIV-infected and non-infected patients, respectively. The prevalence of MRSA was 26.9% and varied among the four hospital based institutions studied. The study showed a higher isolation rate and a significant difference in the prevalence of staphylococcal infections in HIV infected patients compared to the non-infected patients (odds ratio: 2.081, 95% CI 1.347 – 3.215, P < 0.001), while MRSA (odds ratio: 2.174, 95% CI, 0.999-4.732, P=0.046). The isolates from HIV non-infected patients were highly sensitive (100%) to vancomycin while the HIV-infected individuals had 6.8% of the MRSA isolates exhibiting vancomycin intermediate resistance (MIC > 8μg/ml). Up to 19.5% *S. aureus* strains were resistant to oxacillin along with gentamicin, tetracycline, erythromycin, and sulphamethoxazole/trimethoprim. This study shows that HIV is a predisposing factor to staphylococcal infections and that there is no statistical significance in the prevalence of MRSA and the antimicrobial susceptibility profile among the HIV infected and non-infected patients. This raises the concern that treatment with β-lactam antimicrobials may no longer be relied on as the sole empiric therapy for several ill HIV patients whose infections may be staphylococcal in origin. Molecular epidemiology of *S. aureus* in understanding new and emerging trends should be continually carried out.
CHAPTER ONE

INTRODUCTION

1.1 Background information

The genus *Staphylococcus* are gram-positive facultative anaerobic cocci that exist mainly in clusters. The genus comprises 32 species and 15 sub-species (Kloos, 1997), which are widespread in nature and are mainly found living on the skin and mucous membranes of mammals and birds in a benign or symbiotic relationship. The discovery of penicillin in 1940 heralded the era of antibiotic treatment particularly for *Staphylococcus aureus* infections. However, a few years later its therapeutic efficacy ended spurring development of a semi-synthetic penicillin (methicillin) that resists hydrolysis by penicillinase. The development of methicillin in 1960, together with subsequent launch of the first generation cephalosporins proved effective for treatment of these troublesome multi-resistant bacteria (Jevons, 1961).

Shortly after 1960, Jevons reported the first *Staphylococcus* strain resistant to methicillin in Europe giving rise to MRSA which was virtually resistant to all β-lactam antibiotics including penicillins, cephalosporins, monobactams and carbapenems (Jevons, 1961; Oliveira et al., 2002). A steep rise in MRSA incidence in Europe and Japan in 1980 provoked a number of prevalence studies on MRSA and presented a considerable dilemma to clinicians, since therapeutic options of MRSA infections are limited and sub optimal dosing contributed to heightened mortality and increased length of hospital stay (Graffunder et al., 2002).
National Nosocomial Infections Surveillance System (NNISS) recorded an increase of MRSA in US hospitals from 4% in the 1980s to 50% in the 1990s (Wenzel et al., 1991). Point prevalence studies have found MRSA prevalence rates of 23% – 60% with new acquisition rates of MRSA at 4% – 10% per year (Jernigan et al., 1995; Bradley, 1997).

These prevalence rate variations could mainly be due to geographic variation of MRSA strains with different virulence or colonization properties, differences in antimicrobial utilization such as in MIC breakpoints of methicillin/oxacillin (R=\(<2 instead of the 4\mu g/ml currently recommended) practiced by some laboratories and in hospital infection control practices (EARSS, 2002).

In a multivariate logistic regression analysis, methicillin resistance was found to be an independent risk factor for death with a significant mortality rate caused by MRSA and methicillin sensitive Staphylococcus aureus (MSSA) at 49% – 50% and 20% – 32% respectively (Cosgrove et al., 2001). This poor outcome was mainly associated with delayed implementation of appropriate antibiotic therapy, expensive therapeutic agents associated with MRSA infections and expenses related to implementation of recommended infection control measures. The average attributable costs associated with management of MRSA infections have been found to range from $7781 – $34,000 in US and $14,360 in Canada (Rubin et al., 1999; Smith et al., 1999; Kim et al., 2001).

The emerging antibiotic resistant bacterial strains have continued to increase globally, however the frequencies, patterns and distributions vary significantly with geographic regions. The Centre for Disease Control and Prevention (CDC) estimated that there are
about 90,000 deaths per annum related to hospital-acquired infections most commonly due to MRSA (CDC, 2003).

Methicillin resistant *Staphylococcus aureus* is mainly the result of *mecA* gene which encodes an extra penicillin-binding protein (PBP) 2a or PBP 2’ that has decreased affinity for β-lactam antibiotics, thus allowing cell wall synthesis to continue despite inactivation of native PBPs (Daum *et al.*, 2002). To date, five types of *Staphylococcus* cassette chromosome *mec* (SCCmec) element I, II, III, IV, and V and a small number of variants have been characterized (Enright *et al.*, 2000).

There are three stepwise expressions of MRSA beginning with Pre-MRSA, where phenotypic expression of oxacillin resistance does not show though bacteria have *mecA* gene together with its regulator genes, *mecI* and *mecR1*. The second stage is hetero-MRSA in which bacteria are usually susceptible to high concentrations of methicillin whereby *mecI*-mediated repression is released by mutation but the physiological status of the *S. aureus* cell is not yet appropriate to take full advantage of the cell-wall synthesis enzyme coding for the PBP 2’. Lastly, there is the homo-MRSA type where the cell population is uniformly resistant to methicillin with high MIC levels and few treatment options complicating the treatment and management of staphylococcal infections particularly among the HIV patients (Keiichi, 2004).
1.2 Problem statement and justification

The HIV-infected patients have an exceptional vulnerability to invasive bacterial infections in comparison to immunocompetent and HIV non-infected patients (CDC, 1994). Kenya is one of the leading countries with HIV in Southern Sahara, with an estimated 1.2 million people living with HIV/AID (UNAIDS, 2004). At the same time, bacterial isolates such as MRSA, with multiple resistance to the commonly used antibiotics have continued to increase and pose global challenges in management and control of infectious diseases, especially with the advent of HIV and AIDS (Graffunder et al., 2002). Infections due to MRSA present a considerable dilemma to clinicians, since therapeutic options are limited and sub optimal dosing contributes to heightened mortality and increased length of hospital stay particularly among the HIV infected patients. The antimicrobial regimens used in treatment of many bacterial infections in HIV-infected patients are often the same as those used to treat HIV non-infected individuals. Frequencies, patterns, and distributions of resistant bacteria vary significantly with geographic regions.

In Kenya, there is no epidemiological data on the scope and extent of MRSA infections in HIV infected individuals. Given the potential severity of infections caused by MRSA, their recent emergence in settings outside the hospital environment and the challenges in treatment and control measures, it is imperative that an epidemiological study is carried out to provide baseline data on the status of MRSA infections among patients with and without HIV infection. Such data, as presented in this study, will be used as a guide
implementation of appropriate control measures to minimize spread of MRSA and improve healthcare management strategies of patients with staphylococcal infections.

1.3 Hypothesis

MRSA does not pose excess risk among HIV infected patients.

1.4 Objectives

1.4.1 General objective

To assess the prevalence and relative risk of MRSA infections in HIV infected patients.

1.4.2 Specific objectives

i) To determine the prevalence of *Staphylococcus aureus* in HIV infected patients.

ii) To establish the prevalence of MRSA in HIV infected patients.

iii) To compare the antimicrobial susceptibility profile of *Staphylococcus aureus* in HIV infected and non-HIV infected patients.

iv) To determine the relative risk of MRSA in HIV infected patients.

v) To establish the predictive values of common test systems for the identification of MRSA.
CHAPTER TWO
LITERATURE REVIEW

2.1 Staphylococcal infections

Staphylococcal infection occurs when the natural cutaneous barriers are disrupted or damaged, and the organisms gain entry into the host tissues. The infections are often acute and pyogenic and if untreated may spread to surrounding tissues or via bacteraemia to metastatic sites. Infections caused by *S. aureus* may be broadly classified into three; i) superficial lesions such as wounds; ii) deep seated and systemic infections involving the lungs (necrotizing pneumonia), bones (osteomyelitis, septic arthritis), heart (endocarditis), central nervous system (meningitis), bacteraemia and urogenital tract infections and iii) toxemic syndromes such as Staphylococcal scaled skin syndrome, toxic shock syndrome and food poisoning which is of public health significance (Musser *et al.*, 1990).

According to the CDC, MRSA is spread by close contact with infected people, and not through the air. However, it has been stated that airborne transmission of MRSA does occur, as in the case of staphylococcal pneumonia (CDC, 2003). Spreads of MRSA may also occur through indirect contact by touching objects such as towels, sheets, wound dressings and clothes contaminated with MRSA (DHQPN, 2005). MRSA may be carried on the skin or in the nose without causing any disease and the infections commonly occur among persons in hospitals and healthcare facilities and are known as nosocomial infections (CDC, 2003). Community-associated MRSA (CA-MRSA) refers to those cases of illness occurring in persons outside hospitals and healthcare facilities (Hammerschlag,
2003). In 1999, CA-MRSA was documented in the United States as being the cause of the deaths of 4 children in unrelated cases of infection with this organism (Hammerschlag, 2003). Now CA-MRSA skin infections are known to occur in patients of all ages without traditional risk factors for MRSA (Garthwaite et al., 2003.). The strain of CA-MRSA is distinct from that of the nosocomial form of MRSA and has been recently reported as a cause of community-acquired pneumonia (Neil, 2004).

2.2 Colonization sites of *S. aureus*

Colonization of the nasopharynx, perineum, or skin, particularly, can occur shortly after birth or anytime thereafter (Payne et al., 1966) and can last for years (Sanford et al., 1994). Sites of colonization are important not only as potential source of subsequent infection but also as a reservoir for transmission of the organism. Humans are natural reservoirs and asymptomatic colonization is far more common than actual infection. About 20% to 60% of hospitalized patients who become colonized with MRSA eventually get MRSA infections (Jernigan et al., 1995). Studies have indicated colonization rates of MRSA from nasal swabs of normal populace range from 1 to 4% (Heininger et al., 2007; Rim & Bacon, 2007).

2.3 Epidemiology of MRSA

MRSA is a formidable bug responsible for a variety of infections commonly seen in patients of all ages with a fatality rate of 21% compared to 8% seen in Methicillin Sensitive *Staphylococcus aureus* (MSSA) strains (Chambers, 1997). Infections due to MRSA present a considerable dilemma to clinicians, since therapeutic options are limited
and sub-optimal dosing contributes to heightened mortality and increased length of hospital stay (Graffunder et al., 2002; Howden et al., 2004). Vancomycin is considered the only drug that can consistently treat MRSA. However, since the beginning of 1989, hospitals have reported a rapid increase in Vancomycin Resistance Enterococci (VRE) and this is expected to lead to cross-resistance in S. aureus, since genes conferring vancomycin resistance are transferred from VRE (USA report, 1993).

Although alteration of target penicillin-binding proteins is the primary mechanism of resistance to β-lactam antibiotics in Staphylococcus, over the years MRSA strains have gained multiple resistance to several classes of antimicrobials, including macrolides, aminoglycosides, fluoroquinolones, tetracyclines, and lincosamide antibiotics such as clindamycin. In the past, glycopeptide antibiotics, such as vancomycin, have been considered the only agents to which MRSA have not developed resistance. Unfortunately, due to overuse of glycopeptide antibiotics, MRSA with reduced susceptibility to these agents have emerged as well (Rybak et al., 2001). Reports of the emergence of Vancomycin Intermediate S. aureus (VISA) in the USA suggest that S. aureus strains are constantly evolving and full Vancomycin Resistant Staphylococcus aureus (VRSA) may soon develop (USA update, 1997).

According to the National Nosocomial Infection Surveillance System (NNISS), mean prevalence of MRSA in US hospitals as a percentage of all S. aureus strains isolated increased from 2.4% in 1975 to 29% in 1991. Rates as high as 36% were reported in 1999 while rates of 50% to 60% have been reported in intensive care units (Voss et al.,
Regional differences in the epidemiological factors of MRSA appear to be significant. For example, Scandinavian and northern European countries have reported low rates of nosocomial MRSA (<2%), but many other European countries report rates as high as 30% to 60% (Karchmer, 2000). This variability may be due to geographic variation of MRSA strains with different virulence or colonization properties, or it may reflect differences in antimicrobial utilization and hospital infection control practices. In Veterans Affairs-affiliated facilities, point-prevalence studies have found MRSA rates of 23% to 35% and new acquisition of MRSA in 4% to 10% of residents a year (Bradley, 1997). In freestanding nursing homes and other long-term care facilities, MRSA rates appear to be lower, with about 10% to 15% of residents colonized with MRSA at any point in time (Bradley, 1997). In Africa, studies done at Muhimbili Medical Hospital in Tanzania and Kenyatta National Hospital in Kenya, found prevalence of MRSA rates of 0.4% and 39.8% respectively (Omari et al., 1997; Urassa et al., 1999).

2.4 Risk factors to MRSA infection

In the general population, carriage rates are 25% to 50% but higher rates have been documented in injection drug users, persons with insulin-dependent diabetes, patients with dermatological conditions, those with long-term in-dwelling intravascular catheters and health-care workers (Waldvogel, 2000). Although most MRSA illness and death are associated with health-care facilities, isolates from community-associated MRSA (CA-MRSA) infections have been obtained with increasing frequency in the last few years in different countries (Zetola et al., 2005).
At greatest risk are patients who are hospitalised, particularly the elderly, the acutely ill in an intensive care unit, those with surgical wounds or an intravenous catheter, those in physical proximity to a patient with MRSA, and those with prolonged hospital stay and prior exposure to broad-spectrum antimicrobial therapy, as seen in most HIV patients (Boyce, 1992). Although MRSA is usually acquired in the hospital setting, it is also found in residents of long-term care facilities. Since the 1970s, the occurrence of MRSA has increased steadily. Molecular epidemiological studies have shown that a limited number of MRSA strains have spread by clonal dissemination between different hospitals, cities, countries, and even continents and are now the major cause of hospital infections worldwide (Oliveira et al., 2002). MRSA strains are usually introduced into an institution by an infected or colonized patient or by a colonized health-care worker. Thus, epidemiological surveys involving accurate screening and proper identification of MRSA is an elemental procedure in control and preventive measures.

2.5 Laboratory identification of MRSA

Staphylococci are Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes. They are nonmotile, non-spore-forming, facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid. The S. aureus bacteria are catalase-positive and oxidase-negative, produce the enzyme coagulase and β-hemolysis on blood agar.

Conventional method for the laboratory detection of MRSA also used interchangeably with oxacillin-resistant S. aureus (ORSA) (CDC, 2003) include disk diffusion testing for
oxacillin disk (1μg) or cefotaxime (30μg); broth microdilution, agar dilution and E-test for oxacillin or cefotaxime MIC testing and oxacillin agar screen test (6mg/ml of oxacillin in Mueller-Hinton agar) as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2003). Many reports have highlighted difficulties and errors in the laboratory identification of MRSA with use of these and other automated or instrument-based test methods. The performance of these tests may be erratic because factors such as inoculum size and variability in culture conditions may affect phenotypic expression of resistance. Most MRSA strains are heterogeneously resistant to methicillin, so only a small proportion of the S. aureus colonies that grow from a clinical specimen may show resistance in standard laboratory tests and this property also makes detection of MRSA difficult. Moreover, it may take up to three days to get the test result.

Amplification of genetic determinants by polymerase chain reaction (PCR) assays have been used successfully to amplify and detect meca gene from clinical isolates and detect MRSA directly from clinical specimens within a few hours (Vannuffel et al., 1998) and may be used as a tool for diagnosis and management of infectious diseases due to antibiotic-resistant organisms such as MRSA.

2.6 Treatment and prevention of MRSA

Treatment will vary depending on where the infection is located, where the strain originated and the antibiotics to which the strain is sensitive. Minor skin lesions usually do not need treatment and resolve spontaneously. Local lesions and abscesses may
require surgical drainage (or debridement) and antibiotic therapy to prevent dissemination. Systemic infections require vigorous intravenous antibiotic treatment and moderately severe MRSA infections may be treated with sulfamethoxazole-trimethoprim or minocycline if the organism is susceptible to these agents (Merck Manual, 1999).

Penicillin has been replaced as the drug of choice, since most strains (over 90%) are not sensitive to this cell-wall active antibiotic. Synthetic beta lactamase-resistant penicillin types (nafcillin, methicillin, and oxycillin) can be used in this case, but 30-50% of isolates are also resistant to these drugs (Merck Manual, 1999; Murray et al., 2002). The MRSA strains mentioned above and “hyper-β-lactamase producing” strains must be treated using vancomycin, which interferes with cell wall biosynthesis using a non-beta lactam mechanism.

Proper hand-washing among medical personnel is critical in reducing nosocomial infections. Careful handling of contaminated clothing and bedding is important in minimizing the spread of MRSA. Skin decontamination using 4% chlorhexidine gluconate (hibiclens) which is an antiseptic skin cleanser, have been proven effective in eliminating *Staphylococcus* skin colonization (Murray et al., 2002). Mupirocin nasal gel (Bactroban) is effective in reducing nasal carriage of pathogenic *Staphylococcus aureus*, a contributing factor in repeated infections and hyper-contaminated facilities.

Although MRSA and VRSA may be resistant to a whole host of antibiotics, they are not resistant to many of the time-honored infection control measures such as hand washing,
cleaning and disinfection (Murray et al., 2002). Patient-to-patient spread, combined with an ability to favourably mutate, is how MRSA has succeeded where man has failed. Within the modern health care environment, almost half of all patients (and nearly all of those patients in Intensive Care Units) receive antibiotics. If a susceptible strain of an organism is transmitted to a patient receiving antibiotic, it is most likely to survive and proliferate if the bacterium is a resistant strain. As these resistant organisms accumulate and spread from patient to patient via health care providers, the reservoir of antibiotic-resistant pathogens is continually amplified in the hospital (Neil, 2004).

Health care workers spread resistant organisms to patients because their hands, clothing, and equipment become contaminated with resistant organisms as they provide care for infected or colonized patients or as they come into contact with contaminated environmental surfaces (Neil, 2004). Optimal control of transmission involves hand hygiene as well as environmental and equipment decontamination. Besides, it is important to educate the community members and all classes of health care workers on the epidemiological importance and the need of caring for patients colonized or infected with MRSA, in isolation, as a way of minimising the spread. There is no vaccine yet available that stimulates active immunity against staphylococcal infections in humans. However, in 2002, an experimental bivalent vaccine against Staphylococcus aureus was reported to be safe and immunogenic for 40 weeks in a selected group of patients. Further studies are being done to see if this vaccine has a use in patients who are at risk for MRSA, such as those having surgery. Such patients do not require immunologic protection for the rest of their lives, but only for a relatively short period of time while
they are in the hospital. The manufacturers of the vaccine, called StaphVAX, are planning to introduce the vaccine for use once they get approval from the Food and Drug Administration (Todar, 2004).

Although it is not clear which infection control measures are likely to be most effective, several approaches have been used in hospital settings which include screening or surveillance to detect MRSA, precautions to limit the spread of MRSA, and measures to reduce the reservoir of MRSA. The merits and potential limitations of these control strategies have been reviewed (Papia et al., 1999). However, there is accumulating evidence that screening patients for MRSA, careful hand washing by care providers, and isolation of infected or colonized patients are cost effective in controlling the spread of the bug (Chaix et al., 1999).

2.7 Mechanism of MRSA resistance

Methicillin resistant *Staphylococcus aureus* is associated with *mecA* gene which encodes an extra penicillin-binding protein (PBP) 2a or PBP 2’ that has decreased affinity for β-lactam antibiotics, allowing cell wall synthesis to continue despite inactivation of native PBPs (Daum et al., 2002). Given the history of ever-increasing resistance among MRSA strains, such strains are likely to become more prevalent in the future, a situation that would severely restrict treatment options for infections by this virulent pathogen (Susan et al., 2003). Using complementary mechanisms, both β-lactam and glycopeptide antimicrobials inhibit cell wall biosynthesis. β-Lactams bind to and inhibit penicillin-
binding proteins (PBPs), which are the enzymes involved in peptidoglycan synthesis, cell growth, and morphogenesis (Spratt, 1983).

Vancomycin interferes with the action of PBPs by binding to the D-Ala-D-Ala terminus of the peptidoglycan precursor, the substrate on which PBPs act (Geisel et al., 2001). Therefore, an understanding of the factors affecting expression of PBPs might lead to the identification of novel targets for antimicrobial therapy against MRSA (and particularly VISA) isolates, for which few therapeutic alternatives exist. Methicillin-susceptible *S. aureus* isolates produce five PBPs, PBP1, PBP2, PBP2B, PBP3, and PBP4, for which the genes have been cloned and sequenced (Murakami et al., 1994; Henze and Berger, 1995; Wada and Wetanaba, 1998; Komatsuzawa et al., 1999). MRSA isolates have acquired an additional PBP, termed PBP2' or PBP2a that has low affinity for β-lactam antibiotics and substitutes for the other PBPs in cell wall synthesis when they are inhibited by β-lactams (Chambers, 1997). Transcription of the *mecA* gene is induced in some isolates by β-lactams, and such induction is regulated by *MecI* and *MecR1*, a repressor and a signal-transducing protein, respectively (Chambers, 1997). The *mecI* and *mecR1* genes, when present, are carried beside *mecA* on the SCCmec element (Katayama et al., 2000; Ito et al., 2001; Ma et al., 2002). Cross-regulation by *BlaI* and *BlaR1* of *mecA* transcription also occurs, encoded by *blaI* and *blaR1* genes carried on the β-lactamase plasmid along with *blaZ* (Chambers, 1997). Until now, the background PBPs in *S. aureus* have been assumed to be constitutively expressed. Although constitutive *phpB* transcription has been studied recently (Pinho et al., 1998), the factors that control or induce *phpB* gene expression have not yet been explored.
To date, five types of *Staphylococcus* cassette chromosome mec (SCCmec) element I, II, III, IV, and V and a small number of variants have been characterized (Enright *et al.*, 2000). Each SCCmec element integrates at the same site (*attB*<sub>SCC</sub>) at the 3' end of an open reading frame (ORF) of unknown function, designated orfX (Ito *et al.*, 1999). SCCmec consists of the mec gene and cassette chromosome (ccr) gene complexes. Five classes of mec gene complex (A-E), which vary in their genetic structure, have been described (Katayama *et al.*, 2001; Lim *et al.*, 2003). Each mec complex consists of an intact copy of mecA, a copy of IS431mec and when present, complete or truncated mec regulatory genes mecI and mecRI (Lim *et al.*, 2003). The ccr complex consists of the ccr genes ccrA and ccrB in combination with ccrAB or ccrC alone, as well as adjacent ORFs (Katayama *et al.*, 2000; Ito *et al.*, 2004). The ccrAB and ccrC genes encode recombinases necessary for site- and orientation-specific integration and accurate excision of the SCCmec element. Five allotypes of the ccr gene complex have been identified (Ito *et al.*, 2004). The rest of the SCCmec element outside the ccr and mec complex is known as the jankyard (J) region, because it contains genes that are non-essential components of SCCmec (Ito *et al.*, 2003). The five SCCmec types described to date are defined on the basis of the class of mec gene complex and the type of ccr complex they possess, and variants of each type are defined by the J regions.

The stepwise expression of methicillin resistance by *S. aureus* strains may be categorized into 3 forms, the pre, hetero and homo-MRSA. The Pre-MRSA is category of MRSA thought to come as a result of inactivation of mecI gene that encodes for potent repressor of mecA gene transcription and is necessary for the pre-MRSA to become MRSA. *S.*
*S. aureus* does not express methicillin resistance even if it has acquired *SCCmec*. A strain that has acquired *mec A* gene together with its regulatory genes, *mecI* and *mecRI*, remains susceptible to methicillin (Keiichi, 2004).

The *Hetero-MRSA* emerges when *mecI*-mediated repression is released by mutation. This class of MRSA will remains susceptible only to higher concentrations of methicillin. This is explained by the hypothesis that the physiological status of the *S. aureus* cell is not appropriate to take a full advantage of the function of the exogeneous cell-wall synthesis enzyme PBP2’. *Hetero-MRSA* is named as such since the cells of various degrees of methicillin-resistance spontaneously emerged within its cell population (Keiichi, 2004).

The *homo-MRSA* comes as a result of high frequencies of 1 in 10, mutant strains from hetero-MRSA whose cell population is composed of the cells with homogeneous high methicillin-resistance (Katayama *et al.*, 2000; Hiramutsu *et al.*, 2001). Two genes, *hmrA* and *hmrB* whose over-expression raises methicillin resistance from *hetero-* to *homo-* MRSA have been cloned. However, little is known on how they function in raising methicillin resistance (Keiichi, 2004). The five gene elements differ in size, composition, and associated antimicrobial resistance expression (Daum *et al.*, 2002).
Fig 1. Shows the structure of the Staphylococcal gene cassette chromosome *mec*, with the recombinase genes complex upstream of the *mec* complex.

The *mec* complex contains the *mecA* gene responsible for β-lactam resistance in *Staphylococcus aureus*. IS1272 = insertion sequence-like element; *ccrA* and *ccrB* = cassette chromosome recombinase genes A and B that mobilize the *mec* element; *mecR1* = mec sensor transducer and repressor genes that regulate production of PBP-2A, which is responsible for β-lactam resistance; IS431 = integrated plasmid that encodes tetracycline resistance; and *orfX* = open reading frame in which the mobile elements (staphylococcal cassette chromosome) are located.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study sites
The study was conducted at Mbagathi District Hospital with HIV and non-HIV admissions; two children’s homes, Cottolengo and Thomas Barnardos that admit HIV infected and non-infected patients respectively and a rural hospital with HIV adult patients (Alupe sub-District hospital) bordering Kenya and Uganda.

3.2 Ethical consideration
The larger cohort study in which this proposal is an elaborated addendum was given ethical clearance by the KEMRI Ethical Review Committee (SSC No. 704; 741; and 751). The aim of the current study was to provide a more comprehensive analysis of staphylococcal infection among patients enrolled in the original protocols of the KEMRI/JICA program on diagnosis and management of opportunistic infections. Culture and drug susceptibility test results from this study were made available to the clinicians managing the patients for appropriate action. Any publication arising from the study will bear no patient name or identity.

3.3 Study population
The patients in this study were cohorts of three on-going observational studies (KEMRI/JICA project) where children and adults with known HIV status were followed up and examined for the incidence of life threatening clinical events due to opportunistic
infections while being offered standard care. This study narrowed in to suspected cases of bacterial infections and in particularly those found with staphylococcal infections.

3.4 Sample size (n)

The mean prevalence rate was assumed to be 29% MRSA, this was as per the National Nosocomial Infection Surveillance System (Voss et al., 1994). The target number of samples (n) was calculated as shown below.

\[ n \geq \frac{Z^2pq}{d^2} \]

where \( n \) was the minimum sample size required; \( Z = 1.96 \) - the standard error; \( p = 0.29 \) - prevalence of MRSA (Voss et al., 1994.); \( q = 0.71 \) (1-\( p \)); and \( d = 0.05 \) - absolute precision. Therefore \( n \geq (1.96)^2 (0.29) (0.71) / (0.05)^2 = 316 \).

3.5 Study design

This was an analytical, cross sectional study in which patients, clinically suspected of bacterial infections (staphylococcal infections) were taken appropriate specimen for microbiological culture and \( mecA \) gene analysis.

3.6 Sampling

Specimens from both HIV infected and non-infected patients including wound swabs, blood, sputum, lung aspirates and any other suspected of staphylococcal infection were cultured for bacterial isolation. All the \( Staphylococcus aureus \) isolates from Mbagathi District Hospital and the two homes were considered to be from an urban setting while those from Alupe sub-District Hospital were considered as from a rural setting. Bacterial culture and isolation from patients seen at the urban setting was carried out at the
Opportunistic Infections laboratory (OI - KEMRI/JICA) in Nairobi while specimens from Alupe were analysed at KEMRI/JICA annexed laboratory in KEMRI-Alupe. Identification and re-identification of staphylococcal isolates from Busia, detection of \textit{mecA} gene by PCR and PBP 2’ by latex agglutination was conducted in KEMRI/JICA OI laboratory in Nairobi.

3.7 Sample analysis

3.7.1 Specimen transportation

All the specimens were collected by qualified laboratory technologists/clinicians. In case of delay, transport media such as Carry Blair and AMIES, were used and for emergency specimens such as cerebrospinal fluid and blood, the culture was done on site by qualified laboratory technologists.

3.7.2 Specimen culturing

The specimens were carried in Amies transport media and transported immediately to the laboratory. Culture was performed using routine bacteriological procedures on mannitol salt and blood agar media (BA) and incubated aerobically at 35°C for 18 to 24 hours.

3.7.3 Identification of \textit{S. aureus}

The suspected \textit{Staphylococcus} colonies appearing yellowish in mannitol (mannitol fermentation) medium with β-haemolysis on BA was gram stained, set coagulase test and confirmation done using analytical profile index (API) Staph (Biomerieux) (Chambers, 1997).
Quality control of the test system was performed by controlling some of the major factors influencing or affecting drug susceptibility test result such as:

a) Thiamidine and thiamine concentrations
High concentrations of thymine or thymidine can reverse the inhibitory effect (antagonistic) of sulphonamides and Trimethoprim. The batch of the medium was tested against *Enterococcus faecalis* ATCC 29212 with a clear distinct inhibition zone size of ≥ 20 mm in diameter.

b) Divalent cations
Variation of cations, mainly Ca^{++} and Mg^{++} affect results of susceptibility of all bacteria to tetracycline, aminoglycosides and fluoroquinolones. Each batch was therefore tested to ensure a concentration of 20 – 25 mg/L for Ca^{++} and 10 – 12.5 mg/L for Mg^{++}. This was done by testing *P. aeruginosa* ATCC 27853 against gentamicin disk 10mcg and ensuring a zone size diameter of 18 – 19 mm.

c) pH
High pH concentration decreases susceptibility to penicillins and tetracyclines while low pH concentration increases susceptibility to macrolides and aminoglycosides. The pH of the medium was therefore adjusted to 7.2 – 7.4 using appropriate buffer.

d) Concentration of drugs
The concentration of the antimicrobial sensitivity drugs were tested using standard microorganisms ie *S. aureus* ATCC 25923 for disk diffusion and 29213 for the MIC, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 as per the
NCCLS recommendation. *S. aureus* ATCC 25923 and 33592 were used as negative and positive controls for *mec A* gene respectively by the PCR.

### 3.7.5 Preparation of media and test inoculum concentrations

Mueller Hinton medium was prepared by autoclaving at 121°C for 15 minutes and kept at 45°C in water bath. E-test was performed by pouring the medium to a depth of 4.0 ± 0.5 mm and left to set on a clean flat bench. A sterile, non-toxic swab was dipped into the inoculum suspension prepared at 0.5 MacFarland standard and excess fluid removed by pressing the swab against the inside wall of the test tube. The entire agar surface was swabbed three times, rotating the plate approximately 90 degrees each time to ensure an even distribution of the inoculum. Excess moisture was left to dry for about 10 to 15 minutes to ensure the surface of the medium is completely dry before the application of the E-test strips. The strips were then introduced on the agar surface with a clean forceps with the MIC scale facing upward and maximum concentration nearer the rim of the plate.

Disk diffusion was performed by preparing 0.5 MacFarland standard of the test organism and inoculating on the Mueller Hinton plates with the medium poured to a depth of 4.0 ± 0.5 mm and left to set on a clean flat bench. The drugs, impregnated in disks, were then dispensed on the medium surface using an 8-drug-dispenser. The plates were then incubated at 35°C in air for 18 to 24 hours. The zones of inhibition were measured in millimetres using a ruler, recorded and interpreted as per the National Committee for Clinical Laboratory Standards (NCCLS, 2003).
3.7.6 Antimicrobial susceptibility testing

Confirmed *Staphylococcus aureus* isolates were subjected to antimicrobial susceptibility testing using disk diffusion for immediate patient treatment and management before pooled for minimum inhibitory concentration (MIC) testing using E-test and interpreted as per the National Committee for Clinical Laboratory Standards (NCCLS, 2003). MIC of oxacillin, gentamycin, amoxicillin clavulanic acid, chloramphenicol, erythromycin, tetracycline, cefotaxime, sulfamethoxazole trimethoprim and vancomycin were determined. The resistance rate was calculated as the total number of isolates minus those sensitive. Multidrug resistance was defined as resistance to oxacillin and or cefotaxime plus three or more of the drugs (Hong *et al.*, 2004; Jan & John, 2002).

3.8 Determination of *mecA* gene by PCR

3.8.1 DNA extraction

The test organism was grown on Brain Heart Infusion broth (BHI) overnight then centrifuged at 10,000 rpm for 5 minutes at root temperature. The supernatant was poured out and sedimented cells re-suspended in 1 ml of TE buffer and then vortexed to mix. After this, 200μl was transferred to a new sterile tube and boiled for 30 minutes to release the DNA. The suspension was centrifuged at 15,000 rpm. for 10 minutes and then the supernatant used as template DNA for PCR. PuRe Taq Ready-To-Go PCR beads (Amersham biosciences) with a total reaction volume of 25μl, was used in the PCR run using the following primer pair: (F- 5’GGTGGTTACAACGTACAG-3’= 0.2μl; R- 5’GCATTGTAGCTAGCCATTCC-3’= 0.2μl; the template DNA = 1.0μl; with sterile distilled water of 23.6μl (Anne-Merethe *et al.*, 2004).
3.8.2 PCR conditions

The PCR programme consisted of initial denaturation step of 3 minutes at 94°C followed by a further 30-seconds of denaturation at 94°C; annealing step at 55°C for 30-seconds and then extension at 72°C for 30-seconds. The reaction took a complete 35 cycles.

3.8.3 Detection of PCR products

The PCR product was detected through gel electrophoresis using 1.5% agarose (TAKARA) against standard molecular base pair (100 bp) ladder and photographed under ultraviolet rays.

3.9 Detection of penicillin-binding protein 2a (PBP 2a)

Latex agglutination test kit (DenkaSeiken Co Ltd., Japan) was used to detect the penicillin binding protein 2a (PBP 2'). A loopful of *S. aureus* test colonies was obtained fresh from SBA and suspended in 200μl of extraction reagent 1 (0.1M NaOH). The suspension boiled for 3 minutes, cooled and reagent 2 (0.5 M KH₂PO₄) added to each of the test tubes (Reagent 2 neutralizes the alkaline reaction of reagent 1). The mixture was centrifuged at 10,000 rpm for 5 minutes at room temperature. This was followed by placing 50μl of each supernatant on the agglutinating slides and mixing with one drop of anti PBP2' monoclonal antibody-sensitized latex. The slides were then placed on a shaker for three minutes; agglutination when visualized was scored as positive. This test was carried out alongside known MRSA and MSSA as positive and negative control organisms respectively.
3.10 Data analysis

Demographic characteristics of the HIV infected and non-HIV infected patients were entered and analyzed using EPI INFO 2000 (CDC, ATLANTA). Analysis of variance on multiple proportions was performed using Chi-square test.
4.1 Demographic characteristics of respondents

A total number of 436 patients, clinically suspected of staphylococcal infection were recruited from the four study sites. There were 57.1% males and 42.9% females. Out of these patients, 220 were HIV-infected and 216 HIV non-infected. The number of HIV infected and non-infected children were 76 and 160 while for adults was 144 and 56 respectively as shown in Table 1.

Table 1. Number of patients by HIV status

<table>
<thead>
<tr>
<th>Patients</th>
<th>HIV positive</th>
<th>HIV negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>76</td>
<td>160</td>
<td>236</td>
</tr>
<tr>
<td>Adults</td>
<td>144</td>
<td>56</td>
<td>200</td>
</tr>
<tr>
<td>Total</td>
<td>220</td>
<td>216</td>
<td>436</td>
</tr>
</tbody>
</table>

The age of the patients studied ranged from one year to over sixty years with majority (70%) falling below 30 years (Tables 2). The specimens analysed included wound swabs, sputum, blood, urine, cerebrospinal fluid (CSF), broncho-alveolar lavarge (BAL) and lung aspirate which added up to 486. Although the specimens collected had some of the patients giving more than one specimen, all the identified isolates were consecutively
non-duplicated and were either from superficial lesions or cases of systemic infections such as pneumonia, urinary tract, bacteraemia and meningitis (Figure 2).

Figure 2. Percentage *S. aureus* isolates, the MRSA and MSSA by specimen source (superficial lesions and deep seated infections such as cases from pneumonia, bacteraemia, meningitis and urinary tract infections).
4.2 Prevalence of *Staphylococcus aureus*

The prevalence rate of *S. aureus*, based on consecutively non-duplicated isolates was 118/436 (27.1%) among the patients recruited with MSSA accounting for 20% and MRSA 7.1%. The isolation rates of MRSA in males and females were 3.9% and 3.2% respectively, with a prevalence variation of 3.6% to 13.2% by the four institutions where the patients were drawn from (Tables 2 & 3).

### Table 2. *S. aureus* isolates by age group and gender

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Male</th>
<th>MRSA</th>
<th>Female</th>
<th>MRSA</th>
<th>*MSSA</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>01-09</td>
<td>87</td>
<td>9</td>
<td>43</td>
<td>5</td>
<td>35</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(3.2%)</td>
</tr>
<tr>
<td>10-19</td>
<td>73</td>
<td>3</td>
<td>33</td>
<td>1</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.9%)</td>
</tr>
<tr>
<td>20-29</td>
<td>41</td>
<td>4</td>
<td>28</td>
<td>2</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.4%)</td>
</tr>
<tr>
<td>30-39</td>
<td>19</td>
<td>1</td>
<td>45</td>
<td>2</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.7%)</td>
</tr>
<tr>
<td>40-49</td>
<td>21</td>
<td>0</td>
<td>25</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.5%)</td>
</tr>
<tr>
<td>50-59</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.2%)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>249</td>
<td>17</td>
<td>187</td>
<td>14</td>
<td>87</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(3.9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(3.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(20%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(7.1%)</td>
</tr>
</tbody>
</table>

- Total number of MSSA from both male and female.
Figure 3. Shows the distribution of *S. aureus* strains (MRSA and MSSA) by age group in years. Age group between 0-9 had the highest total recovery of MSSA and MRSA (41.5%).
The isolation rate of \textit{S. aureus} in children and adults was 64/281 (22.8\%) and 54/205 (26.3\%) respectively with more than half of the isolates from superficial lesions. Most of the isolates (73.7\%) were recovered from below 30 years age group (Tables 3 & 4).

**Table 3. Number of \textit{S. aureus} isolates by HIV status and the study sites**

<table>
<thead>
<tr>
<th>Source/site</th>
<th>Number of</th>
<th></th>
<th>MSSA</th>
<th>MRSA</th>
<th>HIV status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottolengo Children's home</td>
<td>76</td>
<td>118</td>
<td>24</td>
<td>10</td>
<td>HIV positive children</td>
</tr>
<tr>
<td>Busia sub District hospital</td>
<td>50</td>
<td>50</td>
<td>13</td>
<td>*4</td>
<td>HIV positive adults</td>
</tr>
<tr>
<td>Mbagathi District hospital</td>
<td>94</td>
<td>99</td>
<td>17</td>
<td>**7</td>
<td>HIV positive adults</td>
</tr>
<tr>
<td>Mbagathi District hospital</td>
<td>56</td>
<td>56</td>
<td>11</td>
<td>2</td>
<td>HIV negative adults</td>
</tr>
<tr>
<td>ThomasBenardos children’s home</td>
<td>160</td>
<td>163</td>
<td>22</td>
<td>8</td>
<td>HIV negative children</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>436</strong></td>
<td><strong>486</strong></td>
<td><strong>87</strong></td>
<td><strong>31</strong></td>
<td></td>
</tr>
</tbody>
</table>

* Three of the strains were VISA; ** Two of the strains were VISA.
### Table 4. *S. aureus* isolation by specimens

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Number of specimens</th>
<th>Number of isolates</th>
<th>% MSSA</th>
<th>% MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound swabs</td>
<td>218</td>
<td>68</td>
<td>42.4</td>
<td>15.3</td>
</tr>
<tr>
<td>Sputum</td>
<td>152</td>
<td>34</td>
<td>22.9</td>
<td>5.9</td>
</tr>
<tr>
<td>Blood</td>
<td>57</td>
<td>5</td>
<td>3.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Urine</td>
<td>51</td>
<td>8</td>
<td>4.2</td>
<td>2.5</td>
</tr>
<tr>
<td>CSF</td>
<td>5</td>
<td>1</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>Lung aspirates</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>*1.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>486</strong></td>
<td><strong>118</strong></td>
<td><strong>73.7</strong></td>
<td><strong>26.3</strong></td>
</tr>
</tbody>
</table>

* The isolates had fatal effect on the patients.

The prevalence rate of *S. aureus* in HIV-infected group was significantly higher 75/220 (34.1%) in the HIV non-infected patients 43/216 (20%), odds ratio: 2.081, 95% CI 1.347 – 3.215, P < 0.001 while the MRSA rate in HIV infected was 9.6% as compared to the HIV non-infected 4.6%, odds ratio: 2.174, 95% CI, 0.999-4.732, P=0.046 (Figure 4).
Figure 4. The isolation rate of *S. aureus* by HIV status. The strains in HIV infected patients was 75/220 (34.1%) which is significantly higher than 43/216 (20%) in the HIV non-infected group.

The prevalence of *S. aureus* was higher in HIV infected children 34/76 (44.7%) compared to HIV non-infected children 30/160 (18.8%), p<0.001 with MRSA at
P=0.046. The MRSA prevalence rates in children and adult groups were 7.6% and 6.5%, respectively (Table 4 & Figure 5).

\[ \text{Figure 5. Percentage staphylococcal isolates in children and adults by HIV status. The HIV infected children showed higher isolation rate 34/76 (44.7\%) than in the HIV non-infected children 30/160 (18.8\%) with no difference in the adult population.} \]

The overall prevalence rate of MRSA was 31/118 (26.3\%). Of these, the recovery from superficial lesions was 15.3\% while deep seated wounds or cases of pneumonia, urinary
tract infections, bacteraemia and meningitis added up to 11% (Table 4). Two cases of severe pneumonia in HIV positive children had MRSA with fatal effects on the patients.

4.3 Antibiotic susceptibility profiles of the isolates

The *S. aureus* isolates were found to be highly sensitive to vancomycin with 4.3% intermediate resistance having an MIC of >8 microgram/ml and more than 70% of the isolates concentrated at MIC between one and four microgram/ml (Figure 6). The isolate distributions by other drugs MIC are shown in appendix 3.

![Figure 6. *S. aureus strains* distribution by vancomycin MIC. The majority of the strains concentrated between 1 - 3 μg/ml.](image-url)
The second most effective drug after vancomycin, was amoxicillin-clavulanic acid with a sensitivity of 76.9% of the isolates, followed by chloramphenicol 73.5%. The sensitivity to oxacillin and cefotaxime were both at 70.3% with gentamycin and tetracycline at 60.5% and 54.8% respectively. Low sensitivity rates of the isolates were seen in co-trimoxazole 42.7% and erythromycin 41.1% (Table 5). It was noted that 23 (19.5%) of the isolates were resistant to oxacillin plus three or more of the drugs tested of which 21 were MRSA. Two of the strains were \textit{mecA} gene negative and were resistant to four of the drugs including oxacillin. Of the 21 MRSA, six strains were resistant to 7 drugs, six to 6 drugs, six to 5 drugs and three to four drugs.
Table 5. Drug susceptibility test results of *Staphylococcus aureus* isolates among the HIV infected and HIV non-infected patients

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Breakpoints</th>
<th>No. of isolates</th>
<th>% R</th>
<th>% I</th>
<th>% S</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>Gm</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>S&lt;= 2 R&gt;=4</td>
<td>118</td>
<td>29.7</td>
<td>0.0</td>
<td>70.3</td>
<td>0.5</td>
<td>64</td>
<td>1.60</td>
<td>0.125 - 512</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>S&lt;= 8 R&gt;=64</td>
<td>118</td>
<td>22.0</td>
<td>7.6</td>
<td>70.3</td>
<td>4</td>
<td>192</td>
<td>9.93</td>
<td>0.38 - 512</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>S&lt;= 4 R&gt;=32</td>
<td>117</td>
<td>0.0</td>
<td>4.3</td>
<td>95.7</td>
<td>1.5</td>
<td>3</td>
<td>1.31</td>
<td>0.064 -12</td>
</tr>
<tr>
<td>Amoxicillin clavulanic acid</td>
<td>S&lt;= 8 R&gt;=32</td>
<td>117</td>
<td>6.8</td>
<td>16.2</td>
<td>76.9</td>
<td>4</td>
<td>6</td>
<td>2.70</td>
<td>0.064 - 96</td>
</tr>
<tr>
<td>Trim/Sulphamethoxazole</td>
<td>S&lt;= 2 R&gt;=4</td>
<td>117</td>
<td>55.6</td>
<td>1.7</td>
<td>42.7</td>
<td>4</td>
<td>64</td>
<td>3.57</td>
<td>0.016 - 512</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>S&lt;= 0.5 R&gt;=8</td>
<td>112</td>
<td>36.6</td>
<td>22.3</td>
<td>41.1</td>
<td>1.5</td>
<td>512</td>
<td>4.88</td>
<td>0.047 - 512</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>S&lt;= 8 R&gt;=32</td>
<td>117</td>
<td>17.9</td>
<td>8.5</td>
<td>73.5</td>
<td>4</td>
<td>32</td>
<td>4.71</td>
<td>0.125 - 128</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S&lt;= 4 R&gt;=16</td>
<td>115</td>
<td>37.4</td>
<td>7.8</td>
<td>54.8</td>
<td>2</td>
<td>64</td>
<td>2.69</td>
<td>0.064 - 512</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>S&lt;= 4 R&gt;=16</td>
<td>114</td>
<td>16.7</td>
<td>22.8</td>
<td>60.5</td>
<td>1</td>
<td>16</td>
<td>1.82</td>
<td>0.031 - 256</td>
</tr>
</tbody>
</table>

The table shows percentage resistance (R), intermediate resistance (I) and sensitive (S) with minimum inhibitory concentration at 50 and 90%, a geometric mean (GM) and concentration range. Oxacillin resistance which indicates MRSA among the population studied was at 29.7%.
### Table 6. Drug susceptibility test results of *Staphylococcus aureus* isolates in HIV positive patients

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Breakpoints</th>
<th>No. of isolates</th>
<th>% R</th>
<th>% I</th>
<th>% S</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>Gm</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>S&lt;= 2 R&gt;=4</td>
<td>75</td>
<td>32.0</td>
<td>0.0</td>
<td>68.0</td>
<td>0.5</td>
<td>96</td>
<td>1.84</td>
<td>0.125 – 512</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>S&lt;= 8 R&gt;=64</td>
<td>75</td>
<td>26.7</td>
<td>6.7</td>
<td>66.7</td>
<td>4</td>
<td>192</td>
<td>11.78</td>
<td>2.0 – 512</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>S&lt;= 4 R&gt;=32</td>
<td>74</td>
<td>0.0</td>
<td>6.8</td>
<td>93.2</td>
<td>1.5</td>
<td>3</td>
<td>1.41</td>
<td>0.064 – 12</td>
</tr>
<tr>
<td>Amoxicillin clavulanic acid</td>
<td>S&lt;= 8 R&gt;=32</td>
<td>74</td>
<td>8.1</td>
<td>16.2</td>
<td>75.7</td>
<td>4</td>
<td>6</td>
<td>2.77</td>
<td>0.064 – 96</td>
</tr>
<tr>
<td>Trim/Sulphamethoxazole</td>
<td>S&lt;= 2 R&gt;=4</td>
<td>75</td>
<td>49.3</td>
<td>1.3</td>
<td>49.3</td>
<td>3</td>
<td>64</td>
<td>2.33</td>
<td>0.016 – 64</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>S&lt;= 0.5 R&gt;=8</td>
<td>69</td>
<td>34.8</td>
<td>21.7</td>
<td>43.5</td>
<td>1.0</td>
<td>512</td>
<td>3.95</td>
<td>0.047 – 512</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>S&lt;= 8 R&gt;=32</td>
<td>74</td>
<td>18.9</td>
<td>9.5</td>
<td>71.6</td>
<td>4</td>
<td>32</td>
<td>5.05</td>
<td>0.125 – 128</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S&lt;= 4 R&gt;=16</td>
<td>73</td>
<td>37.0</td>
<td>6.8</td>
<td>56.2</td>
<td>0.5</td>
<td>64</td>
<td>2.35</td>
<td>0.064 – 512</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>S&lt;= 4 R&gt;=16</td>
<td>72</td>
<td>19.1</td>
<td>19.4</td>
<td>62.5</td>
<td>0.5</td>
<td>16</td>
<td>1.69</td>
<td>0.031 – 256</td>
</tr>
</tbody>
</table>

The table shows percentage resistance (R), intermediate resistance (I) and sensitive (S) with minimum inhibitory concentration (MIC) at 50 and 90%, a geometric mean (GM) and concentration range. Vancomycin intermediate resistance is 6.8% among this population.
Table 7. Drug susceptibility test results of *Staphylococcus aureus* isolates in HIV negative patients

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Breakpoints</th>
<th>No. of isolates</th>
<th>% R</th>
<th>% I</th>
<th>% S</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>Gm</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>S&lt;= 2 R&gt;=4</td>
<td>43</td>
<td>25.6</td>
<td>0.0</td>
<td>74.4</td>
<td>0.38</td>
<td>512.0</td>
<td>1.59</td>
<td>0.125 – 512.0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>S&lt;= 8 R&gt;=64</td>
<td>43</td>
<td>18.6</td>
<td>4.7</td>
<td>76.7</td>
<td>4.0</td>
<td>192.0</td>
<td>8.0</td>
<td>0.38 – 512.0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>S&lt;= 4 R&gt;=32</td>
<td>43</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
<td>1.5</td>
<td>2.0</td>
<td>1.15</td>
<td>0.064 – 4.0</td>
</tr>
<tr>
<td>Amoxicillin clavulanic acid</td>
<td>S&lt;= 8 R&gt;=32</td>
<td>43</td>
<td>4.7</td>
<td>16.3</td>
<td>79.1</td>
<td>4</td>
<td>6.0</td>
<td>2.58</td>
<td>0.125 – 12.0</td>
</tr>
<tr>
<td>Trim/Sulphamethoxazole</td>
<td>S&lt;= 2 R&gt;=4</td>
<td>42</td>
<td>66.7</td>
<td>2.4</td>
<td>31.0</td>
<td>64.0</td>
<td>64.0</td>
<td>7.68</td>
<td>0.016 – 64.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>S&lt;= 0.5 R&gt;=8</td>
<td>43</td>
<td>39.5</td>
<td>23.3</td>
<td>37.2</td>
<td>3.0</td>
<td>512.0</td>
<td>6.85</td>
<td>0.125 – 512.0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>S&lt;= 8 R&gt;=32</td>
<td>43</td>
<td>16.3</td>
<td>7.0</td>
<td>76.7</td>
<td>4</td>
<td>32.0</td>
<td>4.18</td>
<td>0.38 – 64.0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S&lt;= 4 R&gt;=16</td>
<td>42</td>
<td>38.1</td>
<td>9.5</td>
<td>52.4</td>
<td>4.0</td>
<td>128.0</td>
<td>3.40</td>
<td>0.094 – 512.0</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>S&lt;= 4 R&gt;=16</td>
<td>42</td>
<td>14.3</td>
<td>28.6</td>
<td>57.1</td>
<td>3.0</td>
<td>16.0</td>
<td>2.07</td>
<td>0.064 – 128.0</td>
</tr>
</tbody>
</table>

The table shows percentage resistance (R), intermediate resistance (I) and sensitive (S) with minimum inhibitory concentration (MIC) at 50 and 90%, a geometric mean (GM) and concentration range. There was no VISA in this group.
### Table 8. Statistical comparison of antibiogram by HIV status

<table>
<thead>
<tr>
<th>Drugs</th>
<th>HIV positive</th>
<th>HIV negative</th>
<th>X² - test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% sensitivity</td>
<td>No.</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>75</td>
<td>68.0</td>
<td>43</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>75</td>
<td>66.7</td>
<td>43</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>74</td>
<td>93.2</td>
<td>43</td>
</tr>
<tr>
<td>Amoxicillin clavulanic acid</td>
<td>74</td>
<td>75.7</td>
<td>43</td>
</tr>
<tr>
<td>Trim/Sulphamethoxazole</td>
<td>75</td>
<td>49.3</td>
<td>42</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>69</td>
<td>43.5</td>
<td>43</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>74</td>
<td>71.6</td>
<td>43</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>73</td>
<td>56.2</td>
<td>42</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>72</td>
<td>62.5</td>
<td>42</td>
</tr>
</tbody>
</table>

The table shows no statistical difference in susceptibility test profile of the isolates from HIV infected and HIV non-infected patients observed.
Figure 7. Antibiogram by HIV status showing susceptibility test result to ox – oxacillin; ce – cefotaxime; va – vancomycin; ac – amoxicillin clavulanic acid; ts – Trimethoprim sulfamethoxazole (co-trimoxazole); er – erythromycin; cl – chloramphenicol; te – tetracycline; ge – gentamycin. There was no statistical difference between the two groups.
Table 9. Drug susceptibility test results of *Staphylococcus aureus* isolates from children

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Breakpoints</th>
<th>No. of isolates</th>
<th>% R</th>
<th>% I</th>
<th>% S</th>
<th>MIC$_{50}$</th>
<th>MIC$_{90}$</th>
<th>Gm</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>S &lt;= 2 R &gt;=4</td>
<td>64</td>
<td>29.7</td>
<td>0.0</td>
<td>70.3</td>
<td>0.38</td>
<td>512.0</td>
<td>1.92</td>
<td>0.125 - 512.0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>S &lt;= 8 R &gt;=64</td>
<td>64</td>
<td>21.9</td>
<td>7.8</td>
<td>70.3</td>
<td>4.0</td>
<td>192.0</td>
<td>10.23</td>
<td>2.0 - 512.0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>S &lt;= 4 R &gt;=32</td>
<td>64</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
<td>1.5</td>
<td>3.0</td>
<td>1.3</td>
<td>0.064 - 4.0</td>
</tr>
<tr>
<td>Amoxicillin clavulanic acid</td>
<td>S &lt;= 8 R &gt;=32</td>
<td>64</td>
<td>10.9</td>
<td>20.3</td>
<td>68.8</td>
<td>4.0</td>
<td>12.0</td>
<td>3.83</td>
<td>0.064 - 96.0</td>
</tr>
<tr>
<td>Trim/Sulphamethoxazole</td>
<td>S &lt;= 2 R &gt;=4</td>
<td>63</td>
<td>65.1</td>
<td>1.6</td>
<td>33.3</td>
<td>64.0</td>
<td>64.0</td>
<td>7.35</td>
<td>0.031 - 64.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>S &lt;= 0.5 R &gt;=8</td>
<td>64</td>
<td>45.3</td>
<td>15.6</td>
<td>39.1</td>
<td>2.0</td>
<td>512.0</td>
<td>8.53</td>
<td>0.19 - 512.0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>S &lt;= 8 R &gt;=32</td>
<td>64</td>
<td>17.2</td>
<td>7.8</td>
<td>75.0</td>
<td>4.0</td>
<td>32.0</td>
<td>4.81</td>
<td>0.38 - 64.0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S &lt;= 4 R &gt;=16</td>
<td>63</td>
<td>44.4</td>
<td>9.5</td>
<td>46.0</td>
<td>8.0</td>
<td>96.0</td>
<td>3.59</td>
<td>0.064 - 512.0</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>S &lt;= 4 R &gt;=16</td>
<td>63</td>
<td>19.0</td>
<td>33.3</td>
<td>47.6</td>
<td>8.0</td>
<td>16.0</td>
<td>2.94</td>
<td>0.125 - 256.0</td>
</tr>
</tbody>
</table>

The table shows percentage resistance (R), intermediate resistance (I) and sensitive (S) with minimum inhibitory concentration (MIC) at 50 and 90%, a geometric mean (GM) and concentration range. Isolates from this group were all susceptible to vancomycin with 29.7% sensitive to oxacillin.
Table 10. Drug susceptibility test results of *Staphylococcus aureus* isolates from adults

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Breakpoints</th>
<th>No. of isolates</th>
<th>% R</th>
<th>% I</th>
<th>% S</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>Gm</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>S&lt;= 2 R&gt;=4</td>
<td>54</td>
<td>29.6</td>
<td>0.0</td>
<td>70.4</td>
<td>0.5</td>
<td>512.0</td>
<td>2.2</td>
<td>0.125 - 512.0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>S&lt;= 8 R&gt;=64</td>
<td>54</td>
<td>24.1</td>
<td>1.9</td>
<td>74.1</td>
<td>4.0</td>
<td>512.0</td>
<td>11.25</td>
<td>0.38 - 512.0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>S&lt;= 4 R&gt;=32</td>
<td>53</td>
<td>0.0</td>
<td>9.4</td>
<td>90.6</td>
<td>1.5</td>
<td>4.0</td>
<td>1.32</td>
<td>0.125 - 12.0</td>
</tr>
<tr>
<td>Amoxicillin clavulanic acid</td>
<td>S&lt;= 8 R&gt;=32</td>
<td>53</td>
<td>1.9</td>
<td>11.3</td>
<td>86.8</td>
<td>3.0</td>
<td>6.0</td>
<td>1.77</td>
<td>0.125 - 96.0</td>
</tr>
<tr>
<td>Trim/Sulphamethoxazole</td>
<td>S&lt;= 2 R&gt;=4</td>
<td>54</td>
<td>44.4</td>
<td>1.9</td>
<td>53.7</td>
<td>0.5</td>
<td>64.0</td>
<td>1.54</td>
<td>0.016 - 512.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>S&lt;= 0.5 R&gt;=8</td>
<td>48</td>
<td>25.0</td>
<td>31.2</td>
<td>43.8</td>
<td>1.5</td>
<td>512.0</td>
<td>2.32</td>
<td>0.47 - 512.0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>S&lt;= 8 R&gt;=32</td>
<td>53</td>
<td>18.9</td>
<td>9.4</td>
<td>71.7</td>
<td>4.0</td>
<td>64.0</td>
<td>4.60</td>
<td>0.125 - 128.0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S&lt;= 4 R&gt;=16</td>
<td>52</td>
<td>28.8</td>
<td>5.8</td>
<td>65.4</td>
<td>0.5</td>
<td>48.0</td>
<td>1.90</td>
<td>0.125 - 512.0</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>S&lt;= 4 R&gt;=16</td>
<td>51</td>
<td>13.7</td>
<td>9.8</td>
<td>76.5</td>
<td>0.5</td>
<td>16.0</td>
<td>1.01</td>
<td>0.031 - 256.0</td>
</tr>
</tbody>
</table>

showing percentage resistance (R), intermediate resistance (I) and sensitive (S) with minimum inhibitory concentration (MIC) at 50 and 90%, a geometric mean (GM) and concentration range. Isolates from this group had 9.4% VISA.

Apart from the 5 (9.4%) strains which showed VISA resistance in the adult patients, sensitivity of the isolates from children and adults population had more or less the same profile (Table 9 and 10).
4.4 MRSA detection

A total of the 118 S. aureus isolates were tested for *mecA* gene and realized 31 positive isolates. The bimodal distribution of MIC and zone diameters by different phenotypic methods and the correlation between phenotypic test results and the *mecA* gene gave 38 MRSA by oxacillin disk diffusion and 40 MRSA by cefotaxime disk diffusion. E-test for oxacillin and cefotaxime had 35 and 26 respectively. The monoclonal latex agglutination test showed 29 PBP 2' positive (Table 11, Figure 8).
Figure 8. Gel photomicrograph of *mecA* gene PCR product showing well number 1 with 100 bp ladder; 2, 3, 7-10 and 12-16 are negative clinical isolates for *mecA*; 4 is a positive *mecA* (*S. aureus* ATCC 33592); 5, 6 and 11 are positive clinical isolates for *mecA*.; well 17 negative control for *mecA* gene (*S. aureus* ATCC 25923).
Table 11. Correlation between the test systems and presence of \textit{mecA} gene for the 118 \textit{S. aureus} strains (\textit{MRSA + MSSA})

<table>
<thead>
<tr>
<th>PCR meca classification</th>
<th>Phenotypic Ox (1\textmu g)</th>
<th>Ox E-test</th>
<th>Cef (30 \textmu g)</th>
<th>Cef E-test</th>
<th>LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive ( (n=31) )</td>
<td>R</td>
<td>31</td>
<td>31</td>
<td>26</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Negative ( (n=87) )</td>
<td>R</td>
<td>7</td>
<td>4</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>80</td>
<td>83</td>
<td>78</td>
<td>87</td>
</tr>
</tbody>
</table>

The gold standard PCR for \textit{mecA} gene analysis gave 31 isolates positive, the oxacillin (Ox) and cefotaxime (Cef) disk diffusion gave 39 and 40 isolates resistant while E-test gave 35 and 26 respectively while latex agglutination test for penicillin binding protein 2' (PBP 2') gave 29 isolates positive.

The performance of the test systems for MRSA was compared with PCR as the gold standard. Sensitivity of oxacillin by MIC and disk diffusion was 100% with a specificity of 95.4% and 92% respectively. Cefotaxime had an MIC sensitivity of 83.9% and specificity of 100% while disk diffusion sensitivity and specificity was 100% and 89.7% respectively. Sensitivity and specificity of the latex agglutination was 93.6% and 100% respectively. The predictive values of disk diffusion for oxacillin and cefotaxime were 94.1% and 92.4% with MIC (E-test) of 96.6% and 100% respectively. The latex agglutination was 100% (Table 12).
Table 12. Performance by test systems against standard PCR for *mecA* gene detection

<table>
<thead>
<tr>
<th>Test Systems</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Predictive value %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin (1 μg) disk</td>
<td>100</td>
<td>92</td>
<td>94.1</td>
</tr>
<tr>
<td>Oxacillin MIC (E-test)</td>
<td>100</td>
<td>95.4</td>
<td>96.6</td>
</tr>
<tr>
<td>Cefotaxime (30μg) disk</td>
<td>100</td>
<td>89.7</td>
<td>92.4</td>
</tr>
<tr>
<td>Cefotaxime MIC (E-test)</td>
<td>83.9</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Latex agglutination (for PBP 2')</td>
<td>93.6</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Disk diffusion (oxacillin and cefotaxime) showed the lowest predictive values followed by E-tests and latex agglutination for the PBP 2'.


CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Bacterial resistance has been shown to be an important threat to antimicrobial treatment of infections in the twenty-first century (Smolinski et al., 2003). *S. aureus* has always been a stumbling block for treatment of numerous infections that are caused by this organism at community and hospital environments. The introduction of new classes of antimicrobial agents is usually followed by the emergence of resistant forms of this pathogen (Hiramatsu et al., 2001; Kim et al., 2004). As such, surveillance on the antimicrobial susceptibility patterns of *S. aureus* is of utmost importance in understanding new and emerging resistance trends and more particularly, in the management of opportunistic infections in the HIV infected patients.

This study showed a prevalence rate of *S. aureus* among cases of suspected staphylococcal infections at 27.1% and from an epidemiological point of view, these isolates are considered hospital based (HA-MRSA) since all the patients in the study were drawn from confined institutions offering medical care for a period of more than three months. It is unlike community acquired strains where the source has to be from outside healthcare settings or isolates drawn two days on hospital admission with no prior history of hospitalization within the preceding two years (Salmenlina et al., 2002). The prevalence of MRSA in this study was 26.3% while multi-drug resistance was at 19.5%. MRSA prevalence rate of greater than 25% is an indication of poor
National infection control practices (McDonald et al., 2003). The high prevalence of MRSA and multi-drug resistant strains are likely to complicate health management of staphylococcal diseases particularly the deep and systemic cases by delaying appropriate treatment in resource poor laboratories in developing countries such as Kenya (Rubin et al., 1999). Besides, there is a likelihood of increased morbidity and mortality as a result of lack of proper infection control measures in most public hospitals which control the largest hospital attendance in Kenya. This finding agrees with MRSA prevalence studies done in South Africa, 26.9% (Adebayo & Johnson, 2006) and 29% quoted by the National Nosocomial Infection Surveillance System (NNISS) in USA (Voss et al., 1994). However, in 1994, higher MRSA prevalence rate of 39.8% was reported in Kenya (Omari et al., 1997). This difference could probably be due to the lower specificity (92%) and predictive value (94.1%) associated with oxacillin disk diffusion used by the author as an indicator of MRSA detection, thereby picking and accounting for even those strains of S. aureus which were merely over-expressing β-lactamase enzymes and not necessarily MRSA (Adebayo & Johnson, 2006) as compared to the gold standard PCR (Vannuffel et al., 1998) used in this study.

Studies from free-standing nursing homes and other long-term health-care settings have shown MRSA prevalence rates ranging from 10% to 15% while reports from Nigeria, Kenya, and Cameroon, quoted rates between 21 to 30%, and below 10% in Tunisia, Malta, Tanzania and Algeria (Urassa et al., 1999; Kesa et al., 2003). Several resistance surveillance studies have been conducted which provide comparable and validated results on the status of MRSA. In Europe, a surveillance study including 27 countries across Europe found an overall 20% MRSA prevalence with the highest
proportion in Southern parts of Western Europe and lowest proportion (5%) in Northern Europe (Grandmann, 2004). Besides the use of different test systems for the detection of MRSA, variability could also be as a result of geographic variation of MRSA strains with different virulence or colonization properties or may reflect differences in antimicrobial utilization and infection control practices.

Several risk factors associated with MRSA infections have been described (Boyce, 1998). This study showed a higher isolation rate and a significant difference in the prevalence of staphylococcal infections in HIV infected compared to the non-infected patients (p< 0.001) and MRSA (p= 0.046). A research done in San Diego in 2005, showed an annual incidence of staphylococcal infections among the HIV infected patients as 8-fold higher than in HIV negative patients (Crum-Cianflone, 2006). This could probably be due to the immunosupression in HIV patients and subsequent vulnerability to this opportunistic pathogen which normally resides on human skin. Besides, it could also be related to the low level of infection control practices by healthcare management group in these institutions.

The study showed an overall staphylococcal resistance to oxacillin at 29.7%. This indicates that there may be very few treatment options left for this group since all beta lactam drugs (as first line treatment of staphylococcal infections) can not be effectively used. This finding agrees with the 26.9% reported in South Africa (Adebayo & Johnson, 2006). Although oxacillin resistance in HIV positive group was 32% compared to 26.5% in the HIV negative group, there was no significant
statistical difference in the finding \( (p = 0.46) \). However, the 6.8% vancomycin intermediate resistance in HIV positive adult group suggests a serious public health implication in treatment of these patients whose immune systems are low and may not afford the cost of the limited treatment options available.

Antimicrobial resistance in bacterial pathogens is a significant problem in many countries with severe consequences including increased medical costs, morbidity and mortality (Bouchillon et al., 2004). Since the emergence of \( S. aureus \) strains with resistance to penicillin and methicillin in 1948 and 1961 (Barber & Rozwadowska, 1948; Jevons 1961), it has become a well-known etiologic agent of a wide variety of infections difficult to treat.

Resistance to \( S. aureus \) is exhibited mainly in three forms. The first is the one mediated by \( blaZ \), the gene that encodes \( \beta \)-lactamase. This predominantly extracellular enzyme, synthesized when staphylococci are exposed to \( \beta \)-lactam antibiotics, hydrolyzes the \( \beta \)-lactam ring, rendering the \( \beta \)-lactam inactive. The \( blaZ \) is under the control of two adjacent regulatory genes, the antirepressor \( blaR1 \) and the repressor \( blal \) (Kernodle, 2000). Recent studies have demonstrated that the signalling pathway responsible for \( \beta \)-lactamase synthesis requires sequential cleavage of the regulatory proteins \( blaR1 \) and \( blal \). When a \( \beta \)-lactam agent binds to the extracellular sensor domain of BlaR1, the cytoplasmic transducer domain is proteolytically cleaved. The transducer is then free to cleave and inactivate the BlaI repressor, and the transcription of the \( \beta \)-lactamase gene ensues (Lewis, 1999). It had been hypothesized that the
cleaved protein functions as a protease that cleaves the repressor \textit{bla}, directly or indirectly and allows \textit{blaZ} to synthesize enzyme itself (Gregory \textit{et al.}, 1997; Zhang \textit{et al.}, 2001).

Another resistance mechanism is the presence of the chromosomally localized \textit{mecA} gene which codes for an additional PBP, termed PBP2' or PBP2a that has low affinity for β-lactam antibiotics and substitutes for the native PBPs during cell wall synthesis when they are inhibited by β-lactams (Chambers, 1997). Transcription of the \textit{mecA} gene is induced in some isolates by β-lactams, and such induction is regulated by \textit{mecI} and \textit{mecRl}, a repressor and a signal-transducing protein, respectively (Chambers, 1997). The \textit{mecI} and \textit{mecRl} genes, when present, are carried beside \textit{mecA} on the SCCmec element (Katayama \textit{et al.}, 2000; Ito \textit{et al.}, 2001; Ma \textit{et al.}, 2002). Cross-regulation by \textit{bla} and \textit{blaRl} of \textit{mecA} transcription also occurs, encoded by \textit{bla} and \textit{blaRl} genes carried on the β-lactamase plasmid along with \textit{blaZ} (Chambers, 1997). Until now, the background PBPs in \textit{S. aureus} have been assumed to be constitutively expressed. Recently, constitutive \textit{phpB} transcription has been studied (Pinho \textit{et al.}, 1998), though the factors that control or induce \textit{phpB} gene expression have not yet been explored.

Lastly, there is the borderline resistance, which is a low-level type of resistance to methicillin exhibited by strains which neither produce \textit{mecA} gene nor over express β-lactamase enzyme. Besides the high level of oxacillin resistance attained through acquisition of the \textit{mecA} gene, another resistance mechanism, such as over-expression of β-lactamase, may have accounted for the 7/118 (6%) oxacillin resistance in \textit{S. aureus} which were \textit{mecA} gene negative as have been indicated elsewhere (Jorgensen, 1991). The mechanism(s) responsible for \textit{mecA} transfer is not known, but evidence
supports horizontal transfer of \textit{mec} DNA between staphylococcal species and of the \textit{mecA} gene between different gram-positive genera (Archer & Niemeyer, 1994).

5.2 Conclusions

HIV is a predisposing factor to staphylococcal infections and the multi-drug resistant MRSA and VISA is threatening to return us to the era that preceded the development of antibiotics. The prevalence of MRSA (26.3\%) is high enough to raise concern and is expected to increase because of poor state of diagnostic and infection control systems in our public hospitals. Since five of the MRSA strains were VISA, all strains with oxacillin MIC greater than or equal to 4 \textmu g/mL should be considered candidate strains for reduced vancomycin susceptibility.

Although there was no difference in drug profile between the HIV infected and non-infected, the rise in incidence and few therapeutic options associated with MRSA infections calls for an informed choice in administration of appropriate antibiotics particularly when dealing with sudden, aggressive, deep-seated or systemic conditions in HIV infected patients.

5.3 Recommendations

- There is need to put in place strategies to strengthen and sustain public health laboratories to be able to diagnose and identify correctly the MRSA, VISA and the 6\% MSSA strains with increased MIC to oxacillin for appropriate therapy.
• Although MRSA prevalence is best predicted by use of PCR in epidemiological studies, oxacillin may be suitable when it comes to immediate treatment and management of Staphylococcal diseases since it will inclusively pick the strains which over express β-lactamase enzymes.

• Infection-control program should be prioritized in all public health facilities in Kenya to avert transmission and spread of the multi-drug resistant bugs and precautions to be strictly adhered to when handling the infected or colonized patients.

• To prevent further emergence of *S. aureus* strains with intermediate glycopeptide resistance, the use of vancomycin should be optimized.

• Continuous surveillance on resistance patterns and molecular epidemiology of *S. aureus* in understanding new and emerging trends should be encouraged.

• The isolation of *S. aureus* with confirmed or presumptive vancomycin resistance should be reported immediately through state and local health departments to the relevant WHO offices.
REFERENCES


Division of Healthcare Quality Promotion National Center for Infectious Diseases Centers for Disease Control and Prevention Atlanta, GA November 15, 2005.


Heininger, V., Datta, F., Gervaix, A., Schaad, U.B., Berger, C., Vaudaux, B.,

protein 4 and intrinsic ß-lactam resistance. Antimicrobial Agents Chemotherapy,


Hong, B.K., Hee-Chang, J., Hee, J., Yeong, S.L., Bong, S., Wan, B., Ki, D., Young,
Activities of 28 Antimicrobial Agents against Staphylococcus aureus Isolates from
Tertiary-Care Hospitals in Korea: a Nationwide Survey Antimicrobial Agents and
Chemotherapy. 48(4): 1124–1127.

infections caused by methicillin-resistant Staphylococcus aureus with reduced
vancomycin susceptibility. Clinical Infectious Disease, 38:521-528.

Novel type V staphylococcal cassette chromosome mec driven by a novel cassette

Ito, T., Okuma, K., Ma, X., Yuzawa, H., Hiramatsu K. (2003). Insights on
antibiotic resistance of Staphylococcus aureus from its whole genome: genomic

Ito, T., Katayama, Y., Asada, K., Mori, N., Tsutsumimoto, K., Tiensasitorn, C.,
Hiramatsu, K. (2001). Structural comparison of three types of staphylococcal
cassette chromosome mec integrated in the chromosome in methicillin-resistant

determination of the entire mec DNA of pre-methicillin-resistant Staphylococcus

aureus isolates from hospitalized patients in Asia-Pacific and South Africa: Results

resistant Staphylococcus aureus at a university hospital: one decade later. Infection
Control Hospital Epidemiology, 16:686-696.


APPENDICES

Appendix 1: Consent explanation

Purpose of the study

People who are infected with HIV and who therefore have reduced body defences are at risk of getting disease caused by germs that ordinarily do not cause problems in those with normal defences. These infections are referred to as opportunistic infections. Understanding which infections are common in Kenya is important because it will help in the development of strategies for preventing and treating these infections. This may help HIV infected persons to live a more healthy life and to prevent deaths. Scientists from Kenya Medical Research Institute (KEMRI) together with your doctors are studying the occurrence of infections in both HIV positive and negative patients in your homes/hospital.

Study procedures

Participation

Scientists from KEMRI are conducting a study to determine the spectrum of infections and drug susceptibility profiles that can be used effectively for your treatment. You will be offered routine care in this home/hospital and doctors will be ready to see you when sick. In the clinical event that you have symptoms that suggest you could have infection; your doctor may require appropriate specimens such as drawing of about 5 ml of blood from adults and about 2 ml from children collected once. Apart from blood, the processes of sampling other specimens involve harmless procedures and require little amount such as 5 ml of urine, 5 grams of stool, and 0.5 – 1 ml of sputum for further investigation that will help in administration of effective
treatment of your infections. You may be asked questions about your health and your
weight, height and clinical signs will be recorded.

Voluntary and right to withdraw from the study

Participation in this study is voluntary. Refusal to participate will involve no penalty
or loss of benefits to which you (your child’s) are otherwise entitled. You (your child)
may discontinue your (your child’s) participation at any time without penalty or loss
of benefits.

Risk

The risks from participation in this study are minimal, mild pain and discomfort may
be experienced where the blood and wound swab will be obtained, but this is only
temporary.

Medical care for research related injury

The phlebotomist will use care when drawing blood and ensure cause of as little pain
as possible during the blood draw. Should you (your child) be injured as a direct result
of participation in this research project, you (your child) will be provided medical care,
at no cost to you (your child) for that injury.

Benefits

You will be offered routine care in this home/hospital and doctors will be ready to see
you when sick. In the clinical event that you have symptoms that suggest you could
have infection, your doctor may require to take an appropriate specimens once for
further investigation that will help in knowing the germ causing your problem and find out the best drug to administer for effective treatment of your infection.

Confidentiality of records

The records relating to your (your child’s) participation in the study will remain confidential. Your (your child) name will not be used in any report resulting from this study. All computerized records and laboratory specimens will contain only a unique study number, not your name. However the investigation result will only be availed to the doctor for the purpose of administering appropriate treatment of your disease.

Persons and places for answers in the event of participant’s related injury

If you (your child) has medical problem related to this study, please report to Tom Turbine Ouko, KEMRI P.O.Box 19464 Nairobi, Phone 0722953194.

Persons and places for answers regarding your rights as a participant

If during the course of this study you have questions concerning the nature of the research or you have sustained a research-related injury, you should contact Tom Turbine Ouko P.O.Box 19464 Nairobi, phone 0722953194 or the doctor on duty at the hospital/clinic. The chairman of the Kenya National Ethical Review Committee, c/o Kenya Medical Research Institute, P.O. Box 54840, Nairobi, Kenya. Telephone number 2722541/ 2722672.
 Appendix 2: Declaration form

I understand the study is investigating the germ causing my disease and the suitable
drug of these germs that will lead to a better understanding and appropriate
management strategies of opportunistic infections.

I am being asked to voluntarily participate (self / child under my care / my child)
because I/child may be suffering from some of these germs.

My/child's participation will involve taking of the blood, sputum, swabs, stool, and
urine samples, and undergoing physical examination to evaluate the presence of other
clinical symptoms.

I understand sample collection is a harmless and is being conducted by a qualified
clinician.

I further understand that my/child's participation in this study is voluntarily and I may
withdraw whenever I choose any time unconditionally.

I have read and understood the information stated and I sign this consent form
willingly.

Name........................................Signature............................Date......................

Thumb print......................................................

In-charge/guardian..............................................
Appendix 3: Percentage isolates by MIC.

3a. Oxacillin
3b. Cefotaxime.

[Bar chart showing the number of S. aureus for different MIC values for Cefotaxime.]

3c. Amoxicillin clavulonic acid

[Bar chart showing the number of S. aureus for different MIC values for Amoxicillin clavulonic acid.]
3d. Sulphamethoxazole trimethoprim.

3e. Erythromycin.
3f. Chloramphenicol

3g. Tetracyclin
3h. Gentamycin.