METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM TOILET AND CLASSROOM DOOR HANDLES IN SELECTED SECONDARY SCHOOLS IN NAIROBI COUNTY, KENYA

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Methicillin Resistant *Staphylococcus Aureus* from toilet and classroom door handles in selected secondary schools in Nairobi County, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this thesis to my late mother Eunice Mbogori who always believed that I can do anything I put in my mind and to my son Ritchie Mbogori for giving me hope and encouragement to pursue my goals in life.

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I would like to acknowledge The Great Almighty God the author of knowledge and wisdom, completion of this work would not have been possible was is not because of Him, I salute him.

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ABBREVIATIONS AND ACRONYMS

ATCC	American type culture collection
CA-MRSA	Community Acquired Methicillin Resistant
CDC	Centres for Disease Control *Staphylococcus aureus*
CLSI	Clinical and Laboratory Standards Institute
DNA-SRIP	Deoxyribonucleic acid strip
EMRSA	Epidemic Methicillin Resistant *Staphylococcus aureus*
ERC	Ethical Review Committee
HA-MRSA	Hospital Acquired Methicillin Resistant *Staphylococcus aureus*
KEMRI	Kenya Medical Research Institute
MoE	Ministry of Education
MoH	Ministry of Health
MRSA	Methicillin Resistant *Staphylococcus aureus*
PBP2	Penicillin-binding protein Two prime
PCR	Polymerase Chain Reaction
PVL	Pantone-Valentine leukocidin
SCC	*Staphylococcus* Cassette Chromosome

SSC	Scientific Steering Committee
SSSS	*Staphylococcus* Scalded Skin Syndrome
STATA	Statistics and data
TSS	Toxic Shock Syndrome
VISA	Vancomycin Intermediate *Staphylococcus aureus*
VRSA	Vancomycin Resistant *Staphylococcus aureus*
UK	United Kingdom
US	United States

ABSTRACT

Staphylococcus aureus is found on most surfaces especially in public areas like hospitals and schools and on frequently touched areas like toilet and class room door handles. Methicillin resistant *S. aureus* (MRSA) is a strain of *S. aureus* which is resistant to methicillin. There are two types of MRSA; Community acquired methicillin resistant *S. aureus* (CA-MRSA) and hospital acquired methicillin resistant *S. aureus* (HA-MRSA). MRSA in the community presents a significant reservoir that could enter into healthcare facilities and spread among patients and also is a health risk for immunosuppressed populations in the community. The study aimed at determining the prevalence of MRSA isolated from toilet and classroom door handles as a potential source of infection to the students and the workers in selected schools in Nairobi, Kenya. The study also compared the prevalence of MRSA between day and boarding girls, boys and mixed secondary schools. Twelve secondary schools in Nairobi County were randomly selected and 306 samples from both the toilet and classroom door handles were collected using sterile swabs and transported to the National microbiology reference laboratory (NMRL) at the National Public Health Laboratories (NPHL) for *Staphylococcus* isolation. Sample collection was done over a duration of one month. Isolation of *S. aureus was* done by use of selective media Mannitol salt agar, antibiotic susceptibility of MRSA isolates was done by disk diffusion method and Polymerase chain reaction (PCR) was used to detect genes for methicillin resistance *Mec*A and PVL genes. The prevalence of *S. aureus* was 20% and 19.6% of the *S. aureus* isolated were MRSA positive by both antimicrobial susceptibility test (AST) and PCR detection. MRSA in this study was only found in girls' schools and mixed schools and none in the boys' schools. Twenty percent of *S. aureus* showed the presence of Pantone Valentine Leukocidin (PVL) virulence genes while 8% showed the presence of both genes. Fifty six percent of isolates which contained mec*A* gene had also PVL genes. The presence of both genes in this study indicates that surveillance and researches on MRSA that carry both PVL and *mec*A gene should continue to provide a significant insight into the prevalence and epidemiology of

these important resistant pathogens. The findings also emphasise the need to formulate hygiene measure in the schools including hand washing and disinfecting of all services that could act as a source of infections on MRSA. This could prevent possible spread of MRSA and other related pathogens to students and workers in the schools especially the food handlers in the school's kitchen and the community.

CHAPTER ONE

INTRODUCTION

1.1. Background information

1.1.1. Taxonomy of Staphylococcus aureus

Staphylococcus aureus (*S.aureus*) is a Gram positive coccus, catalase positive, mainly coagulase positive, non-motile bacterium (Ryan & Ray, 2004). *S. aureus* is named for its yellow-pigmented colonies (*aureus* means golden) (Jawetz *et al.*, 2010). The yellow pigment confers some protection from the antimicrobial effects of sunlight (Jawetz *et al.*, 2010). Members of this species are facultative anaerobes. They grow well under conditions of high osmotic pressure and low moisture, making them able to grow and survive in nasal secretions and on the skin (Ryan & Ray 2004). This ability also helps the bacteria to be able to grow in some foods with high osmotic pressure such as ham and other cured meats or in low-moisture foods that tend to inhibit the growth of other organisms (Ryan & Ray 2004).

Staphylococcus aureus is found in a wide range of habitats including environmental surfaces, in nasal nares of domesticated animals like dogs, cats and horses and on human body surfaces as part of normal microflora (Cimolai, 2008)). The human body is a natural reservoir for this bacterium, and studies have shown that the anterior nares are where it is often found than in the other parts of the body. (Maina *et al*., 2012). Carriers may be divided into three groups: persistent, intermittent, and non-carriers (Dora, 2011). Persistent carriers usually carry only one strain and make up 20% of the population. Sixty percent of intermittent carriers harbor multiple strains of *S. aureus* for weeks at a time. Twenty percent are non-carriers and may yield negative results in the laboratory cultures on repeat swabs over time (Dora, 2011). *S. aureus* appears as cocci in clusters if examined microscopically as shown in Figure 1.1.

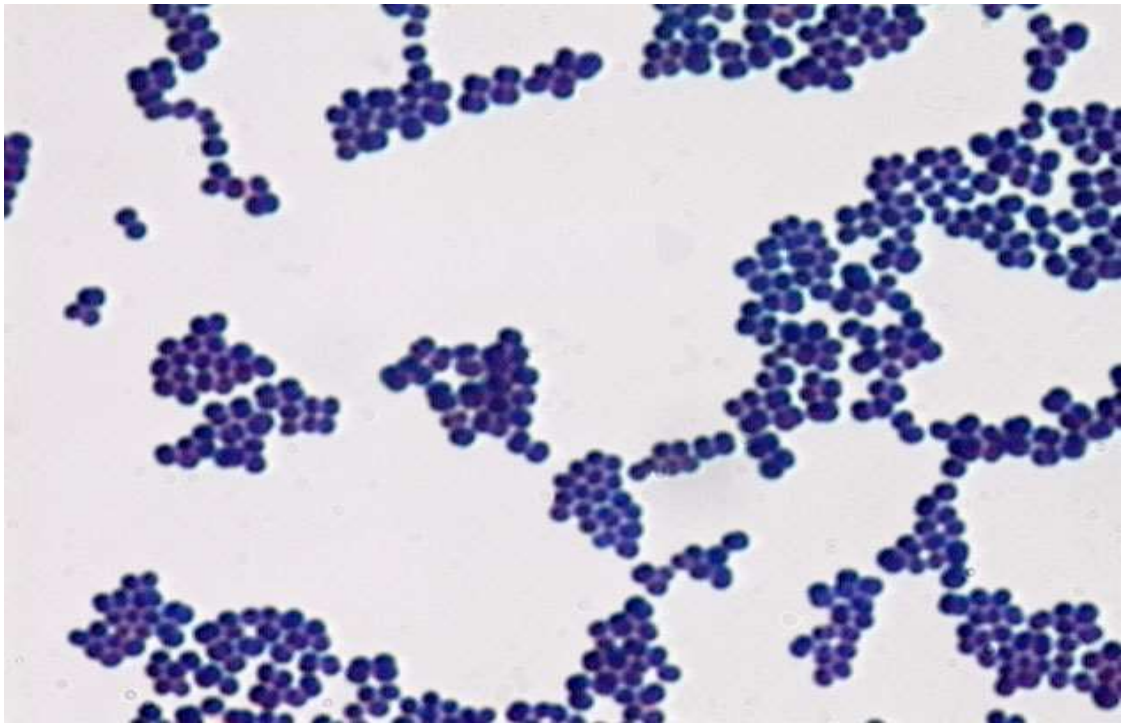

Figure 1.1 Staphylococcus aureus – Cocci in clusters (Dora, 2011)

1.1.2. Discovery of Staphylococcus aureus

Staphylococcus aureus was discovered in 1880 (Giancarlo, 2013). The discovery was made by a Scottish surgeon Sir Alexander Ogston (1844/ 1929) in pus from a surgical abscess in a knee joint. Ogston was not happy with the high rate of postoperative mortality and he was not willing to accept death of a patient as one of the outcome of a surgery (Chan *et al.*, 2013).

Joseph Lister (1827/1912) was also a surgeon who believed that air was the source of putrefaction. He hypothesised that by applying carbolic acid (phenol) dressings to surgical wounds, the air was excluded and thereby preventing suppuration, a form of putrefaction. Ogston was on the other hand convinced that the abscesses were caused by some special germ. Ogston made a stained smear of pus abscess of one of his patients and examined it under a microscope. The stained smear showed chains of many round organisms among the pus cells and debris. In 1882 Ogston named the clustered micrococci "*staphylococci*," from the Greek *staphyle*, meaning bunch of grapes (Giancarlo, 2013).

Later in 1884, two strains of *staphylococci* were isolated by Anton J. Rosenbach (1842/ 1923). He named the two strains after their pigmented appearance of their colonies: *Staphylococcus aureus*, from the Latin aurum for gold, and *Staphylococcus albus* (now called *epidermidis*), from the Latin albus for white (Chan *et al.*, 2013).

1.1.3. Distribution of Staphylococcus aureus

Staphylococcus aureus are widespread in nature although they are mainly found in living organisms colonizing the skin, skin glands and mucus membranes (Grundmann, *et al.*, 2013). They can also live freely in inanimate environment such as medical instruments, school environment like desks, on telephone booths, door handles, sits in public transport buses surfaces (Brook *et al*. 2009). *S. aureus* also can be found in food and can cause food poisoning from foods which are not prepared well (Grundmann, *et al.*, 2013). In United States of America *S. aureus* is the leading cause of food borne illnesses (Chambers & DeLeo, 2009). *S. aureus* has been recognized as the bacteria that cause most of nosocomial infections. In United States of America, approximately out of two million patients with health care related infections, 230,000 have infections which are due to *S. aureus* (Tammy & Bannerman, 2003).

S. aureus is also widely distributed in animals and can colonize animals and cause infections in animals such as mastitis, endocarditis, wound infections, eye infections and osteomyelitis (Boucher *et al.* 2010). Due to its ability to colonize the human and animals, S. *aureus* can also cause zoonotic infection which is an infection that can be spread from animals to human beings (Tammy & Bannerman, 2003).

1.1.4. Diseases caused by Staphylococcus aureus

Staphylococcus aureus in humans is found on the mucus membranes like the nasal passages and the human skin of around a third of the population (Burian *et al.*, 2010). *S. aureus* is well adaptable to antibiotic pressure therefore it is able to colonize healthy individuals which can be a source of infections and spread among people (Chambers & DeLeo, 2009).

Anyone can develop an infection due to *S. aureus,* although certain groups of people are at greater risk of acquiring *S. aureus* infection. These groups of people include new-born infants, breastfeeding women and people with chronic conditions such as diabetes, cancer, and lung disease (Burian *et al.,* 2010). Another group of people at a risk of acquiring *S. aureus* infection are injecting drug users, those with skin injuries or disorders, those with intravenous catheters and surgical incisions are also at higher risk of *S. aureus* infection. Persons with a weakened immune system as a result of other diseases like Human Immunosuppression viruses (HIV) and cancers, or use of immune suppressing medications have also increased chances of *S. aureus* infection (Maina *et al*., 2012).

Staphylococcus aureus is able to combine a number of bacterial immuno-evasive strategies to invade humans. These strategies include production of virulence factors like carotenoid pigment a Staphyloxanthine responsible for the golden colour of *S. aureus* colonies on a culture media. This factor helps the bacteria to evade reactive oxygen species which the host uses to kill the pathogens. In the 1940s and 1950s, *S. aureus* was the primary cause of nosocomial infections (Jawetz *et al*., 2010). *Staphylococcus aureus* can cause three major groups of infections, these groups include; Superficial lesions infections, toxicosis infections and systemic infections (Sousa & Lencastre, 2004).

1.1.4 .1. Superficial lesions infections caused by *Staphylococcus aureus*

The lesions caused by *S. aureus* includes infections of the skin. This lesions can progress to conditions such as impetigo (crusting of the skin) or cellulitis (inflammation of the deeper layers of skin and connective tissue under the skin (Sousa & Lencastre, 2004). In rare situations, a serious complication known as scalded skin syndrome can develop. In breastfeeding women, the infection can result in mastitis (inflammation of the breast) or abscess of the breast (Sousa & Lencastre, 2004).

1.1.4.2. Toxicosis infections caused by *Staphylococcus aureus*

Staphylococcus aureus can cause toxicosis conditions like food poisoning due to ingestion of enterotoxin produced by the bacteria. The enterotoxin is a superantigen that affects the intestines by causing the cells to secrete large amounts of fluids and electrolytes, this leads to normal muscular contractions that leads to diarrhoea accompanied by vomiting(Boucher *et al.,* 2010). Food poisoning due to *S. aureus* is one of the most common food poisoning (Boucher *et al.,* 2010).

Toxic shock syndrome (TSS) is also another form of toxicosis condition caused by *S.aureus* (Sousa & Lencastre, 2004). The infection is severe and it is characterized by high fever, muscle aches and vomiting, followed by low blood pressure (hypotension) which can lead to shock and death. Toxic shock syndrome is a community acquired infection mainly found among young menstruating women using highly absorbent tampons (Sousa & Lencastre, 2004). The syndrome may also be found in men and non-menstruating women (Boucher *et al.,* 2010). TSS is associated with strains that produce and secrete the exotoxin shock syndrome type 1(TSS-1) (Boucher *et al.,* 2010).

1.1.4.3. Systemic infections caused by *Staphylococcus aureus*

Systemic infections can also be caused by *S. aureus*. This kind of infection is as a result of the bacteria entering the bloodstream and spreading to other organs, this causes a number of serious conditions such as bacteraemia or sepsis, *Staphylococcal* pneumonia which mainly affects people with underlying lung disease and may lead to abscess formation within the lungs. Infection of the heart valves (endocarditis) which can lead to heart failure.

Spread of the bacteria to the bones can result in severe inflammation of the bones known as osteomyelitis. Septic arthritis can occur when the bacteria infect a joint space. Thrombophlebitis due to *S. aureus* can also occur when the bacteria infect a vein (Sousa & Lencastre, 2004).

Diagnosis of the *S. aureus* infection in cases of minor skin infections, are commonly diagnosed by their appearance without the need for laboratory testing. More serious *staphylococcal* infections such as infection of the bloodstream, pneumonia, and endocarditis require culturing of samples of blood or infected body fluids or tissues in the laboratory to identify the bacteria and determine the antibiotics to be used for treatment (Tacconelli *et al.,* 2008).

1.1.4.4. *Staphylococcus aureus* infections in animals

Staphylococcus aureus can also cause a variety of infections in animals, for instance mastitis in dairy cows, septicaemia and arthritis in poultry and genital tracts infections of animals. Colonization of *S. aureus*, including both nasal and rectal carriage, has been reported in animals without causing an infection. Carrier animals may serve as reservoirs for disease and may transmit *S. aureus* to other animals or people. The large polysaccharide capsule of the organism protects it from recognition by the immune system of the animal (Cenci-Goga *et al.*, 2003). Treatment of the animal infection with MRSA can be through antibiotics and topical treatments. Prevention of infections in the animals can be through screening targeted animal populations such as animals not responding to antibiotics, non-healing or nosocomial infections, and animals belonging to healthcare workers or known MRSA-positive households.

1.1.5. Social economic impact of Staphylococcus aureus

Staphylococcus aureus has a social economic and clinical significance in a community. *S. aureus* in the hospital related infections is one of the major cause of morbidity and mortality as the infections are both acute and pyrogenic and if untreated can spread to other parts of the body causing death (Chambers & DeLeo, 2009).

Staphylococcus aureus is one of the organisms that have shown resistance to more than one antibiotic. It is estimated that between 2014 and 2050 the world may expect to lose between 60 and 100 trillion USD worth of economic output if antimicrobial

drug resistance is not addressed (O'Neill, 2014). *S. aureus* also causes community-acquired infections like osteomyelitis and septic arthritis, skin infections, endocarditis, and meningitis (Tammy & Bannerman, 2003). *S. aureus* presence in food presents a potential public health hazard. It can cause food poisoning that can lead to vomiting and diarrhea and may lead to hospitalization (Jawetz *et al.*, 2010). *S. aureus* is also known to resist treatment by some antibiotics such as methicillin drug. This resistance leads to use of other alternative antibiotics leading to overuse of antibiotics and eventual resistance of antibiotics in other bacterial infections (Tacconelli *et al.*, 2008).

Control of MRSA infection is important as this will reduce the rate of treatment of the infected person that might lead to more resistance of the bacteria and other bacterial infections (Tacconelli *et al.*, 2008). Medical costs attributed to *S.aureus* infection such as professional fees incurred during hospitalization, treatment and the costs of other infection-related medical services provided after discharge is high (Boucher *et al*., 2010).

1.1.6. Prevention of *Staphylococcus aureus* infections

Prevention of the *S. aureus* infection is diverse and may include hand washing, use of disposable aprons and gloves in the hospitals to reduce skin contact (Tacconelli *et al.*, 2008). Hand hygiene is associated with a 47% decrease in *S. aureus* infection rate (Angelis *et al.*, 2013). Use of disinfectants like ethanol, quaternary compounds and sodium hypochlorite are used to disinfect surfaces in the hospitals such as floors, laboratory surfaces and theatre surfaces. The efficacy of the disinfectant depends greatly on the correct dilutions of the disinfectants and the contact time of the disinfectant on the contaminated surface (Tacconelli *et al.*, 2008). Screening of patients for MRSA can be done to every patient upon hospital admission; this will prevent the cohabitation of MRSA carriers with non-carriers, and exposure to infected surfaces. The screening test used can be nasal culture and rapid molecular method (Tacconelli *et al*., 2008). Control of MRSA in the community involves cleaning and disinfecting shared equipment such as gym equipment, telephone surfaces, doors, avoiding sharing of personal items like towels, seeking medical

attention promptly if skin lesion occurs, covering skin lesions, nasal decolonization with agents such as 2% mupirocin ointment and short term implementation of bathing with antiseptic solutions such as 4% Chlorohexidamine (Tacconelli *et al*., 2008).

1.1.7. Resistant genes produced by *Staphylococcus aureus*

The mechanism of action of antibiotics used for *S. aureus* infections is mainly focused on inhibiting its cell-wall synthesis (Tammy & Bannerman, 2003). Peptidoglycan chains are the strongest structure in the cell wall and are transported extracellulaly by lipid carriers present in the cytoplasmic membrane (Dora, 2011). Penicillin-binding protein (PBP) is the enzyme responsible for linking newly formed peptidoglycan chains inside the cell. Beta-lactams covalently bind to PBP and inhibit cross-bridge formation of the peptidoglycan chains. Without a strong extracellular membrane, the cell ruptures, and *S. aureus* is no longer viable (Dora, 2011).

Methicillin-resistant *S. aureus* has been known to possess antibiotic resistance genes, these genes includes *mec*A gene which leads to methicillin resistance (Tammy & Bannerman, 2003). *MecA* gene transcription is regulated by regulatory regions *mecI* and *mecR*1 genes located 5' to *mecA* gene (Jensen & Lyon, 2009) The gene complex consisting of *mecI*, *mecR*1 and *mecA* has been referred to as the *mec* complex (Jensen & Lyon, 2009). *S. aureus* was one of the first bacteria in which penicillin, resistance was found in 1947 four years after the drug started to be produced. *S. aureus* is also resistance to a number of antibiotics which includes beta lactam antibiotics like penicillin, amoxicillin and oxacillin (Jawetz *et al*, 2010). Treatment of *S. aureus* infections has been difficult due to its ability to resist the commonly used drugs in the hospitals; methicillin was the drug of choice but has been replaced by oxacillin due to significant kidney toxicity (Tammy & Bannerman, 2003). Half of the *S. aureus* infections in USA are resistant to penicillin, methicillin, tetracycline and erythromycin leaving vancomycin as the only drug of choice (Jawetz *et al*, 2010). Vancomycin is considered to be the last line of antibiotic defense for treatment of *S. aureus* infections that are resistant to other antibiotics. The widespread use of vancomycin to treat MRSA has led to the appearance of vancomycin-resistant

enterococci (VRE) (Jawetz *et al*, 2010). Strains of *S. aureus* which are resistant to vancomycin have been identified and are called vancomycin intermediate resistance *S. aureus* (VISA) and vancomycin resistant *S. aureus* (VRSA) (Tacconelli *et al.*, 2008). This appearance of vancomycin-resistant pathogens, leaving few effective alternatives, is considered a medical emergency (Jawetz *et al.*, 2010). *S. aureus* can also have other antibiotic genes such as gentamicin resistant genes (*aac(6')/aph(2''), aph(3')-IIIa, ant(4')-Ia*), *aph3'-III* gene encoding resistance to neomycin, amikacin and isepamycin, erythromycin resistant genes (*ermA*, *ermB*, *ermC*, and *msrA*), kanamycin resistance gene *aadD*, tetracycline resistant genes (*tetK, tetM*), *far-1* gene for fusidic acid intermediate isolates and penicillin (*blaZ*) (Jensen & Lyon, 2009).

1.1.8. Methicillin Resistant *Staphylococcus aureus*

Methicillin Resistant *Staphylococcus aureus* belongs to the large group of gram positive cocci bacteria known as *S. aureus* (Ryan & Ray, 2004). MRSA acquire a gene that makes them resistant to all beta-lactam antibiotics including methicillin, amoxillin and penicillin. MRSA first appeared in the hospitals and other health institutions especially among the elderly, the very sick and those with open wounds (CDC, 2003). This type of infection is known as Hospital Associated MRSA (HA-MRSA). MRSA can also be found in the community set up; this type of MRSA is known as community associated MRSA (CAMRSA). CAMRSA strains occur in people who have not been hospitalized or recently had invasive procedures (CDC, 2003). CAMRSA first appeared in high-risk populations like the intravenous drug users and people with chronic illnesses but are now found even in healthy children (Jensen & Lyon, 2009).

1.1.9. Distribution of Methicillin Resistant *Staphylococcus aureus*

Methicillin Resistant *Staphylococcus aureus* is widely distributed in nature. MRSA can be found in both human and animals. Almost all the continents have reported cases of MRSA. Methicillin was introduced in 1959 to treat penicillin resistant *S aureus* infections and by 1961 reports in US indicated isolates of *S aureus* which

were resistant to methicillin. Later MRSA was discovered from European countries, Australia and Japan (CDC, 2003).

1.1.9.1. Worldwide distribution of Methicillin Resistant *Staphylococcus aureus*

An estimated two billion people worldwide carry *S. aureus* and 53 million (2.7%) of carriers carry MRSA (CDC 2003). MRSA is found in all the continents. In America the prevalence ranges from 10% percent to over 50% while in Asia the prevalence is from unknown to 50%. The prevalence of MRSA in Africa ranges from unknown in some parts of Africa and up to 50% in other parts of Africa. A prevalence of over 50%% were noted in Thailand, Vietnam and Japan as shown in Figure 1.2

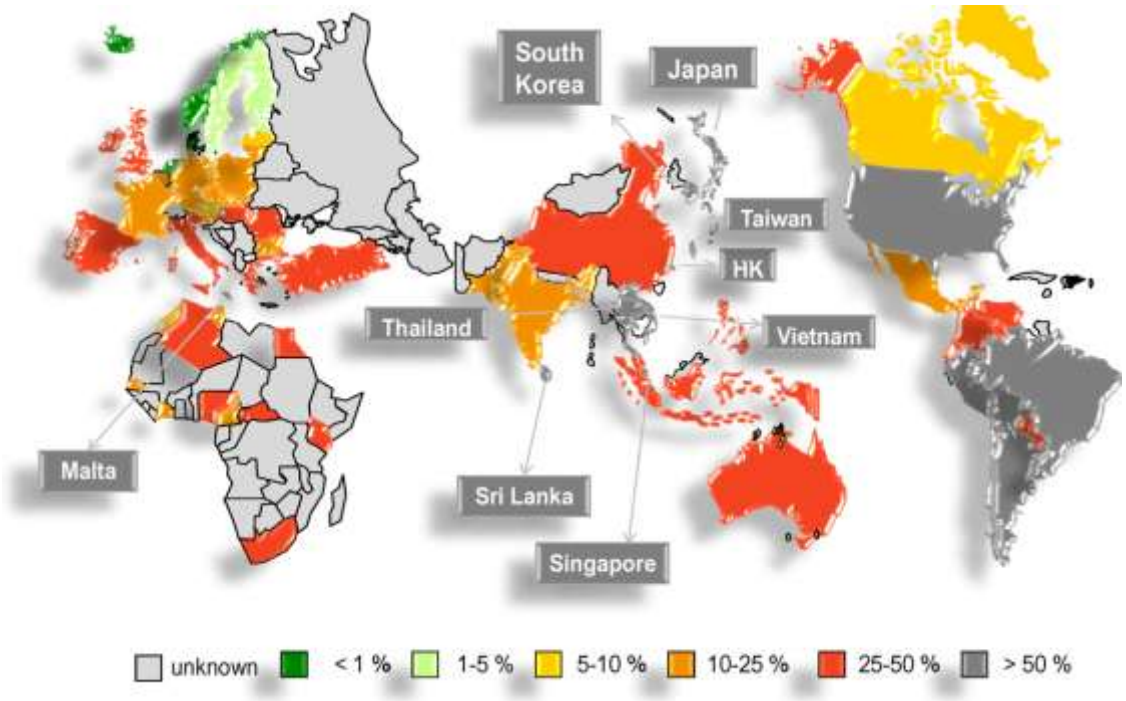

Figure 1.2: Global Prevalence and Epidemiology of MRSA (Otter & French, 2010**)**

1.2. Statement of the problem

Staphylococcus aureus are widespread in nature although they are mainly found in living organisms colonizing the skin, skin glands and mucus membranes. They can also live freely in inanimate environment such as medical instruments, school environment like desk. Research has shown that the highest concentration of bacteria found in toilets and washing rooms are located in and around the inside door handles and tap handles. Educational facilities provide ideal conditions for spreading infectious microbes. MRSA can easily be spread from one person to another through direct contact from infected persons and contaminated surfaces.

MRSA have many virulence factors that enable them to cause disease in normal hosts. MRSA are an emerging cause of community-associated infections, especially skin and soft tissue infections and necrotizing pneumonia. *S. aureus* presence in food can cause food poisoning that can lead to vomiting and diarrhoea which may lead to hospitalization.

High rates of outbreaks in schools are increasing in the US and emerging reports of Community Acquired MRSA (CA-MRSA) in Scandinavian countries. In a study done in Midwestern United States Urban University, *S. aureus* was isolated from computer keyboards, telephones and elevator buttons. Their presence in the school's surfaces can lead to infections of CA-MRSA which could find their way to health institutions leading to HA-MRSA. This could increase morbidity and death from MRSA, increased treatment costs as a result of alternative medicine, prolonged hospitalization and costs to patients and government.

1.3. Justification of the study

The presence of MRSA in a community set up such as schools has both social and economic impact to the Country. The cost incurred for treatment and managing the infection of MRSA by both the individual and the government is high and should be avoided at all cost. Good surveillance of the presence of MRSA in community would greatly benefit both the parties. Determination of the presence of the MRSA

will help the government in determining the measures to take in order to control and prevent the MRSA infections from occurring. This study is one of the many ways the government can do regular surveillance to determine not only the presence of MRSA but also other infections of public health. Data on prevalence of MRSA in secondary school in the whole Kenya as a possible reservoir in community is lacking and yet students are more likely to transfer the MRSA from schools to their homes. The secondary schools were chosen to represent type of community set up where a large population of community gather at a particular time. This study has provided critical information on the burden of MRSA in this subset of the population which is a representative of a community set up. This will help the concerned ministries in establishing informed measures such as the use of the right treatment in case of MRSA infections and prevention strategies such as hygiene in schools.

1.4. Null Hypothesis

There is no presence of methicillin resistant *Staphylococcus aureus* on toilet and classroom door handles in selected secondary schools in Nairobi County, Kenya.

1.5 Research Questions

1. Is Methicillin resistant *Staphylococcus aureus* present on the classroom and the toilet door handles in selected secondary schools in Nairobi County, Kenya?
2. What are the factors associated with MRSA contamination in the classroom and the toilet door handles in selected secondary schools in Nairobi County Kenya?
3. Is there presence of PVL virulence and *mec*A resistance genes on the *Staphylococcus aureus* strains isolated from the toilet and classroom handles in the selected secondary schools in Nairobi County, Kenya?

1.6. Objectives

1.6.1. General Objective

To detect methicillin resistant *Staphylococcus aureus* and determine the factors associated with contamination of Methicillin resistance *Staphylococcus aureus* on toilet and classroom door handles in selected secondary schools in Nairobi County, Kenya.

1.6.2 Specific Objectives

1. To determine the prevalence of *Staphylococcus aureus* on toilet and classroom handles in selected secondary schools in Nairobi County, Kenya.
2. To determine the antibiotic susceptibility profiles of *Staphylococcus aureus* isolated from the toilet and classroom handles in selected secondary schools in Nairobi County, Kenya.
3. To determine the factors associated with *Staphylococcus aureus* (MRSA) contamination.
4. To detect PVL virulence genes and *mec*A resistance genes of MRSA strains isolated from the toilet and classroom handles in selected secondary schools in Nairobi County, Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1. Epidemiology of methicillin resistant *Staphylococcus aureus*

MRSA first appeared in the hospitals and other health institutions especially among the elderly, the very sick and those with open wounds (CDC, 2003). The type of infection found in the hospital setup is referred as Hospital Associated MRSA (HA-MRSA) while the infection found in the community setup is known as Community Acquired MRSA (CA-MRSA). About two billion people worldwide carry *S. aureus* and 53 million (2.7%) of carriers carry MRSA (CDC, 2003).

2.1.1. Epidemiology of methicillin resistant *Staphylococcus aureus* in Europe

Methicillin-resistant *S. aureus* (MRSA) was first reported in the UK in 1961, soon after the introduction of methicillin into clinical practice (Borg *et al.,* 2009). MRSA reportedly spread to European hospitals in the early 1960s. Generally in Europe, a north–south gradient is observed, MRSA strains being rare in Scandinavian hospitals (<2%) and far more prevalent in Mediterranean hospitals (>40%) (Stefani & Varaldo, 2003).

A study done in 25 university hospitals, in 15 countries in central and southern Europe indicated that 25% of 3051 of *S. aureus* isolates were MRSA (Fluit *et al.,* 2001). The highest prevalence was seen in hospitals in Portugal (54%) and Italy (from 43% to 58%), whereas the lowest prevalence was observed in hospitals in Switzerland and The Netherlands (2%). In Europe prevalence of Infection in intensive care (EPIC) study, the highest prevalence of MRSA strains was found in Italy (81%) and in France (78%) (Fluit *et al.,* 2001).

The prevalence of MRSA in Europe is about 26% (Dora, 2011). In a study which collected 15,439 *S. aureus* isolates from all over the world from 1997-1999, showed that among the

regions under investigation, Europe was the region with the most variations (Dora, 2011). This study also showed that MRSA rates were highest in countries from southern Europe (Greece, Italy, Portugal, and Turkey) (Dora, 2011). The most common strain found in United Kingdom is epidemic MRSA (EMRSA15 and EMRSA16) (Johnson & Saravolatz, 2005).

2.1.2. Epidemiology of methicillin resistant *Staphylococcus aureus* in United states of America

The first human case of MRSA in United States of America was reported in 1968 (Graham *et al*., 2006). Ninety five million people in United States of America carry *S. aureus* in their noses and of these 2.5 million (2.6%) of the carriers have MRSA (Borg *et al.,* 2009)). Research done in Midwestern United States Urban University, on 70 frequently used environmental surfaces in a university of 25,000 individuals, contamination of *S. aureus* was noted. *S. aureus* was isolated form desktops, computer keyboards, telephone mouthpieces, water fountains, photocopy keypads, vending machines and elevators buttons(Brook *et al.,* 2009).

A study done in all the states of America in 2010 on the prevalence of MRSA showed that out of 4,476 MRSA-colonized/infected patients in 67,412 inpatients, the overall MRSA prevalence rate was 66.4 per 1,000 inpatients (25.3 infections and 41.1 colonization per 1,000 inpatients) (Jarvis *et al.,* 2010).

2.1.3. Epidemiology of methicillin resistant *Staphylococcus aureus* in Asia

In Asia, particularly the Asia-Pacific region that includes Taiwan, Singapore, Japan, and Hong Kong, the prevalence rates of *S. aureus* are at above 60%. A study done on the spread of MRSA between the community and the hospitals in Asian countries showed that MRSA infections in the community have been increasing in Asian countries (Borg *et al.,* 2009).

Data also suggest that various MRSA clones have spread between the community and hospitals as well as between countries. MRSA was also highly prevalent in hospitals in many Asian countries. In the study MRSA accounted for 25.5% of

community acquired *S. aureus* infections and 67.4% of hospitals acquired infections (Song *et al.*, 2013).

Predominant clones of CA-MRSA isolates were ST59-MRSA-SCCmec type IV-spa type t437, ST30-MRSA-SCCmec type IV-spa type t019 and ST72-MRSA-SCCmec type IV-spa type t324. Previously established nosocomial MRSA strains including sequence type (ST) 239 and ST5 clones were found among CA-MRSA isolates from patients without any risk factors for HA-MRSA infection. CA-MRSA clones such as ST59, ST30 and ST72 were also isolated from patients with HA infections (Song *et al.*, 2013).

Another Sentry program that focused only on South East Asia and Africa showed a prevalence rate of between 5% and 73.8%. The lowest being Philippines (5%) and the highest Hong Kong with prevalence of 73.8% (Dora, 2011) Figure 2.1.

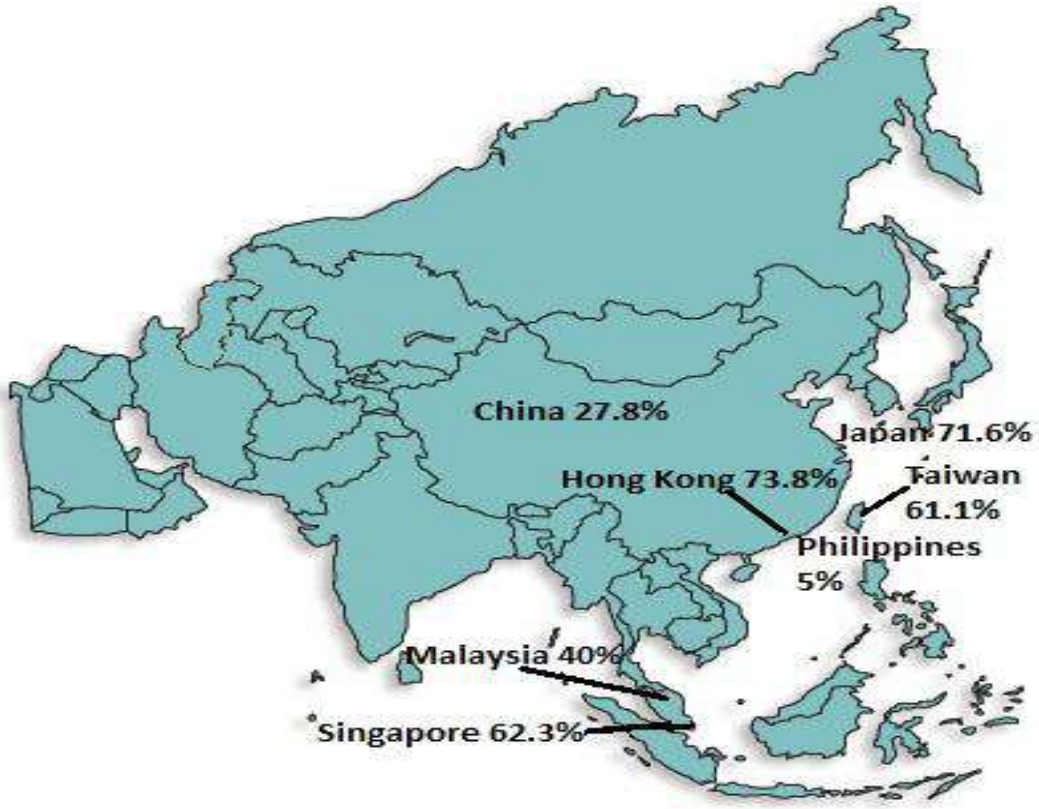

Figure 2.1: Prevalence of MRSA in Asia (Dora, 2011**)**

2.1.4. Epidemiology of methicillin resistant *Staphylococcus aureus* in Africa

One of the first cases reported in the continent was in South Africa in 1978. The same factors that aggravate the challenge of growing MRSA rates in the developed world, i.e. increasing antibiotic consumption, inadequate coverage of the antibiotics and inaccurate antibiotics sensitivity tests done in the laboratories are also noted in Africa (Borg *et al.,* 2009).

Data of MRSA in Africa are limited however from data available, the prevalence of *S.aureus* ranges from 5% to 45% (Dora, 2011). Presence of MRSA was reported in almost all parts of the Continent except the South west part of Africa, Madagascar had the least incidences (5%) while Algeria had the highest incidences of MRSA with Prevalence of 45%. Incidences in North Africa ranged between 8.1% in Tunisia and 45% in Algeria. In East Africa the Ranges were between 16% in Tanzania to 27, 7% in Kenya. In the south of the Continent the prevalence was up to 40%. Most countries had prevalence of 20% and above indicating the magnitude of the MRSA problem in the Africa Continent (Dora, 2011).

A survey done in eight African countries between 1996 and 1997 showed a prevalence of (21-30%) of MRSA in Nigeria, Kenya and Cameroon (Kesah *et al.,* 2003). In East African region, high prevalence of *S. aureus* (28.7%) and MRSA (31.5%) was found in surgical site infections in Mulago National Referral Hospital, Kampala Uganda (Ojulong *et al.,* 2008). This prevalence is shown in Figure 2.2.

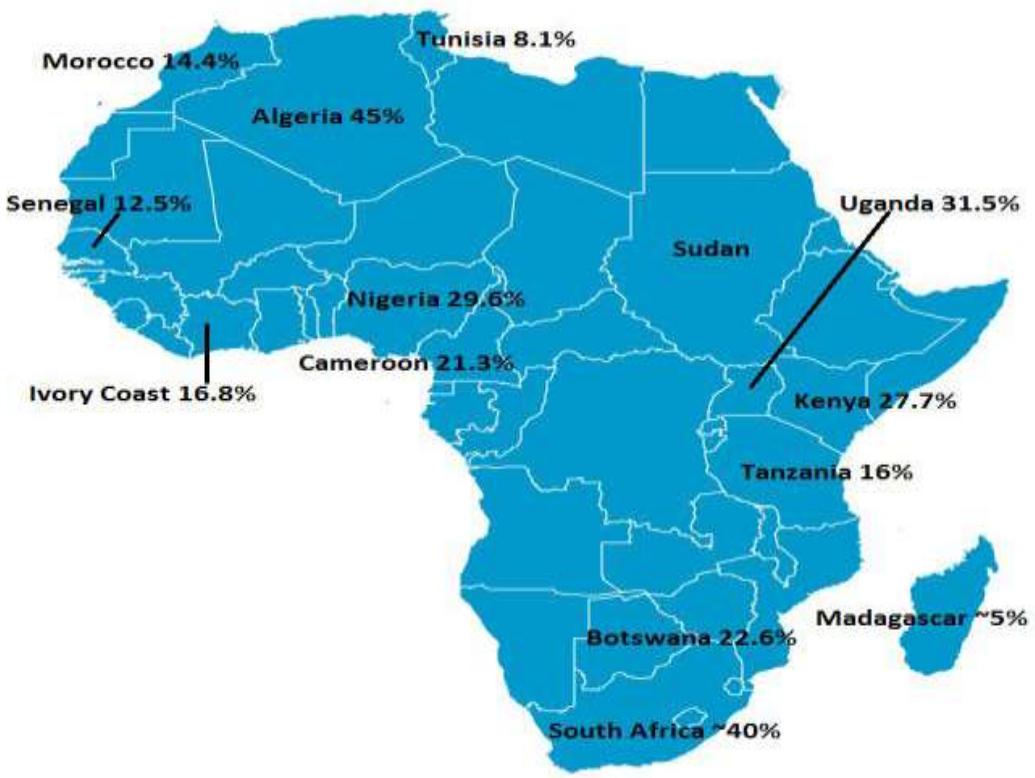

Figure 2.2: Prevalence of MRSA in Africa (Dora, 2011).

2.1.5. Epidemiology of methicillin resistant *Staphylococcus aureus* in Kenya

In Kenya there are not many studies done for CA-MRSA, however a study carried out in Nairobi showed a prevalence of 41% of *S. aureus* isolated from community acquired infections (Malonza *et al.*, 1997)**.**

A study done of MRSA from skin and soft tissue infections in patients in Nairobi, Kenya showed that 69 of 82 (84.1%) *S. aureus* isolates were MRSA (Maina *et al*., 2012). Eight point nine percent (8.9%) of *S. aureus* was found in Thika level five hospitals which is a typical mid-sized Kenyan government hospital (Aiken *et al*., 2014). In all these studies done in Kenya the prevalence ranges from 8.9% to 41%.

MRSA can be divided into community acquired methicillin resistant *S. aureus* (CA-MRSA) and hospital acquired methicillin resistant *S. aureus* (HA MRSA) (Richard, 2010).

2.2. Community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA)

CA-MRSA is found in the community outside of the hospitals (Ellis *et al.*, 2004). CA-MRSA is associated with people with recent antibiotic use and living in crowded settings such as in schools (Hidron *et al*., 2005). CA-MRSA is more easily treated than HA-MRSA although it is more virulent than HA-MRSA (Ellis *et al.,* 2004).

Characteristics of CA-MRSA infections include hospital associated risk factors, distinct genotypes, susceptibility to non-beta lactam antibiotics and distinct determinant of virulence; the PVL toxin (Ellis *et al.,* 2004).

CA-MRSA is known to possess Pantone Valentine Leukocidin virulent (PVL) genes which cause necrotizing infections on the skin and in the lungs. Anyone can get infections from CA-MRSA including healthy patients, people with no recent history of hospitalizations and young people are mostly infected (Richard, 2010). HA-MRSA do not contain this gene but contain various Staphylococcal cassette chromosomes that cause infections in blood stream, surgical sites and site of implant.

CA-MRSA can cause a variety of infections known as community acquired infections, they include simple skin infections, such as impetigo, boils, abscesses, folliculitis, and cellulitis. Rarer, but more serious, manifestations can occur, such as necrotizing fasciitis and pyomyositis (most commonly found in the tropics), necrotizing pneumonia, infective endocarditis (which affects the valves of the heart), and bone and joint infections ((Herchline, 2010). The United State Centres for Disease and Prevention (CDC) estimates that about 12% of MRSA infections are now community associated unlike in the past where it was known only to be found in the health care settings like hospitals. This prevalence can vary by community and patient population (CDC, 2005). The first cases of CA-MRSA appeared in mid-1990

in Australia, New Zealand, United States, United Kingdom, France, Finland and involved people exposed to healthcare settings (Raygada & Levine, 2009).

2.2.1. Global epidemiology of CA-MRSA

Global outbreaks of CA-MRSA were seen in North America, Latin America, Western Europe, Saudi Arabia, India, Australia, Asia and New Zealand in 1997 to 2000. It is worth noting that CA-MRSA was not seen in Africa in that period as shown in Figure 2.3. CA-MRSA outbreaks have also been reported among military recruits, prison in mates' intravenous drug users, athletes and among men who have sex with men (Cook *et al.*, 2007).

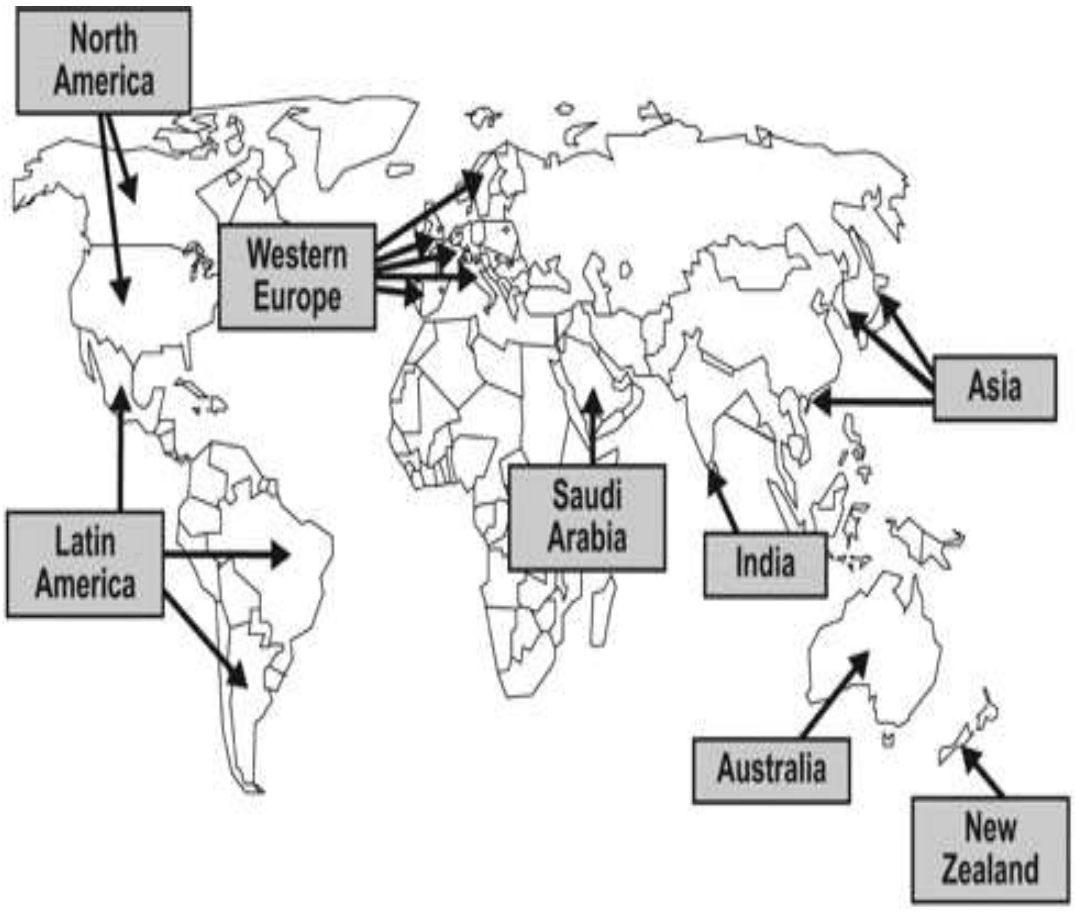

Figure 2.3: Global outbreaks of community-associated methicillin-resistant *Staphylococcus aureus* infection, 1997–2000 (Cook *et al.*, 2007)

2.3. Hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA)

HA-MRSA is commonly found affecting people who are immunocompromised, those in long term care facilities, recently hospitalized persons, dialysis patients and persons who have had a recent surgery (Richard, 2010). HA-MRSA can cause health care-associated infections which include blood stream infections, surgical site infections and pneumonia. MRSA accounts for up to 40% of nosocomial *S. aureus* infections in large hospitals and 25%-30% in smaller hospitals in the USA (Herchline, 2010).

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

2.4. Types of infections caused by MRSA

The common types of infections caused by MRSA include skin infections like impetigo (crusting of the skin), boils, abscesses, folliculitis, cellulitis (inflammation of the connective tissues), scalded skin syndrome and boils (Ellis *et al.*, 2004). When the bacterium gains entry into the blood stream it leads to bacteraemia, which can cause conditions like endocarditis, necrotizing pneumonia, necrotizing fasciitis and pyomyositis arthritis and osteomyelitis (Herchline, 2010).

MRSA can also cause food poisoning leading to nausea, violent vomiting, and abdominal cramping; with or without diarrhoea. The disease is usually self-limiting and typically resolves within 24–48 hours after its onset (Le Loir *et al.*, 2003).

MRSA can also cause toxic shock syndrome characterized by sudden onset of high fever, vomiting and muscle aches which may lead to shock. This condition is mostly common in menstruating women using tampons (Herchline, 2010).

2.5. Prevention and control of MRSA infections

2.5.1. Prevention of MRSA infections in a health care setup

In Health care setup, prevention of MRSA includes screening programs in patients upon admission, health workers and on surfaces. Surface sanitization in the hospitals and public areas should be done regularly. Proper hand washing should be a routine to health workers, patients and everyone in the community. Use of surgical respirator, proper disposal of hospital gowns, and isolation of infected persons can reduce the infections (Tacconelli *et al.,* 2008). Proper disposal of hospital gowns should be done since this has been associated with MRSA hospital infections (CDC, 2005). Any drainage from the wounds should be disposed off very carefully. All infectious lesions should be kept covered with a dressing. Mupirocin (Bactroban) 2% ointment can be effective at reducing the size of lesions (Angelis *et al.,* 2013).

Restriction of antibiotics use by patients can reduce resistance of antibiotics. Cephalosporins and glycopeptides particularly quinolones are associated with an increased risk of colonisation of MRSA. Reduced use of these antibiotic classes is recommended (Tacconelli *et al.,* 2008). Use of mupiricon (Bactroban) 2% ointments can be applied inside each nostril for 7 days. Treatment of the infected patients is important so as to prevent the spread of MRSA from the infected to the ones who are not infected (Tacconelli *et al.,* 2008).

2.5.2. Prevention of MRSA infections in a community setup

Prevention and control of infection in a community setup such as in school involves many strategies like early treatment of infected students to stop infections from causing serious harm and to prevent spread. There are several ways of reducing MRSA infections as recommended by (CDC, 2003).

Firstly by practicing good hygiene like keeping hands clean by washing with soap and water or using an alcohol-based hand sanitizer and showering immediately after participating in exercise. Secondly by covering skin trauma such as abrasions or cuts with a clean dry bandage until healed. Thirdly by avoiding sharing personal items

(e.g., towels, razors) that come into contact with bare skin; using a barrier (e.g., clothing or a towel) between the skin and shared equipment such as weight training benches. Fourthly by maintaining a clean environment by establishing cleaning procedures for frequently touched surfaces and surfaces that come into direct contact with people's skin.

Other ways include covering draining wounds. Other persons or children should not come into contact with an employee's or child's infection or wound. Contact activities should be suspended until the wound is completely healed. Utensils, dishes, clothes and other laundry should be washed normally with hot water and normal detergents. Laundry should be dried on the hottest setting. Clean non-sterile gloves should be used by employees caring for the child's wound or infection. Change gloves when moving from one body site to another or from one child to another. Use liquid soap instead of shared bar soap that is mild and nonirritating.

Discourage the use of extended artificial nails especially when caring for wounds. Keep nails neatly trimmed short and free of debris under the nail. Use moisturizers or hand lotions to keep skin healthy. Transport soiled items in a plastic bag or other waterproof container. Inform laundry workers of contaminated articles and pre rinse/ wash grossly soiled items. Clean the facility and used recreational equipment daily with a commercial disinfectant or a daily prepared solution of 1:100 bleach and water mix (1 tablespoon bleach in 1 quart of water). Clothes should always be dried completely.

2.6. Genetic basis of resistance to Methicillin (*Mec*A gene)

Methicillin resistance is mediated by a penicillin-binding protein (PBP2A). This protein has a low affinity for beta lactam antibiotics such as penicillin, cephalosporins and carbapenems and it is encoded by the *mec*A gene (Robinson *et al.,* 2005).

PBP2A is an altered penicillin binding protein and has a low affinity for binding with β lactam antibiotics which leads to methicillin resistance in *S. aureus*. PBP2a is

encoded in the *mec*A gene whose transcription is regulated by regulatory regions *mecI* and *mecR*1 genes located 5' to *mec*A gene. The gene complex consisting of *mecI*, *mecR*1 and *mecA* has been referred to as the *mec* complex (Jensen & Lyon, 2009).

The *mec*A gene is carried in SCC*mec* cassette, which can accommodate a number of genetic elements for antibiotic resistance (leading to multiple resistant strains) and many other virulence factors. These chromosome mediated genes have been widely distributed among many *Staphylococcal* species. MRSA is produced when methicillin sensitive *S. aureus* (MSSA) acquires the genetic element, *staphylococcal* cassette chromosome, SCC*mec* (Robinson *et al.*, 2005).

Evolution of methicillin sensitive *S. aureus* (MSS) to MRSA is postulated to occur due to horizontal transfer of virulence genes such as PVL genes and acquisition of SCC mec allotypes (Jensen and Lyon, 2009).

2.7. MRSA antimicrobial Susceptibility Patterns

Current AST patterns of MRSA have shown to resist most first line drugs used for treatment like oxacillin, flucloxacillin and gentamicin. In a study done among primary school children and prisoners in Jimma Town, Southwest Ethiopia, MRSA displayed multiple drug resistance (MDR) of two to 10 different antibiotics. The most frequent MDR was resistance to ampicillin, bacitracin, erythromycin, penicillin, and cefoxitin (Kejela & Bacha, 2013).

Approximately 10% of *S. aureus* isolates in the United States of America are susceptible to penicillin. However, many *S. aureus* strains, while resistant to penicillin, remain susceptible to penicillinase-stable penicillins, such as oxacillin and methicillin. Strains that are oxacillin and methicillin resistant, historically termed methicillin-resistant *S. aureus* (MRSA), are resistant to all ß-lactam agents, including cephalosporin and carbapenems.

Healthcare-associated MRSA isolates often are multiple resistant to other commonly used antimicrobial agents, including erythromycin, clindamycin, and tetracycline, while CA- MRSA isolates are often resistant only to ß-lactam agents and erythromycin (CDC, 2005).

A study done on the prevalence of methicillin-resistant *S. aureus* in eight African hospitals and Malta (Nigeria, Tunisia, Ivory Coast, Senegal, Kenya, Cameroon, Morocco, Algeria and Malta), showed that all MRSA strains tested in this study were susceptible to vancomycin, fusidic acid, co-trimoxazole, rifampin and ciprofloxacin exhibited moderate efficacy, while high rates of resistance were obtained for gentamicin and erythromycin. Multiresistance was noted to be relatively higher in Morocco, Kenya, Nigeria and Cameroon, with more than 60% of the isolates exhibiting resistance to at least three antibiotics (Kesah *et al*., 2003).

(Maina *et al.*, 2013) noted that all 82 *S. aureus* isolates in the study done on MRSA from skin and soft tissue infections infections in patients in Nairobi, Kenya were susceptible to vancomycin and resistant in high numbers to macrolides, aminoglycosides, and quinolones. Bacterial isolates were mostly susceptible to vancomycin, ciprofloxacin and co-trimoxazole, and none was resistant to vancomycin.

A resistance of 27.7% was seen in community-acquired methicillin-resistant *S. aureus* carrying *mecA* and Panton-Valentine leukocidin (PVL) genes isolated from the holy shrine in Najaf, Iraq (Al-Mohana *et al.*, 2012). In a study done in South Africa all the isolates were susceptible to vancomycin. Penicillin and ampicillin were the least effective antibacterial agents. In addition, 30% were resistant to erythromycin, clindamycin, trimethoprim and tetracycline, 28.6% to gentamicin and 20.3% to rifampicin (Shittu & Lin, 2006). In a study done for residential indoors in Texas, 54.59% of *S. aureus* isolates showed resistance to ampicillin (Gandara *et al.*, 2007).

In an analysis done for antibiotic resistance worldwide, different variations in antibiotics resistance are noted (Dora, 2011). In this analysis, the countries in southern Europe showed the highest patterns of antibiotic resistance. Africa and Asia showed antibiotic resistance patterns, on average, that were higher than in the United States and Europe. All Continents reported high resistance of erythromycin (Up to 95%), gentamycin, ciprofloxacin and clindamycin also were resistant in all the Continents. Asia and Uganda reported high resistance of chloramphenicol. In USA, Europe and Asia high resistance was seen in all the antibiotics used in the study except for the chloramphenicol in USA with resistance of 4.7% as shown in Table 2.1.

Table 2.1: Worldwide antibiotics resistance patterns (Dora, 2011)

	Erythromycin	Gentamycin	Ciprofloxacin	Clindamycin	Bactrim	Chloramphenicol	Rifampin	Tetracycline
USA	92%	35%	88%	79%	26%	4.7%	7.7%	15.7%
Europe	87%	72%	90%	74%	23%	9.4%	44%	57%
Denmark	1%	0%	1%	0%			0%	3%
Norway	3%	0%	1%	1%			0%	3%
Sweden	3%	0%	4%	2%			0%	11%
Finland	4%	1%	8%	0%			1%	5%
Germany	13%	7%	9%	1%			1%	10%
Lithuania	22%	7%	8%	8%			0%	34%
France	24	4%	23%	6%			0%	11%
England	20%	11%	21%	10%			3%	10%
Spain	38%	26%	32%	30%			11%	19%
Belgium	42%	24%	30%	26%			5%	29%
Poland	29%	29%	26%	28%			17%	73%
Greece	70%	35%	64%	68%			25%	53%
Africa								
S. Africa	39%	35%	29%	39%			23%	34%
Botswana	+			+				+
Nigeria	69%	53%	79%		64%		78%	
Uganda	88%	58%	70%	82.4%	88%	88.2%		
Madagascar	33%	11%	14%		39%	23%	14%	75%
Asia	95%	74%	88%	79%	36%	96%	10%	82%
New Zealand	52%	2%	3%	1%			1%	1%
Australia	10%	0%	3%	0%			0%	2%
Malaysia	55-92%	48-76%	30-94%	2-18%	73%		12%	47-55%
Japan	+	+	+	+				+
Hong Kong	+	+	+	+				+
China	+	+	+	+				+
Thailand	94-96%			37-69%				

2.8. Factors associated with MRSA infections

It is acknowledged that there are several factors associated with MRSA infections. These include the Virulence factors and the physical risk factors of MRSA transmission (Fischetti *et al.,* 2006).

2.8.1. Virulence Factors of MRSA

Methicillin Resistant *Staphylococcus aureus* are resistant to many antibiotics unlike the fully sensitive *S. aureus*, this fact can lead to severe infection (Borg *et al.,* 2009). *S. aureus* is very common in the environment and can spread easily to susceptible people or even animals. *S. aureus* possess a cell wall that is encapsulated; the capsule makes it easy to evade host immune system by resisting phagocytosis (Fischetti *et al.,* 2006).

S. aureus is capable of secreting several exotoxins categorized into three groups; The first group are Pyrogenic toxin super antigens that induce toxic shock syndrome (TSS) associated with tampon characterised by fever, erythromatous rash, hypotension, shock, multiple organ failure and skin desquamation (Fischettti *et al*., 2006).

The second group are the enterotoxins causing food poisoning characterised by vomiting and diarrhoea. The third group are exotoxins and are exfoliative toxins implicated in *Staphylococcal* scalded skin syndrome (SSSS) occurring mostly in infants and young children. It can also occur as epidemics in hospitals nurseries (Vandenesch *et al.,* 2003).

Protease activity of the toxin causes peeling of the skin seen with *Staphylococcus* Scalded Skin Syndrome (SSSS). Other toxins which act on cell membranes including alpha-toxins, beta-toxins, delta-toxins and bicomponent toxins Panton-Valentine leukocidin (PVL) associated with severe necrotizing pneumonia in children (Borg *et al.,* 2009).

MRSA virulence factors include Pantone-Valentine leukocidin (PVL), Staphylococcal enterotoxins A-E and exfoliative toxins A and B and toxic shock syndrome toxin. CA- MRSA has unique genes that distinguish them from HA-MRSA. These genes encode for enterotoxins H and O which have high binding affinities for major histocompatibility complex II and are not found in HA-MRSA. PVL toxin is a virulence factor specific to CA-MRSA encoded by genes *lukS-PV* and *lukF-PV* carried by bacteriophage incorporated into *S. aureus* chromosome. The toxin is associated with necrotic skin lesions and severe necrotizing pneumonia in children and adults (Vandenesch *et al.*, 2003).

Protein A is a another virulence factor found in *S. aureus*, the factor is an IgG binding protein that binds to the Fc region of an antibody leading to resistance of the bacteria from the host immune system (Borg *et al.*, 2009).

Some *S. aureus* can produce staphyloxanthine, a pigment which acts as a virulence factor by acting as an antioxidant that helps it to evade death by reactive oxygen species produced by the host immune system (Clauditz *et al.*, 2006).

S. aureus can also produce a number of enzymes that helps it to invade the host cells; these enzymes include DNase, coagulase, and hyaluronidase that promote bacterial spread in tissues leading to conditions like osteomyelitis (Clauditz *et al.*, 2006).

2.8.1.1. Panton Valentine leukocidin (PVL) virulence factor

One of the virulence factors associated with MRSA infection is Panton Valentine leukocidin (PVL). PVL virulence factor is seen in CA-MRSA and not in HA-MRSA. PVL is a cytotoxin, one of the β-pore forming toxins and it is encoded by *luk*S-PV and *luk*F-PV genes (Panton & Valentine, 1932). PVL is produced from the genetic material of a bacteriophage which infects *S. aureus*, making it more virulent. Presence of PVL is associated with increased virulence of certain strains of *S. aureus*. PVL is present in the majority of community associated methicillin resistant *S. aureus* (CA-MRSA). PVL creates pores in the membranes of infected cells and is the cause of necrotic flesh eating lesions, an aggressive condition that often kills

patients within 72h (Voyich *et al.,* 2007). PVL may also contribute significantly to particular types of infections, such as severe lung infections and osteomyelitis (Vandenesch *et al*., 2003).

PVL-positive CA-MRSA clones have spread throughout the world (Garnier *et al.,* 2006). In a study done in Indonesia on Indonesian population, up to (10.6%) of MRSA contained PVL (Juliëtte *et al.,* 2002). Prevalence of PVL-positive CA-MRSA varies considerably from one continent to another. In the USA, CA-MRSA was isolated from 50% of patients presenting to emergency departments of 11 cities with skin and soft-tissue infections (Moran *et al.,* 2006). The prevalence of PVL-positive CA-MRSA in Europe is lower, 1-3% (Naas *et al.,* 2005).

In the Netherlands an increasing tread of presence of PVL in MRSA was noted in consequent studies, The PVL genes were detected in two of the 216 MRSA isolates (1%) selected from the first study period (1987 through 1995). PVL genes were detected in five of the 99 MRSA isolates (5%) selected from the year 2000 and in 15 of the 98 MRSA isolates selected from 2002 (Wannet *et al.,* 2002).

International travel is likely related to the worldwide spread of PVL-positive CA-MRSA. For instant ST80 isolates recovered in France were mainly detected in patients originating from Algeria. Algeria reported a high rate of community- and hospital-acquired infections due to ST80 isolates in 2006 (Ramdani *et al.,* 2006). Strains of ST22 were recovered from Turkish migrants in Germany (Maier *et al., 2006*). In this study the strain of the MRSA that was isolated was CA-MRSA. PVL virulence gene is one of the virulence genes seen mostly in CA-MRSA and less common in HA-MRSA. The prevalence of the gene was tested in this study.

2.8.2. Physical Risk factors of MRSA transmission

Transmission of CA-MRSA is largely from people with active skin infections and spread by direct physical contact and indirect contact by touching objects like towels, workout areas, surfaces like door handles contaminated by the infected person (Raygada & Levine, 2009).

Patients who have recently been hospitalized within the past year and patients in long-term health care facilities like nursing homes and dialysis centers, are at a higher risk for HA-MRSA infection. Medical conditions that weaken the immune system like HIV/AIDS, recent invasive medical procedures like surgery, catheterization and dialysis and recent use of antibiotics also increases the risk for HA-MRSA. Health care workers and people who are in close contact with health care workers are at increased risk for developing MRSA infections. Children have a higher risk for infection, possibly because their immune systems are not fully developed.

Risk factors for CA-MRSA infection include participation in contact sports like football, sharing contaminated items like towels and poor hygiene. People exposed to crowded conditions such as schools, childcare, military barracks and prison also are at increased risk for CA-MRSA infection (Raygada & Levine, 2009).

The Centers for Disease Control and Prevention developed a conceptual model incorporating epidemiological risk factors of basis of CA-MRSA outbreak data. This "Five Cs of CA-MRSA transmission" model suggests that MRSA infection results from contact; direct skin to skin, lack of cleanliness, compromised skin integrity, contaminated object surfaces and items and crowded living conditions (Miller & Diep, 2008).

Research has also recognized the household as a potentially important transmission setting for *S. aureus*, this leads to the spread of CA-MRSA within households and the potential for these strains to cause recurrent infections among family members (Jones *et al.,* 2006). Household members who have a skin infection may also increase the risk of transmission due to close personal contact and young children appear to be particularly important as reservoirs for CA-MRSA (Knox *et al.,* 2012). Increase in nasal and extra-nasal colonization with CAMRSA strains has also been noted (Miko *et al.,* 2012).

2.9. Laboratory Diagnosis of MRSA

Methicillin resistance *Staphylococcus aureus* is diagnosed by detecting the mec*A* gene (Enright *et al.*, 2000). The samples that are analysed in the laboratory may include clinical samples such as blood, urine, sputum, or other body-fluid samples and environmental samples such as food, swabs from the surfaces such as theatre, schools surfaces such as door handles, gym equipment and telephone booth.

The bacterium is first cultured in a culture media to obtain sufficient numbers to do the confirmatory tests. The culture media could be enrichment broth or agar media like blood and nutrient agar media or a selective media for *S. aureus,* like Mannitol salt agar which selects only the *S. aureus*. The isolate is then identified by use of biochemical tests like catalase test, coagulase test and Gram staining to identify the morphology characteristics of the bacterium. Commercial biochemical tests which use automated instruments can also be used to identifyy *S. aureus*.

Disc diffusion or Minimum inhibitory concentration (MIC) test is used to test MRSA against drugs like cefoxitin. Resistance to this drug is considered to be MRSA (CLSI, 2013). The confirmatory tests include rapid tests, real time PCR, and quantitative PCR to detect MRSA clones for *S. aureus* (Enright *et al.*, 2000). Other tests include Latex agglutination test used to detect the PBP2a protein a variant protein that gives *S. aureus* the ability to resist oxacillin. Automated systems including Vitek/Vitek2 (bioMe´rieux), Phoenix (Becton Dickinson) and Microscan (Dade Behring) can be used to tests for methicillin/oxacillin susceptibility (Enright *et al.*, 2000).

2.10. Treatment of MRSA infections

MRSA infections are treated with topical, oral, or intravenous antibiotics, depending on the type of the infection (Blot *et al., 2002).* Minor skin infections are usually treated with an antibiotic ointment such as a nonprescription triple-antibiotic mixture. In some cases, oral antibiotics may be given for skin infections. Additionally, if abscesses are present, they are surgically drained. More serious and life-threatening infections are treated with intravenous antibiotics (Blot *et al., 2002).*

The recommended drugs for treatment of *S. aureus* infections in a clinical setup include azithromycin, clarithromycin, erythromycin, clindamycin, oxacillin, cefoxitin, penicillin, trimethoprim-sulfamethoxazole, ceftalothine, linezolid, doxycycline, minocycline, tetracycline, vancomycin, chloramphenicol, ciprofloxacin, levofloxacin, gentamicin, norfloxacin, nitrofurantoin and rifampin (CLSI, 2013).

Treatment of MRSA is limited as many standard antibiotics do not work effectively. The duration and the choice of treatment depend on the type of infection as well as the drug resistant pattern of the particular bacterial type. The first line therapy is a penicilinase-resistant beta lactam antibiotic like oxacillin or flucloxacillin (Blot *et al.,* 2002). Serious infections like endocarditis can be treated by the combination therapy with gentamycin (Blot *et al.,* 2002). Resistance to antibiotics has led to use of broad spectrum anti gram positive antibiotics such as linezolid. Linezolid belongs to the newer oxazolidinones class and is effective against both CA-MRSA and HA-MRSA especially with patients with MRSA pneumonia. Serious invasive infections due to MRSA can be treated by using vancomycin as the first line of treatment (Mongkolrattanothai *et al.,* 2003). Teicoplanin and daptmycincan can also be used for treatment of MRSA infections. Tetracyclines (like doxycycline and minocycline) and clindamycin can also be used to treat osteomyelitis (Mongkolrattanothai *et al*., 2003). Treatment with glycopeptides is not recommended as the drug does not penetrate very well into infected tissues especially the brain and the meninges (Blot *et al.,* 2002).

Mupirocin (Bactroban) can sometimes be effective for treating and eliminating MRSA from the nose of healthy carriers, but decolonization is usually not recommended unless there has been an outbreak of MRSA or evidence that an individual or group of people may be the source of the outbreak (Mongkolrattanothai *et al*., 2003).

Staphylococcal food poisoning typically resolves on its own without long-term complications no antibiotics are required since the toxin produced by *S.aureus* is the one that affects an individual and not the bacteria itself. Supportive treatment is given in such cases. No vaccine is available against *S. aureus*.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

This was a cross sectional prospective study detecting and characterizing MRSA in selected secondary schools in Nairobi County, Kenya. Questionnaires were administered to the school's Principals or the head teachers in each individual school Appendix 5. The school Principal/head teacher who participated in the study answered the questions asked by the researcher and signed the consent forms to allow the study to be done in their respective schools Appendix 3.

3.2. Study area

The study was done in Nairobi County, Kenya. Nairobi is the capital city of Kenya and it is surrounded by Thika, Machakos, Kajiado and Kiambu Counties. Figure 3.1.shows the map of Nairobi County.

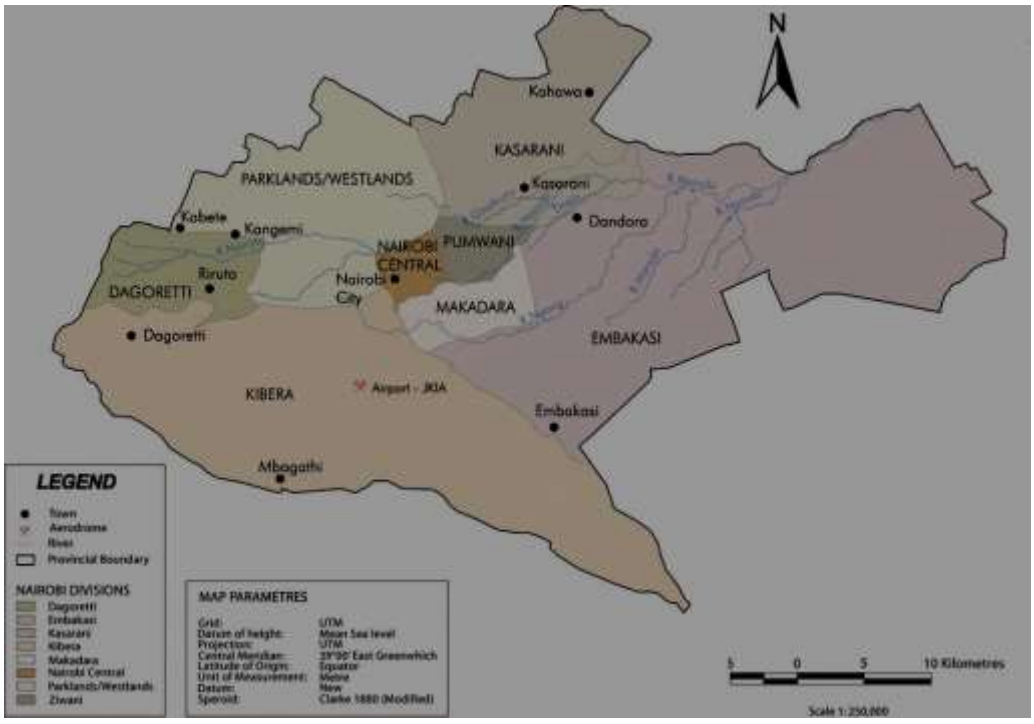

Figure 3.1: Map of Nairobi County (Google Maps)

3.3. Study population

The target population were surfaces of classroom and toilets door handles of the selected secondary schools. The sampled surfaces included the inside and the outside door handles of both the classrooms and the toilets. The surfaces of the handles of the doors were swabbed using a sterile moistened swab. The study population also included the head teachers and the principals of the selected schools. The head teachers and the principals were interviewed and the data populated in the questionnaires.

3.4. Sampling technique

Three characteristics were used to select the schools, these characteristics included, Boarding girls and boys schools, Day girls' and boys' schools and the mixed girls' and boys' schools. On each characteristic of school, a systematic random sampling was used to select two schools. A total of 12 schools were selected; four boarding schools (two girls and two boys' schools). Four day schools (two girls and two boys schools), four mixed schools (two boarding and two day school).

3.4. Study variables

The variables in the study were the category of the school (day or boarding, girls or boys or mixed school), whether the surfaces were disinfected or not, samples from either the inside or outside the door handles and the positive and negative sample results. A total of eight variables were analysed.

3.5. Sample size Determination

The sample size was calculated according to Fisher *et al*, 1998 and the expected prevalence of condition of interest was 21 % (Kesah *et al.*, 2003).

$$n = \left[\frac{Z^2_{1-\alpha/2} P (1 - P)}{D^2} \right] DEFF$$

Where n = Minimum sample size

$Z_{1-\alpha}$ = 1.96 standard error

P = Expected prevalence of condition of interest (21%)

D = 0.05 (inverse of 95% allowable error)

DEFF = 1.2 for systematic design effect in systematic random sampling

$$= \left[\frac{1.96^2 \times 0.21(0.79)}{0.05^2} \right] 1.2 = 306$$

A minimum of 306 samples included in the study. These 306 samples were collected in 12 schools. A minimum of 25 samples was collected in each school. The schools differed in the numbers of the classrooms and the toilets because some schools had large numbers of students. The number of classrooms ranged from four to 17 in some other schools. The toilets ranged from six to 30 in the selected schools. The sample collection area included the surfaces of the door handles in all the toilets and the classrooms in those schools.

3.6. Ethical approval for the study

Clearance to carry out the study was obtained from Kenya Medical Research Institute (KEMRI) Scientific Steering Committee and Ethical Review Committee of KEMRI (SSC/ERC protocol Number-2360 (Appendices 1 and 2). All procedures were carried out in accordance to the CMR/ KEMRI Biosafety guidelines and waste disposal. Informed consent was also obtained from the head teachers of the schools that agreed to be included in the study Appendix 3.

3.7. Inclusion criteria

- The secondary school in Nairobi County, Kenya whose management agreed to participate in the study.
- Head teachers and principals interviewed in the schools.

3.8. Exclusion criteria

Secondary school in Nairobi whose management was not willing to allow the school to participate in the study.

3.9. Collection of samples

The samples were collected using sterile swabs moistened using buffered peptone water by swabbing the toilet and the classroom door handles. The swabs were collected by gently rubbing the moistened swab on the door handles surface and rotating the swab round to 360 degrees. Samples were collected from both inside and outside door handles of each door that was sampled. Two samples from each site were collected to ensure recovery of *S. aureus*. A total of 306 in samples in duplicates were collected.

The samples were then put in a sterile tubes containing one millilitre of peptone water to avoid drying and transported in a cool box to the National Public Health Laboratory in Nairobi. The samples were properly labelled using reference numbers so that the identity of the school was anonymous. Student's toilets, school workers

toilets, class rooms and visitors' toilets in each school were swabbed randomly. A total of 306 swabs were collected from door handles of the both the class rooms doors and the toilets in randomly selected secondary schools in Nairobi, Kenya.

3.10. Laboratory procedures

3.10.1. Culture of the *S. aureus*

In the laboratory the samples were thoroughly mixed to suspend the microorganisms into the phosphate buffered peptone water solution. The suspension was inoculated into Mannitol salt agar media (Oxoid, Cambridge, UK) by use of sterile inoculating loop. The samples were then incubated at 35^{o}C for 24 h. The isolates in the Mannitol salt agar were then sub cultured into Tryptase supplemented with 5% sheep blood. The identity of the isolates was confirmed by standard laboratory methods which included colonial morphological appearance, biochemical reaction tests such as catalase test and coagulase test and Gram staining.

3.10.2. Identification of *Staphylococcus aureus*

3.10.2.1. Colonial appearance

The samples were inoculated in culture media and their colonial morphologies of *S. aureus* were observed as described in the results section

S. aureus ATCC 25923) was used as positive control organism by subjecting the bacteria under the same conditions as the test organism (Enright *et al.*, 2000). The standard organism showed the same culture characteristic as the bacteria under examination.

3.10.2.2. Gram staining

Gram stain is used to classify bacteria on the basis of their forms, cellular morphologies and Gram reactions. One drop of sterile normal saline was placed on a labelled glass slide. A small portion of colony was picked from the culture media using a sterile applicator stick and mixed gently to emulsify on

the slide. The slide was left to air dry and then fixed by passing the slide over a flame three times. The slide was left to cool before staining. The slide was stained by flooding the fixed smear with crystal violet stain for 30 seconds. The stained slide was then washed gently with running water and blotted with paper towel before Gram's iodine was added and left on the slide for 30 seconds.

The iodine was then rinsed off gently with flowing tap water. Decolourization was done by use of acetone and the slide then rinsed off with water. Counter staining was done by flooding the slide with Safranin and allowed to remain on the slide for two minutes. The Safranin was then washed off with tap water. The slide was left to air dry. The slide was examined on microscope for the Gram stain reaction.

S. aureus ATCC 25923 was used as positive control organism by subjecting the bacteria under the same conditions as the test organism (Enright *et al.,* 2000). The standard organism showed the same Gram stain reaction as the bacteria under examination.

3.10.2.3. Catalase test

Thirty percent hydrogen peroxide was used as the reagent to test for catalase production.

A colony of the bacteria was picked from the blood culture media by using a sterile applicator stick and placed on a clean glass slide. One drop of the reagent was placed on the colony on the slide. Effervescence was observed indicating presence of *S.aureus.*

S. aureus ATCC 25923 was used as positive control organism by subjecting the bacteria under the same conditions as the test organism (Enright *et al.,* 2000). The standard organism showed the same catalase test reaction as the bacteria under examination.

3.10.2.4. Coagulase test

Zero point five millilitres (0.5ml) of rabbit plasma were put into a test tube and the test tube was inoculated with one colony of the test organism growing from the sheep blood agar. Incubation of the tube was done at 37˚C for 4 hours and observed hourly for clot formation and also 24 hours for the colonies that form weak agglutination in 4 hours.

Positive test was a complete clot formation or any degree of clot formation while the negative was lack of clot formation.

S. aureus ATCC 25923 was used as positive control organism by subjecting the bacteria under the same conditions as the test organism (Enright *et al.*, 2000). The standard organism showed the same coagulase test reaction as the bacteria under examination.

3.10.2.5. Deoxyribonuclease test (DNase test)

Confirmation of *S. aureus* was also done by doing a DNase test. The test organism was inoculated on a DNase agar containing a green dye (Scharlau, Barcelona, Spain). The plate was then incubated for 24 hours at 37°C. *Staphylococcus epidermidis* was used as a negative control. A positive reaction was seen by the medium turning colourless around the test organism while the Negative reaction was seen by the media remaining green around the negative control.

3.10.3. Preservation of *Staphylococcus aureus* isolates

Colonies of the isolates were diluted in 1 ml of sterile tryptic soy broth. High concentration (2 Mac Farland standards) of the organism was prepared to enable high recovery of the organism. One millitre (1ml) of the organism was put into a cryovial containing 1ml of sterile tryptic soy broth with 15% glycerine, then sealed with a cock and stored at -80^{o}C.

Fifteen percent (15%) of glycerol was used as a cryoprotective agent to protect the organisms from damage during freezing, storage and thawing. The preserved isolates were used for molecular characterisation of the methicillin resistance gene *mecA* and PVL virulent genes

3.10.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by use of Kirby Bauer disk diffusion method under Clinical Laboratory Standards Institute (CLSI, 2013) guide lines. Five colonies of the organism were emulsified in 5mls of sterile normal saline and mixed well; the turbidity was compared to 0.5 Mac Farland standards.

A sterile cotton swab was used to inoculate the sample into Mueller-Hinton agar plates (Oxoid, Cambridge, UK) and allowed to dry. The following antibiotics were used; oxacillin (30 µg cefoxitin), 20/10 µg amoxicillin/clavulanic acid,10 µg gentamycin, 30 µg ceftazidime, 30µg vancomycin, 5 µg Levofloxacin,10 µg ampicillin, 30 µg tetracycline, 1.25/23.75 µg trimethoprim-sulfamethoxazole and 15 µg erythromycin. The choice of the antibiotics was done according to CLSI guideline of 2013.

Zone of inhibitions were determined by measuring the size of clear zones with a graduated ruler. The measurements were done in millimetres and the zones were compared with the CLSI standards for interpretation (CLSI, 2013). The reporting was done by indicating Resistant, Intermediate or Sensitive (CLSI, 2013).

S. aureus ATCC 25923 was used as positive control organism by subjecting the bacteria under the same conditions as the test organism (Enright *et al.*, 2000). The standard organism showed the expected zones of inhibition of the drugs as indicated in the CLSI standards. The positive control was used verify that the drugs used for the susceptibility were working correctly.

3.10.5. Detection of MRSA by disk diffusion

Methicillin resistance was determined by testing using 30 μg cefoxitin, if the inhibition zone was equal or less than 21 mm, the culture was considered as positive for MRSA. The result was reported as oxacillin susceptible or resistant based on the cefoxitin result. The results indicated the presence of MRSA (CLSI 2013).

The Clinical and Laboratory Standards Institute (CLSI), recommends the cefoxitin disk screen test (CLSI, 2013 Guidelines). Methicillin is no longer commercially available in the many countries due to its toxicity and does not maintain its activity during storage. Cefoxitin is a better inducer of the *mec*A gene, and disk diffusion tests using cefoxitin give clearer endpoints and are easier to read.

3.10.6. Detection of mecA gene and PVL genes

3.10.6.1. DNA extraction

The DNA isolation was done using Spin protocol method using the QIAGEN kit (QIAGEN Inc, Hilden-Germany). The test was based on the DNA-SRIP technology and permits the identification of the *S. aureus* strains and at the same time SRIP detecting Methicillin- mediating *mec*A gene and the bicomponent cytotoxin virulence factor PVL (Lifescience, 2009). The extraction for each sample was done in duplicates to ensure recovery of the DNA.

The cultured *S.aureus* was used for the identification of MRSA and PVL genes. Twenty microlitres of QIAGEN protease (proteinase k) was mixed with 200 μl of the sample. 200 μl of lysis buffer was added and the mixture incubated at 56^{o} C for 10 min. Proteinase k was used to digest proteins from the bacteria cells while lysis buffer was used to lyse the bacterial cells.

200 μl of Ethanol (for precipitation of the DNA) was added and mixed and then centrifuged at 800 rpm for 1 minute. Buffer Washing was done by adding 500 μl Wash buffer (AW1) and then centrifuging at 800rps for 1 min. The second wash was done by adding 500 μl of Wash buffer (AW2) and then centrifuging for 14,000 rpm

for 3min. The elution of the DNA was done by adding 200 µl of distilled water and incubating at room temperature for 1 min and then centrifuging at 800rpm for 1 min. The extracted DNA was then subjected to PCR as per the manufacturer's instructions shown in the next step below. The other vials containing extra DNA were stored at -20^{o}C for downstream analysis.

3.10.6.2. Polymerase chain reaction (PCR)

The amplification was done using GenoTypeMRSA assay kit. Thermocycler ABI 2720 was used for amplification. Primers supplied in the kit for *S. aureus*, *Staphylococcus epidermidis*, *mec*A and PVL genes were used. The kit did not provide sequences for primers, so it was used as per the manufacturer's instruction.

The amplification mix was prepared in in the pre PCR room. Each PCR tube (0.2ml) for each sample contained a total of 45 µl of the mix; the mix was made for 63 tubes, 61 for the samples, one for positive and one for negative controls. The negative control contained distilled water and the positive control contained the DNA from known MRSA isolate. The mix contained 35 µl of PNM solution (Deoxynucleotide triphosphates dNTPs,), 5 µl of polymerase incubation buffer, 2 µl MgCL2 solution (cofactor and catalyzer in PCR), 1 µl of HotStarTaq DNA polymerase (used to synthesize new strand of DNA) and 2 µl of water. Five micro litres (5 µl) of extracted DNA of each sample was added into the reaction tube containing 45 µl of the amplification mix. A thermal cycler was used for amplification. Denaturisation was done at 95^{o}C for 15 min, then annealing was done at 60^{o}C for 30 sec and extension at 60^{o}C for one (1) min. A total of 22 cycles were done to obtain the required DNA product. The amplified DNA was then subjected to hybridization for identification of *mecA* gene and PVL gene.

3.10.6.3. Hybridization

The identification of the *mecA* gene and PVL gene was done through reverse hybridization using GenoTypeMRSA assay kit. The test strips are coated with probes for *mec*A gene, PVL gene, *S. aureus* and *S. epidermidis.* If the sample

contained *mec*A gene, PVL gene, *S. aureus* and *S. epidermidis*, the reaction will be seen by the formation of clear lines of reaction on the test strip, Plate 4.2. The test kit is provided with reaction wells for each sample. Each well was filled with 20 µl of denaturisation solution (<2% NaOH) and 20 µl of the amplified sample. Denaturation was done for 5 min. One millilitre of Hybridization buffer (8-10% anionic tenside) was added into the denatured DNA and mixed. The reaction strip for each sample was added into each sample's well. The tray holding the wells was placed into the TwinCubator at 45^{o}C and incubated for 10min.

After incubation the hybridization buffer was completely aspirated by using a sterile pasture pipette. One millilitres (1ml) of Stringent wash solution (>25% quatinary ammonium compound and <1% anionic tenside) was added into the wells and incubated for 5 min and then the solution poured out. One ml of Rinse solution (buffer, <1% NaCl, <1% anionic tenside) was used to rinse the strips for 1min and then the solution was poured out. One millilitre of conjugate (streptavidin-conjugated alkaline phosphatase, an enzyme that binds to the individual probs/DNA complex during the hybridization step) was added and incubated for 10 min.

The strips were then washed using 1ml of Rinse Solution for 1min and then 1ml of substrate (dimethyl sulfoxide, <1% MgCl2, <1% NaCl used for the color development on the reaction lines on the strips) was added to each sample well and incubated in the dark for 20min. The reaction was stopped by washing the strips with distilled water. The strips were then removed from the tray and left to dry for evaluation and interpretation.

3.10.6.4. Evaluation and interpretation of results

An evaluation sheet was used to determine if the samples contained Mec*A*, PVL genes and *S.aureus*. The evaluation sheet is aligned with the conjugate control band (CC), universal control band (UC) directed against highly conserved DNA region appearing in all bacterial species tested for and the specific bands of each specific probes.

Both the CC and UC must show the reactions in all the tests for both positive and negative samples. The probes include *mec*A gene, PVL gene, *S. aureus* and *S. epidermidis,* Appendix 4.

3.11. Statistical analysis

Data generated from the laboratory experiment and the questionnaire was put in Ms Excel and the analysis was performed using Stata 10. Descriptive statistics were used on quantitative data. Frequencies and cross tabulation were used on categorical data. Chi-square test, odds ratio and p values were used as a measure of association and significance. A P value of less than 0.05 was considered to be statistically significant. The data was presented in form of tables and charts.

CHAPTER FOUR

RESULTS

4.1. Characteristics of sampled schools

Different characteristics were used to sample the 12 schools. The characteristics included six boarding school and six day schools with a ratio of 1:1. Four boys schools, four girls schools and four were mixed boys' and girls' school with a ratio of 1:1:1. The numbers of classes were ranging from four classes to 17 classes with a mean of eight classes. The total number of students in all the schools ranged from 140 to 600 students with a mean of 370. The number of toilets ranged from 6 to 40 with a mean of 12 toilets. On the characteristic of the classroom doors that were opened and closed each time a person entered and left the classroom, seven classrooms were not opened and closed each time a person entered and left the classroom while five kept on being opened and closed each time a person entered and left the classroom. Three schools in the study indicated that the toilets were disinfected while nine schools did not disinfect the toilets, Table 4.1.

Table 4.1: Characteristics of sampled schools

Characteristics of schools		Frequency	Percentage
School type		6	50
Boarding			
		6	50
School type		4	33.3
Boys			
		4	33.3
Number of classes:	Median,(Min,	8, (4, 17)	
Max)			
Number of students:	Median, (Min,	369.5,	
Max)		(140,600)	
Number of toilets:	Median, (Min,	12, (6, 40)	
Max)			
Classroom doors that were opened and closed each time a person entered and left the classroom:			
Yes		5	41.7
Toilets disinfected		3	25
Yes			

4.2. Prevalence of *Staphylococcus aureus*

4.2.1. Laboratory identification and characterization of MRSA

S. aureus (MRSA) were identified as Gram positive grapelike cocci in clusters showing deep violet colour. The *S. aureus* were coagulase, catalase and DNase

positive. S. *aureus* were also identified by observing their colonial characteristics. Strains of MRSA exhibit the same morphological and biochemical characteristics as other *S. aureus.* The colonies were yellow, smooth, slightly raised and translucent. In blood agar media the colonies were whitish in colour and shows beta haemolysis as a result of lyses of the red blood cells in the culture media, Plate 4.1.

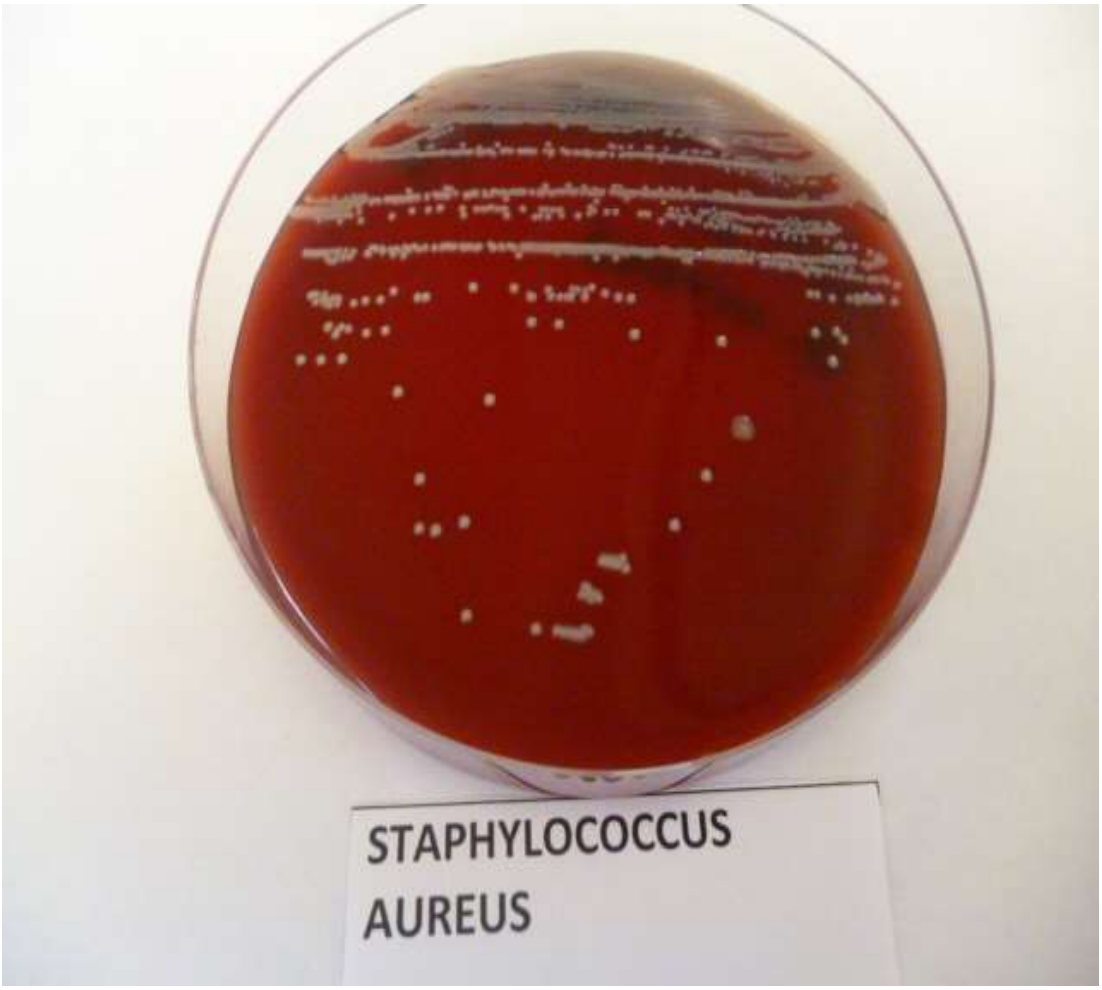

Plate 4.1: Showing a picture of *S. aureus* on a culture plate

The samples which contained the *mec*A and PVL genes showed reactions as indicated in the hybridization strips. Some samples showed presence of both genes depicting the presence of both genes tested while some showed the presence of only one tested gene, Plate 4.2.

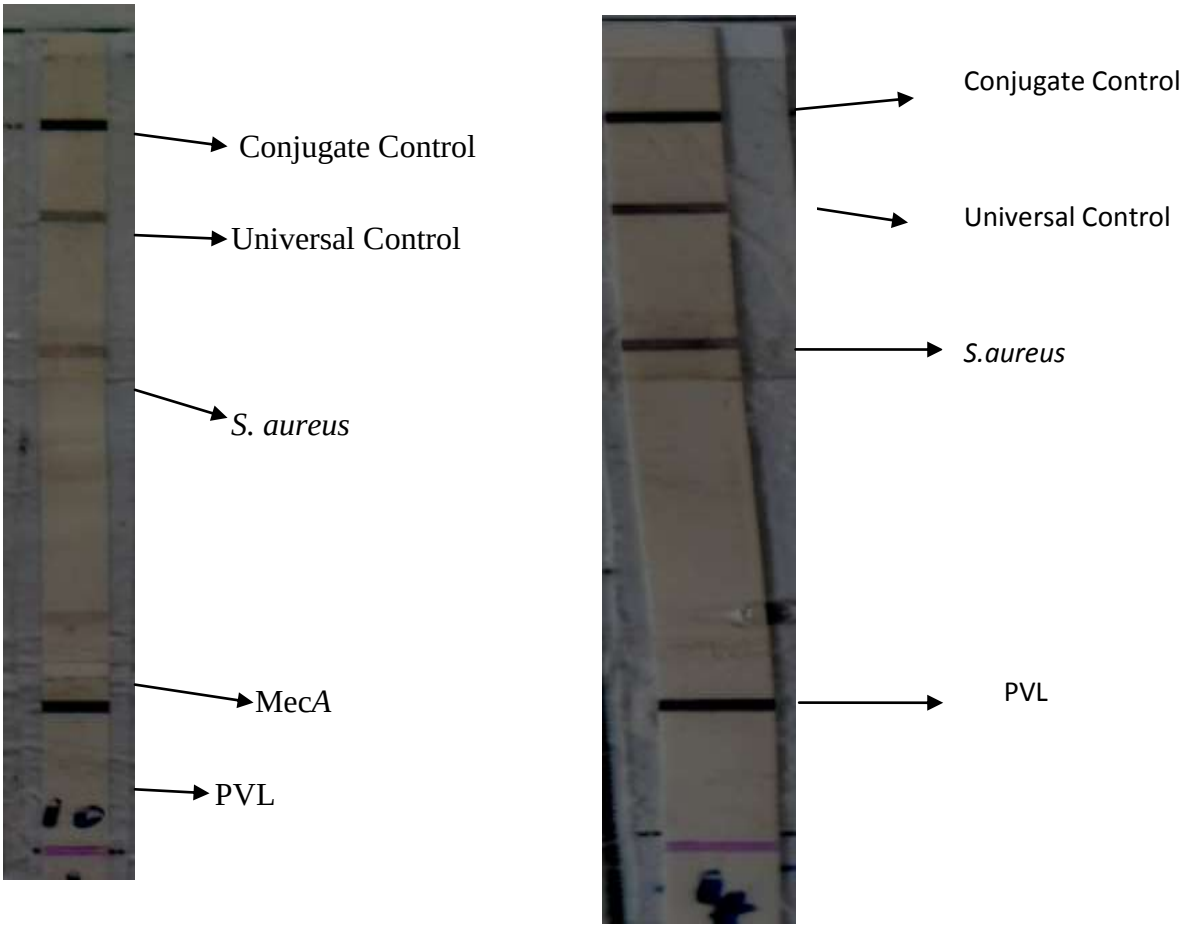

Plate 4.2: Hybridization reactions from the samples

4.2.2 Prevalence of *Staphylococcus aureus* isolated from the study

A total of 306 samples were collected from selected secondary schools. Sixty one (19.9%) samples were positive for *S. aureus.* The highest numbers of *S. aureus* positive samples were from boarding girls' schools 41.5%, followed by day girls' schools 38.5%, 20% in mixed day schools and 14% in mixed boarding schools as in Figure 4.3.

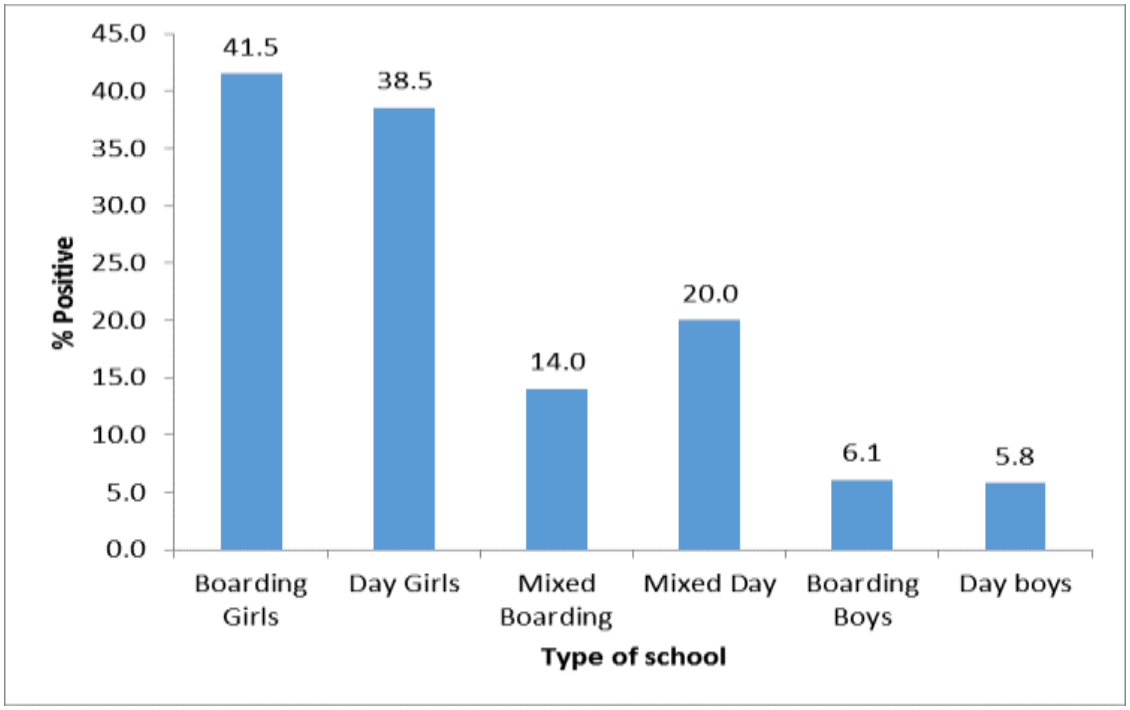

Figure 4.1: Prevalence of *Staphylococcus aureus* isolated

4.3. *Staphylococcus aureus* distribution

Distribution of *Staphylococcus aureus* was found in various surfaces as seen in the samples that were collected. In the classrooms sampled, 34 samples were positive for *S. aureus* while in the toilets 27 samples were positive with a P value of 0.2 and

chi square of 1.6578. Samples from the inside of the door handles, 24 were positive for *S.aureus* while the outside door handles were 37 with a P value of 0.03 and a chi square of 4.6078. This indicated that *S. aureus* was four times likely to be isolated from the outside door handles than the inside door handles. The term inside door indicates whether the sample was taken from the handles on the inside handle of the door or the outside handle of the door.

The classroom doors that were opened and closed each time a person entered and left the classroom harboured more *S. aureus* isolates(48) than the classrooms that were not opened and closed each time a person entered and left the classroom(13) with a P value of 0.026 and a chi square of 4.955. The presence of *S. aureus* was higher in the toilets that were not being disinfected than the toilets which were disinfected (9) with the p value of 0.003 and a chi square of 8.55 as in Table 4.2.

Table 4.2: *Staphylococcus aureus* distribution according to the source of the school sampled

Source		**Positive N(%)**	**Negative**	**Chi Square**	**P-Value**
Source:	Classroom	34(55.7)	114(46.5)	1.6578	0.2
	Toilet	27(44.3)	131(53.5)		
Surface (Door handles):	Inside door	24(39.3)	134(54.7)	4.6078	0.03
Classroom doors that were opened and closed each time a person entered and left the classroom:	No			4.955	0.026
	Yes	13(21.3)	89(36.3)		
Toilets disinfected:	No	55(90.2)	177(72.24)	8.55	0.003

4.4. Antibiotic susceptibility testing of *Staphylococcus aureus* isolates and prevalence of MRSA

Antibiotic susceptibility testing (AST) was performed on all the 61 *S. aureus* isolates. The resistance of the antibiotics was evaluated by use of CLSI guideline 2013. The rate of resistance to each antibiotic was calculated as the number of resistant isolates divided by the total number of isolates. The choice of drugs selected for AST was guided by the CLSI guidelines 2013.

The proportions of isolates were classified as susceptible, intermediate or resistant to the antibiotics that were tested. The zones of inhibition of the antibiotics were measured then the interpretation of the drugs was done according to the CLSI guideline 2013 to indicate susceptible, intermediate or resistant, Appendix 6.

Twelve out of 61(19.6%) *S. aureus* isolates were resistant to cefoxitin and 49(80.4%) out of 61 *S. aureus* isolates were sensitive to cefoxitin. Sixty one percent *S. aureus* isolates exhibited resistance to trimethoprim/sulfamethoxazole, 16% showed intermediate and 23% were sensitive to the drug. Fifty one out of the 61 *S.aureus* isolates (83.6%) were susceptible to amoxicillin/clavulanic, 1.6% was intermediate and 14.8% were resistant. Ninety three percent of *S.aureus* isolates were sensitive to ampicillin and seven percent were resistant. Prevalence of MRSA based on the disc susceptibility testing with cefoxitin was 19.9% as in Figure 4.2.

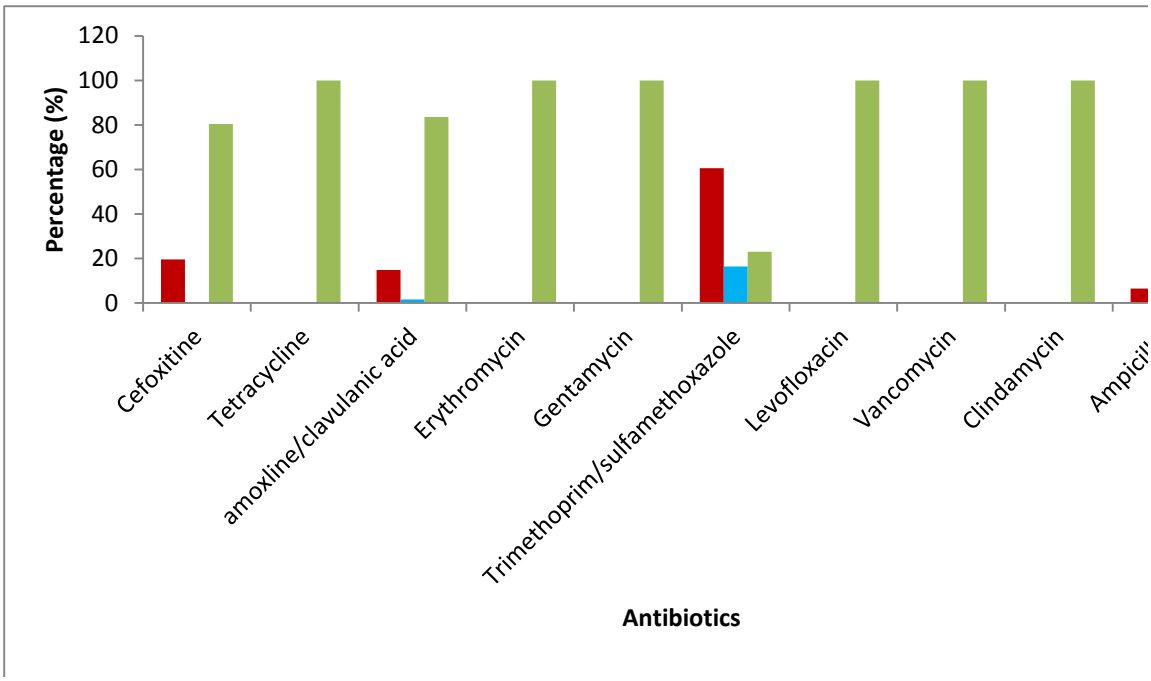

Figure 4.2: Antimicrobial susceptibility patterns of *Staphylococcus aureus* strains isolated

4.5. Factors associated with *Staphylococcus aureus* contamination

Contamination with *S. aureus* was significantly associated with position of door handles (inside or outside) (95% CI 1.8; P 0.03). The results also showed that gender was associated with the presence of *S. aureus* ($\chi 2$ 71.9; P < 0.001), similarly disinfection of the toilets also was significantly associated with contamination of *S. aureus* (95% CI; P0.003). Classroom doors that were opened and closed each time a person entered and left the classroom contributed to the presence of *S. aureus* (95%; 0.026) as in Table 4.3.

Table 4.3: Factors associated with *Staphylococcus aureus* contamination

Variable		Positive (%)	Negative (%)	OR (CI)	P Value
Source	Class	34 (55.7)	114 (46.5)	1.45 (0.82-2.54)	0.2
	Toilet	27 (44.3)	131 (53.5)		
Surface(**Handles**	Outside door	37(60.7)	111(45.3)	1.8 (1.02-3.16)	0.03
	Inside door	24 (39.3)	134 (54.7)		
Type of school:	Boarding	32 (52.5)	120 (49.0)	1.15 (0.66-2.02)	0.63
	Day	29 (47.5)	125 (51.0)		
Gender	Boys	6 (5.8)	101 (41.2)	$\chi 2$ 71.9	<0.001
	Girls	48 (78.7)	57 (23.3)		
	Mixed	13 (21.3)	87 (35.5)		
Classroom doors that were opened and closed each time a person entered and left the classroom					
Yes		48 (78.7)	156 (63.7)	2.11 (1.08-4.1)	0.026
		13 (21.3)	89 (36.3)		
No					
Disinfected:	No	55 (90.2)	177 (72.2)	3.52 (1.45-8.56)	0.003
	Yes	9 (9.9)	68 (27.7)		

4.6. Presence of *Mec*A gene and PVL gene

Out of 61 isolates of *S. aureus,* 9 (14.7 %) isolates contained *mec*A gene. Five of the *S.aureus* isolates containing *mec*A gene were from classrooms while four were from toilets with a P value of 0.971. Twelve (20%) of *S.aureus* isolates had the PVL genes. The number of *S.aureus* containing PVL genes from the toilets was 6 while 7 were from the classroom with a P value of 0.925. Five (8%) *S.aureus* isolates had both *mec*A and PVL genes; four were from the toilets while two were from the classrooms with a P value of 0.261. Three *S.aureus* isolates containing mec*A* genes did not contain PVL genes, Table 4.4.

Table 4.4: Percentage of Staphylococcus aureus isolates carrying mecA and PVL gene detected by PCR analysis

Gene	Source	No. Positive	No. negative	Percentage	Chi square	P value
Mec A	Classroom	5	**33**		**0.0013**	**0.971**
	Toilet	4	28			
	Total	**9**	**61**	**14.7%**		
PVL	Classroom	7	**33**		**0.0089**	**0.925**
	Toilet	6	28			
	Total	**12**	**61**	**20%**		
Both PVL & Mec A	Classroom	2	**33**		**1.2645**	**0.261**
	Toilet	4	28			
	Total	**5**	**61**	**8%**		

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1. Discussion

5.1.1 Prevalence of *Staphylococcus aureus* in schools

A previous study carried out in Nairobi showed a prevalence of 41% of *S. aureus* isolated from community acquired infections (Malonza *et al.,* 1997**).** These findings were much higher than the ones found in this study done in secondary schools in Nairobi, Kenya. The findings by Malonza *et al.,* (1997) could be higher because the samples were taken from outpatient's clinics at the Kenyatta National hospital which raises the probability of the patients harbouring the *S. aureus*. In comparison, the prevalence of *S. aureus* could be lower in the schools in this study because the samples were gotten from students who were presumed to be healthy unlike in samples collected from the patients in the hospitals.

A survey done in eight African countries between 1996 and 1997 shows a prevalence of MRSA in Nigeria, Kenya and Cameroon (21-30%) (Kesah *et al.,* 2003**).** In an urban university in Mid-western United states, *S. aureus* was isolated from computer keyboards, telephones and elevator buttons. The study aimed at checking for presence of *S.aureus* in 70 frequently used surfaces (Brook *et al.,* 2009). All these studies shows that *S. aureus* is quite common in environmental surfaces such as public places like in schools and that CA-MRSA is associated with people living in crowded settings (Ellis *et al.,* 2004).

5.1.2. *Staphylococcus aureus* distribution in different types of schools and surfaces

Staphylococcus aureus was more common in class room door handles (34%) than the toilet door handles (27). The data shows that it was 1.6578 times more likely for the classroom doors to have *S. aureus* than the toilets, however with a P value of 0.2 the difference is small and the overall chance of classrooms having *S. aureus* is the

same as the toilets. More *S. aureus* on the class door handles could be as result of the likelihood of more students touching the same door handle when entering the class room and the fact that the boys' school did not have door handles in the urinals which reduced the contamination from the classrooms to the toilets and *vice versa.*

The prevalence of *S. aureus* was also noted on the classroom door handle where the students kept on opening and closing the classroom doors every time one entered and left the classroom (78.7%) as compared to 21.3% were the door was not opened every time a student entered and left the classroom. This valuable showed a likelihood of more than four times (chi square 4.955) of isolating *S. aureus* on doors which were being opened and closed every time a student entered the class room. In this case, contamination is due to person touching the door handle when opening and when closing the door.

S. aureus was more on the outside part of the door handles (60.7%) than the inner part of the door handles (39.3%). This could be as a result of the door handle on the outside being touched more frequently as compared to the inner side of the class room. This agrees with the study done by Tammy & Bannerman (2003) that *S. aureus* can live freely in inanimate environment such as medical instruments, school environment like desks, on telephone booths, door handles and in public transport surfaces like sits.

CA-MRSA is associated with people living in crowded settings (Ellis *et al.,* 2004). A school is ones setup of crowded areas in the community. CA-MRSA infections can also occur in healthy people with no recent history of hospitalizations and young people are mostly infected (Richard 2010).

5.1.3. Role of disinfection and the risk of *Staphylococcus aureus* infection

Contamination of the *S. aureus* was noted on the toilets which were not disinfected than the ones being disinfected and a likelihood of more than eight times of getting contaminated by *S. aureus* from the toilets which were not disinfected than the ones disinfected. Disinfection of the toilet handles will kill most bacteria in the surfaces

(Gebel *et al., 2013).* It was noted that disinfection of the toilets was done only in the boy's schools and none in girls' schools. This could explain why there was less presence of *S.aureus* in boys' schools. Disinfection is known to kill bacteria on the contaminated surfaces; CDC recommends disinfection of surfaces with bleach. The effectiveness of a disinfectant depends on the surface to be disinfected, the application of the disinfectant including the concentration, formulation, water solubility and pH, and the microorganism present *(*Gebel *et al.,* 2013).

5.1.4. Antimicrobial susceptibility pattern

Antimicrobial resistance is one of the paramount microbial threats in the twenty-first century (Smolinski *et al.,* 2003). Anti-microbial chemotherapy and the introduction of new classes of antimicrobial agents are usually followed by the emergence of resistant forms of *S. aureus* (Hiramatsu *et al.,* 2001). Surveillance on the antimicrobial susceptibility patterns of *S. aureus* is of utmost importance in understanding new and emerging resistance trends. In this study different antibiotic susceptibility patterns were observed.

Trimethoprim/sulfamethoxazole:

A resistance rate (61%) of trimethoprim/sulfamethoxazole was observed in this study. This is lower than the percentage observed in Lagos, Nigeria, where 100% resistant to trimethoprim/sulfamethoxazole was noted in isolates from telephone handles (Smolinski *et al.,* 2003). A resistance of 27.7% was seen in CA-MRSA carrying *mecA* and Panton-Valentine leukocidin (PVL) genes isolated from the holy shrine in Najaf, Iraq (Al-Mohana *et al*., 2012). Thirty percent resistance was found in a study in South Africa (Shittu & Lin 2006). According these studies, the resistance of trimethoprim/sulfamethoxazole ranges from 27.7% to 100% and the resistance differs geographically

Erythromycin

Resistance of 0% of erythromycin was seen among the isolates in this study. CA-MRSA is only resistant to erythromycin and beta lactams (Tammy & Bannerman, 2003). This is in

line with 100% resistance of the same drug done in a study in Lagos, Nigeria, isolates from telephone handles (Smolinski *et al.,* 2003). A resistance of 37% was seen in CA-MRSA carrying *mecA* and Panton-Valentine leukocidin (PVL) genes isolated from the holy shrine in Najaf, Iraq (Al-Mohana *et al*., 2012). Thirty percent resistance was also found in a study in South Africa (Shittu & Lin 2006).

Resistance of erythromycin differs from one Continent to the other (Dora, 2011). In the study the resistance ranged from as low as 1% in Denmark to as high as 95% in Asia with most countries having a resistance of 20% and above.

Tetracycline

No resistance was found in tetracycline all the isolates were sensitive to tetracycline. In Lagos, Nigeria, only 33% of the isolates from telephone handles were sensitive to tetracycline (Smolinski *et al.,* 2003). Thirty percent resistance was also found in a study in South Africa (Shittu & Lin, 2006).

Resistance of tetracycline differs diversely from one Continent to the other (Dora, 2011). In the study the resistance ranged from as low as 1% in New Zealand, Asia to as high as 75% in Madagascar in Africa. More studies should be done to find out why some countries have high resistance and others very low resistance.

Amoxyllin/Clavulanic acid

Fourteen point eight percent (14.8%) isolates were resistant to amoxyllin/clavulanic acid, this was lower compared to 59.3% resistance in a study conducted in Nigeria on the handles of telephone booths (Smolinski *et al.,* 2003). Resistance of 48.1% was also noted in the CA-MRSA carrying *mecA* and Panton-Valentine leukocidin (PVL) genes isolated from the holy shrine in Najaf, Iraq (Al-Mohana *et al*., 2012). According to the current study, the resistance of the drug is not as high as compared to other studies

Ampicillin

Six point six percent (6.6%) resistance was noted in this study. In a study done for residential indoors in Texas, 54.59% showed resistance to ampicillin (Gandara *et al.,* 2007). Penicillin was among the least effective antibacterial agents in a study done in South Africa but all the isolates were susceptible to most other antibiotics (Shittu & Lin 2006). From these studies, the resistance pattern of ampicillin varies from one region to the other.

Gentamycin

All the isolates were susceptible to gentamycin in this study. In a study done in Lagos, Nigeria, isolates from telephone handles were also 100% sensitive to Gentamycin (Smolinski *et al.,* 2003). In a study done in South Africa 28.6% resistance to gentamicin was noted (Shittu & Lin 2006). Resistance of Gentamycin differs diversely from one Continent to the other (Dora, 2011**).** In the study the resistance ranged from as low as 0% in some European countries to 74% in Asia.

Cefoxitin

Nineteen point six isolates (19.6) were resistant to cefoxitin . Cefoxitin in this study was used to test for MRSA. A study done among primary school children and prisoners in Jimma Town, Southwest Ethiopia, 23.08% of *S. aureus* was found resistant to cefoxitin (Kejela & Bacha 2013). The resistant patterns in both studies show almost the same prevalence.

Clindamycin

Hundred percent (100%) of the isolates were susceptible to clindamycin in this study. Resistance of clindamycin also differs from one Continent to the other (Dora, 2011). In the study the resistance ranged from as low as 0% in some European countries to 79% in Asia and America.

Levofloxacin and Vancomycin

Hundred percent (100%) of the isolates were susceptible to levofloxacin and vancomycin seen in this study. A study done on the Prevalence of methicillin-resistant *S. aureus* in eight African hospitals and Malta (Nigeria, Tunisia, Ivory Coast, Senegal, Kenya, Cameroon, Morocco, Algeria and Malta), showed that all MRSA strains tested in the study were susceptible to vancomycin (Kesah *et al*., 2003).

Maina *et al*., 2012) noted that all 82 *S. aureus* in the study done on MRSA from skin and soft tissue infections infections in patients in Nairobi, Kenya were susceptible to vancomycin.

In another study done in South Africa all the isolates were susceptible to vancomycin (Shittu & Lin 2006). This is good because vancomycin now is the drug of choice for treatment of MRSA infection.

5.1.5. Factors associated with *Staphylococcus aureus* contamination

Contamination with *S. aureus* was significantly associated with position of door handles (inside or outside), therefore a student is more likely to get contaminated if he/she touches the handles on the outside of the door. *S. aureus* is spread by direct physical contact and indirect contact by touching objects like towels, workout areas, surfaces like door handles contaminated by the infected person (Raygada & Levine, 2009). The probability of getting contamination from both the boarding and day school was almost the same, however a closed community such as schools and prisons which are a form of crowded living conditions could be a source of contamination (Miller & Diep, 2008). Day school students are a source of spread of infections from schools to homes. Research has shown the household as a potentially important transmission setting for *S. aureus*, this leads to the spread of CA-MRSA within households and the potential for these strains to cause recurrent infections among family members (Jones *et al.*, 2006).

Gender was associated with the presence of *S. aureus*, *S. aureus* was more in girls' school (78.7%) and in mixed school (21.3%). This could be unhealthy since girls are known to use tampons. The tampons are known to cause toxic shock syndrome a community acquired infection mainly found among young menstruating women using highly absorbent tampons (Tammy & Bannerman, 2003).

Whether the students kept on opening and closing the door of the classrooms all the time (78.7%) had a difference with the door that was not open all the time (21.3%). The door that kept on being opened and closed during the class room had more likelihood of contamination, this is because a door handles is touched by different persons. This agrees with the fact that *S. aureus* is spread by touching surfaces like door handles contaminated by the infected person (Raygada & Levine, 2009). The infected student can touch the door and contaminate the door handle with *S. aureus*, this will then be transmitted to the other students as they continue to touch the door as they enter and leave the classroom.

In the toilets where disinfection was done the rate of contamination was minimal (9.9%) as compared with the toilets which were not disinfected (90.2%).Disinfection of the infected surfaces has been shown to prevent the spread of the pathogenic organisms such as *S. aureus* (Gebel *et al.,* 2013)

5.1.6. Prevalence of Mec*A* gene

In this study done among the schools in Nairobi, Kenya and the study done by Malonza *et al* (1997) indicates presence of CA-MRSA in Kenya. It is well noting that the presence of *mec*A genes in girls' in this study is of great significance, since *S. aureus* causes toxic shock syndrome a condition found in young menstruating women and can cause death. *S. aureus* can also cause food poisoning in school set up. The presence of MRSA in the school is of concern since treatment of MRSA is difficult as a result of resistance to most drugs used for treatment of MRSA (Voyich *et al.,* 2007).

The prevalence of MRSA differ worldwide, an estimated two billion people worldwide carry *S. aureus* and 53 million (2.7%) of carriers carry MRSA (CDC 2003). In Europe the prevalence of MRSA is about 26% while in Asia, particularly the Asia-Pacific region that includes Taiwan, Singapore, Japan, and Hong Kong, the prevalence rates of *S. aureus* are at above 60% and in Africa, the prevalence of *S.aureus* ranges from 5% to 45% (Dora, 2011).

5.1.7. Prevalence of PVL gene

Presence of PVL in this study (20%) is of importance since PVL contribute significantly to particular types of infections, such as severe lung infections and osteomyelitis (Vandenesch *et al.,* 2003). PVL is present in the majority of CA-MRSA and is the cause of necrotic flesh eating lesions, an aggressive condition that often kills patients within 72h (Voyich *et al.,* 2007).

Like in this study, a study done in France showed that most CA-MRSA contain the PVL gene (Dufour *et al.,* 2012). There is evidence that PVL-positive CA-MRSA clones have spread throughout the world (Garnier *et al.,* 2006) as seen in this study where we find a prevalence of 20% PVL containing CA-MRSA No studies of PVL has been done in Kenya so far.

5.1.8. Clinical significance of these findings

The presence of MRSA in schools in this study signifies the presence of a pathogen that is resistant to most drugs used for treatment of infections caused by the pathogen. MRSA can easily spread within the community and cause multiple infections and especially to persons who are immune compromised. The CA-MRSA can also spread into health care through patients coming to the health facilities and through health workers.

5.2. Conclusion

Sixty one (20%) samples were found to contain *S. aureus* in this study. Mixed Boarding school had a higher number of positives compared to mixed day school.

The positive samples were from six schools out of the 12 schools sampled. The class rooms had a higher number of positives than the toilets. Similarly *S. aureus* was more prevalent in boarding schools than in day schools in girls.

Twelve out of 61(19.6%) *S. aureus* isolates were resistant to cefoxitin. Cefoxitin was the antibiotic used in this study to test for MRSA. All the 61 isolates were sensitive to tetracycline, Gentamycin, erythromycin, levofloxacin, vancomycin and clindamycin.

On factors associated with *S. aureus* contamination, *S. aureus* was more prevalent in classroom than toilets doors. Contamination was more on inside door handles than the outside door handles. Prevalence was more in boarding schools than in day schools. Based on gender prevalence, *S. aureus* was more in girls' school than in boys' school. Contamination was more on the classrooms that kept on being opened and closed every time a person entered and left the classroom. Toilets which were disinfected did not have any contamination.

Out of 61 isolates of *S. aureus* 9 (15 %) isolates contained *Mec*A gene and 12(20%) had the PVL genes. Five (8%) isolates had both *mec*A and PVL genes. Not all the MRSA had PVL genes; Three MRSA did not contain PVL genes, while six of isolates containing PVL did not have *Mec*A genes

The study does not agree with the hypothesis that there is no presence of methicillin resistant *S. aureus* on toilet door and classroom door handles in selected secondary schools in Nairobi County, Kenya. MRSA was found in toilet and classroom door handles in selected secondary schools in Nairobi County, Kenya.

5.3. Recommendations

In line with the findings of this research i recommend the following.

1. Hygiene measures should be done in schools to prevent the spread of MRSA. The measure include; Practicing good hygiene like washing of hands, maintaining a clean environment for frequently touched surfaces

and surfaces that come into direct contact with people's skin like class room and toilets door handles. Cleaning and disinfecting the classrooms and toilets with commercial disinfectant or a daily prepared solution of 1:100 bleach and water mix (1 part of bleach and 99 parts of water).

2. Health education on MRSA by the Ministry of Health to the schools should be done at regular bases.
3. Surveillance programs by the Ministry of Health and Ministry of Education, Science and technology should be done regularly to monitor the presence of MRSA in schools.
4. Researches by the Ministry of Health should be done regularly to monitor the antibiotic patterns of MRSA to curb the problem of developing resistant to antibiotics and more so resistance to methicillin.

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APPENDICES

Appendix i: Scientific steering committee letter of approval

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NAIROBI

Forwarded to Caroline on 10/08/2012
Dr. Bm. Ruga for D/CMR

REF: SSC No. 2360 (Revised) – Detection and characterization of methicilin resistant *Staphylococcus aureus* from toilet and classroom door handles in selected secondary schools in Nairobi County

This is to inform you that the above-mentioned protocol in which you are the PI was reviewed by KEMRI SSC and it was recommended that you revise this proposal in view of the following issue(s) raised by our reviewers.

1. Kindly note your proposal has some typographical errors. A few references have been poorly sited.

Kindly address the above and enclosed minor issues and submit the revised protocol (with changes highlighted) and a cover letter responding to each of the issues raised by the reviewer(s) within one (1) month from the date of this letter i.e., 7th September, 2012.

You are advised to submit **5 copies** of the revised proposal.

Sammy Njenga, PhD
SECRETARY, SSC

Encl(s)

In Search of Better Health

Appendix ii: Ethical approval

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
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KEMRI/RES/7/3/1 October 31, 2012

TO: Ms. CAROLINE KARAMBU MBOGORI (PRINCIPAL INVESTIGATOR
STUDENT No. TM302-1060/2011

Forwarded on 06/11/2012

THROUGH: DR. SAMUEL KARIUKI,
THE DIRECTOR, CMR,
NAIROBI

Dear Madam,

RE: SSC PROTOCOL No. 2360 – REVISION 2 (*RE-SUBMISSION*): DETECTION AND CHARACTERIZATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM TOILET AND CLASSROOM DOOR HANDLES IN SELECTED SECONDARY SCHOOLS IN NAIROBI COUNTY
(*VERSION 02 DATED OCTOBER 25, 2012*)

Reference is made to your letter dated October 23, 2012. The ERC Secretariat acknowledges receipt of the revised proposal on October 29, 2012.

This is to inform you that at the Committee determines that the issues raised at the 208th ERC meeting of 9th October 2012 are adequately addressed. Consequently, the study is granted approval for implementation effective this **31st day of October 2012** for a period of one year. Please note that authorization to conduct this study will automatically expire on **October 30, 2013**.

If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to the ERC Secretariat by **September 18, 2013**. The regulations require continuing review even though the research activity may not have begun until sometime after the ERC approval.

You are required to submit any proposed changes to this study to the SSC and ERC for review and the changes should not be initiated until written approval from the ERC is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of the ERC and you should advise the ERC when the study is completed or discontinued.

Work on this project may begin.

Sincerely,

DR. CHRISTINE WASUNNA,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

In Search of Better Health

Appendix iii: Information Sheet and consent form

DETECTION AND CHARACTERIZATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM TOILET AND CLASSROOM DOOR HANDLES IN SELECTED SECONDARY SCHOOLS IN NAIROBI COUNTY

Your School has been randomly selected as one of the secondary schools which shall be involved in the above study.

What will the study involve?

The study involves determining the risks of acquiring MRSA in the school set up.

We would like to ask for your help in our study by answering to a few questions and ask for your permission to collect some samples from your school

If you agree for your school to take part in this study, we will request to take some samples from the classroom and toilets door handles to examine if these places might be harbouring the bacteria we are going to study.

Are there any or advantages to the school involved in the study?

The risks involved in this study will be social harm resulting from bad publicity in case there are positive *S.aureus* isolated. However risk will be minimized by use of identification numbers instead of the use of names of the school.

Health education will be provided to all the participating secondary schools on hygiene measures including proper washing of the hands.

What happens if I refuse for my school to take part in the study?

Taking part in the study is voluntary, if you agree now to take part in the study; you can still change your mind at any time during the process and pull out of the study.

Who will have access to the information about my school research?

All information will be stored in a password protected database and only the Principal Investigator will access the data.

What if I have any Questions?

You may ask any questions or concerns about the study or in the event of a study related injury. Contacts of the Principal Investigator for this research:

Caroline K. Mbogori

ITROMID KEMRI

Telephone number O723 324 038

P.O. Box 20750- 00202

Nairobi

carombogori@yahoo.com

Any questions pertaining rights as a research participant, Contact

The secretary

KEMRI Ethical review committee

P. O Box 54840- 00200

Nairobi

Telephone 020-2722541, 0722205901 and 0733400003

E-mail address erc@kemri.org

Consent Form

I, being the Principal/Head Teacher of..... (Name of the School) have had the research explained to me. I have understood all that has been read and had my questions answered satisfactorily. I understand that I can change my mind at any stage and it will not affect the benefits due to the school:

I agree to allow my school to take part in this research.

Principal/Head teacher signature: .....

Principal/Head teacher name.....

Date.....

I certify that I have followed all the study specific procedures for obtaining informed consent.

Investigator's signature: .....

Investigator'name: .....

Time .....

Date.....

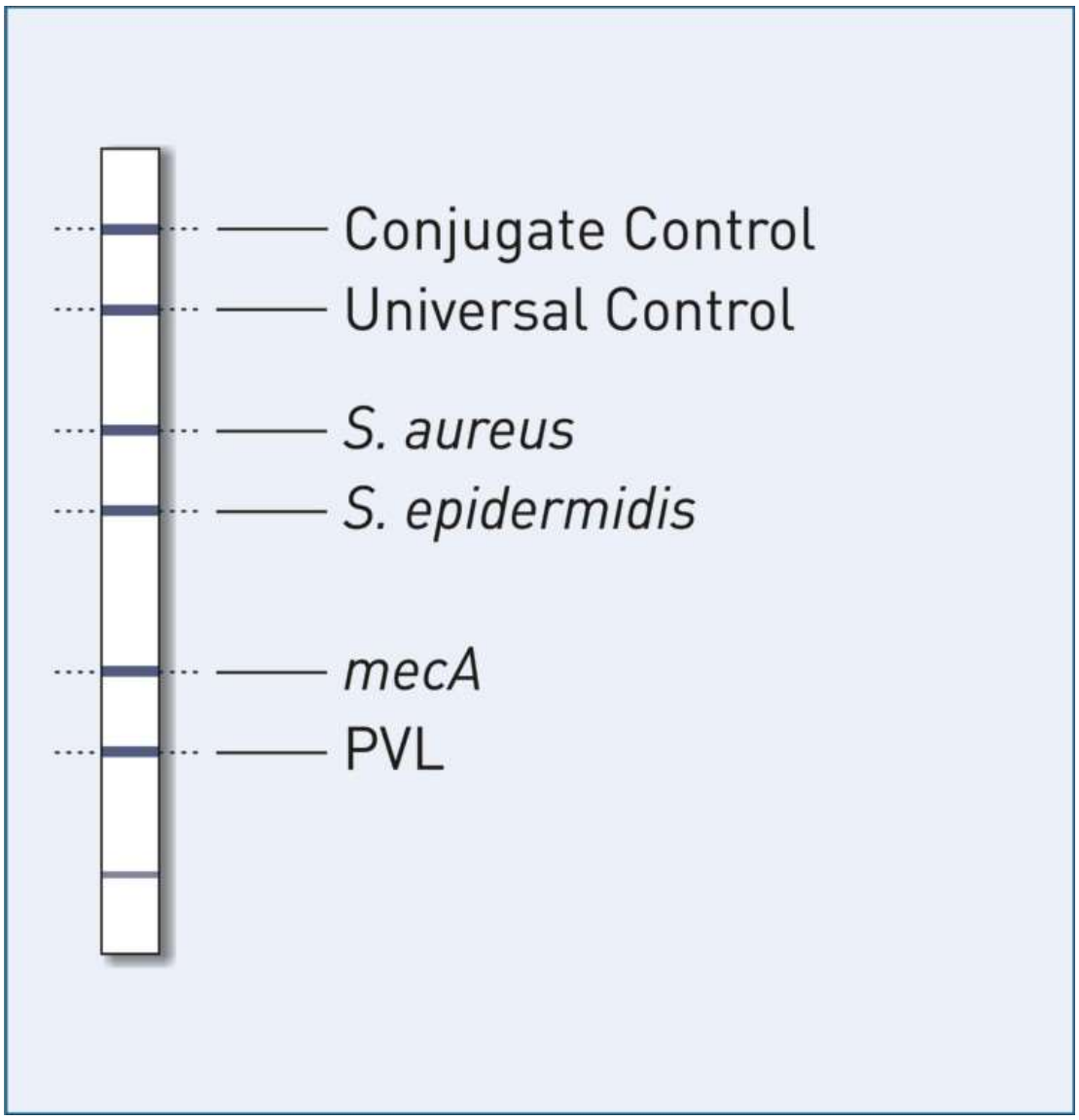
The information concerning this research was accurately explained to and understood by the Principal/ Head teacher and that informed consent was freely given by the Principal/ Head teacher.

Witness' signature .....Date.....

Witness' name .....Time.....

*A witness is a person who is independent from the trial or a member of staff who was not involved in gaining the consent.

Appendix iv: Evaluation sheet for Hybridization



Appendix v: Questionnaire

DETECTION AND CHARACTERIZATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM TOILET AND CLASSROOM DOOR HANDLES IN SELECTED SECONDARY SCHOOLS IN NAIROBI COUNTY

Name of the school.....

Is the school a mixed, girls or boys school? .....

Type of the School (boarding or day School).....

How many students do you have in your school? .....

How many classrooms do you have in the school? .....

Are the classroom doors opened all time? .....

If not how many times is the door shut in a day.....

How many toilets are there in the school? .....

Are the toilets disinfected each day? .....

Are there different toilets for the classrooms and the dining hall? .....

Appendix vi: CLSI guideline for *Staphylococcus aureus* antimicrobial testing

Table 2C. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm) S	I	R	MIC Interpretive Criteria (µg/mL) S	I	R	Comments
PENICILLINASE-LABILE PENICILLINS									
(8) Penicillin-susceptible staphylococci are also susceptible to other **β-lactam agents with established clinical efficacy for** staphylococcal infections. Penicillin-resistant **staphylococci** are resistant to penicillinase-labile penicillins, **including ampicillin, amoxicillin, azlocillin, carbenicillin, mezlocillin, piperacillin, and ticarcillin.**									
A	Penicillin	10 units	≥29	–	≤28	≤0.12	–	≥0.25	(9) Penicillin should be used to test the susceptibility of all staphylococci to all penicillinase-labile penicillins. **Penicillin-resistant strains of staphylococci produce β-lactamase.** Perform test(s) to detect β-lactamase production on staphylococci for which the penicillin MICs are ≤ 0.12 µg/mL or zone diameters ≥ 29 mm before reporting the isolate as penicillin susceptible. Rare isolates of staphylococci that contain genes for β-lactamase production may appear negative by β-lactamase tests. Consequently, for serious infections requiring penicillin therapy, laboratories should perform MIC tests and β-lactamase testing on all subsequent isolates from the same patient. PCR testing of the isolate for the *blaZ* β-lactamase gene may be considered. See Table 2C Supplemental Tables 1 and 3 at the end of Table 2C. (10) For oxacillin-resistant staphylococci report penicillin as resistant or do not report.
A	Oxacillin For *S. aureus* and *S. lugdunensis*.		–	–	–	≤2 (oxacillin)	–	≥4 (oxacillin)	For use with *S. aureus* **and *S. lugdunensis*.** **(12) Oxacillin disk testing is not reliable. For disk testing see cefoxitin and comment (13) for reporting oxacillin when using cefoxitin as a surrogate test.**
		30 µg cefoxitin	≥22	–	≤21	≤4 (cefoxitin)	–	≥8 (cefoxitin)	(13) Cefoxitin is used as a surrogate for oxacillin; report oxacillin susceptible or resistant based on the cefoxitin result. (14) If both cefoxitin and oxacillin are tested against *S. aureus* or *S. lugdunensis*, and either result is resistant, the organism should be reported as oxacillin resistant. See comments (4), (8), **and (11).**
CEPHEMS (PARENTERAL)									
B	Ceftaroline	30 µg	≥24	21–23	≤20	≤1	2	≥4	(16) For use with *S. aureus* only, including MRSA. (17) Interpretive criteria are based on a dosage regimen of 600 mg every 12 h.
AMINOGLYCOSIDES									
(24) For staphylococci that test susceptible, aminoglycosides are used only in combination with other active agents that test susceptible.									
C	Gentamicin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
O	Amikacin	30 µg	≥17	15–16	≤14	≤16	32	≥64	
O	Kanamycin	30 µg	≥18	14–17	≤13	≤16	32	≥64	
O	Netilmicin	30 µg	≥15	13–14	≤12	≤8	16	≥32	
O	Tobramycin	10 µg	≥15	13–14	≤12	≤4	8	≥16	

MACROLIDES									
(25) Not routinely reported on organisms isolated from the urinary tract.									
A A A	Azithromycin or clarithromycin or erythromycin	15 µg 15 µg 15 µg	≥18 ≥18 ≥23	14–17 14–17 14–22	≤13 ≤13 ≤13	≤2 ≤2 ≤0.5	4 4 1–4	≥8 ≥8 ≥8	
O	Telithromycin	15 µg	≥22	19–21	≤18	≤1	2	≥4	
O	Dirithromycin	15 µg	≥19	16–18	≤15	≤2	4	≥8	
LINCOSAMIDES									
A	Clindamycin	2 µg	≥21	15–20	≤14	≤0.5	1–2	≥4	(29) Inducible clindamycin resistance can be detected by disk diffusion using the D-zone test or by broth microdilution (see Table 2C Supplemental Tables 2 and 3, and Section 12 in M02-A11, and Section 13 in M07-A9). See comment (25).
FOLATE PATHWAY INHIBITORS									
A	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	11–15	≤10	≤2/38	–	≥4/76	
U	Sulfonamides	250 or 300 µg	≥17	13–16	≤12	≤256	–	≥512	(30) Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
U	Trimethoprim	5 µg	≥16	11–15	≤10	≤8	–	≥16	

Appendix vii: Publication copy

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Published Online December 2013 (http://www.scirp.org/journal/ojmm)

Scientific Research

Detection and Characterization of Methicillin Resistant *Staphylococcus aureus* from Toilet and Classroom Door Handles in Selected Secondary Schools in Nairobi County

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Received**********2013

ABSTRACT

Background: *Staphylococcus aureus* is found on all surfaces especially in public areas like hospitals and schools and on frequently touched areas like toilet and class room door handles. Methicillin resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* which is resistant to methicillin. There are two types of MRSA: Community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) and hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA). MRSA in the community presents a significant reservoir that could enter into healthcare facilities and spread among patients and also a risk for immune compromised persons in the community. **Methodology:** The study aimed at determining the prevalence of MRSA isolated from toilet and classroom door handles as a potential source of infection to the students and the workers in selected schools in Nairobi, Kenya. The study also compared the prevalence of MRSA between boarding and non-boarding girls, boys and mixed (both girls and boys in the same school) secondary schools. Twelve secondary schools in Nairobi County were randomly selected and 306 samples from both the toilet and classroom door handles were collected using sterile swabs and transported to the laboratory. Isolation of *Staphylococcus aureus* was done by the use of selective media Mannitol salt agar, antibiotic susceptibility of isolates was done by disk diffusion method, and molecular detection of *mecA* and PVL genes were done by polymerase chain reaction (PCR). **Results:** 15% of the isolates were resistant to Cefoxitin. MRSA in this study was only found in Girls schools and mixed schools and none in the boys' schools. 15% of the *S. aureus* isolates were mec*A* gene positive, 20% showed the presence of PVL virulence genes, while 8% showed the presence of both genes. **Conclusion:** The presence of MRSA in this study emphasis the need to formulate hygiene measures to prevent possible spread of MRSA and other transmissible pathogens to students and workers in the schools.

Keywords: Methicillin Resistant *Staphylococcus aureus*; Secondary Schools; Antibiotic Patterns

1. Introduction

Staphylococcus aureus is a gram positive coccus, catalase positive, mainly coagulase positive, non motile bacterium [1]. The bacterium is found in a wide range of habitats including environmental surfaces, in nasal nares of domesticated animals like dogs, cats and horses and on human body surfaces [2] as a part of normal microflora. However, *S. aureus* can also cause a variety of infections in animals, for instance, mastitis in dairy cows, septicaemia and arthritis in poultry and genital tracts infections of animals. The large polysaccharide capsule of the organism protects it from recognition by the immune system of the cow [3]. *S. aureus* in humans is found on the mucus membranes, for example, the nasal passages and the human skin of around a third of the population and, it is well adaptable to antibiotic pressure, therefore it is able to colonize healthy individuals which can be a source of infections and spread among people [4]. People with suppressed immunity due to the use of suppressive drugs and other diseases that cause immune diseases are at higher risk of acquiring *S. aureus* infections. *S. aureus* is able to

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