CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1 Introduction

Staphylococcus aureus is one of the most important human pathogens. It is a common cause of hospital and community acquired infections worldwide (Kaplan, 2005). Humans are the major reservoirs of *S. aureus* and its colonization are mainly found in the anterior nares which is about 30% of normal individuals (Moselio *et al.*, 2008).

 Carriage of *S. aureus* in the nose appears to play a key role in the epidemiology and pathogenesis of the infections. Nasal carriage of *S. aureus* has being identified as a risk factor for community-acquired and nosocomial infections (Muge *et al.*, 2008). The level of *S. aureus* in the nares may be influenced by the nature of the tissue. Some domestic animals also carry *S. aureus* but the animal species do not cause diseases in humans (Methas, 2002). Nasal carriage among hospital personnel and patients (60-70%) are much higher as compared to those among community carriers (Agraval, 2006). Young children have a higher carriage of *S. aureus,* the sebum, a secretion of the sebaceous gland usually found in adults acting as a mild antiseptics but the sebum is lacking in young children (Tanaka *et al.,* 2002). *S. aureus* spread is mostly by direct contact, they can also spread in the environment by handkerchief, clothing and secretions from the anterior nares and upper respiratory tract (Tanaka *et al.*, 2002).

S. aureus has been found to be resistant to most antiseptics and disinfectants (Toder, 2008). They are resistant by virtue of plasmid encoded β-lactamase and other mechanisms. Antimicrobial resistance of *S. aureus* especially methicillin resistant *Staphylococcus aureus* (MRSA) continues to be a problem for clinicians worldwide (Lu *et al.*, 2008). Due to an increasing number of infections caused by MRSA strains

which are often multidrug-resistant, therapy has become problematic. Therefore, prevention of Staphylococcal infections has become more important than ever before. The purpose of this study is to determine the prevalence *S. aureus* and MRSA nasal carriage among school children in Omdurman City and to evaluate the antibiotics susceptibility pattern of the isolates. It was necessitated by high level of nasal carriage of this bacterium among children reported in different developing countries. This study was carried out at February 2014.

1.2 Rationale

After increase level of *Staphylococcus aureus* nasal carriage among children and presence of resistant strains to several antibiotics, detection of MRSA nasal carriage is important for children care and appropriate utilization of infection control resources.

1.3 Objectives

1.3.1 General Objective

This study aimed to determine the prevalence of MRSA nasal carriers among healthy school children in Omdurman City community.

1.3.2 Specific Objectives

1. To isolate *Staphylococcus aureus* from anterior nares of primary school children.
2. To perform antibiotic Susceptibility testing for isolated bacteria
3. To identify risk factors effect on *S. aureus* nasal colonization.

CHAPTER TOW

LITERATURE REVIEW

2.1 Definition

Staphylococci are spherical Gram-positive bacteria, which are non motile and form grape like clusters. Staphylococci are facultative anaerobes. They mainly grow by aerobic respiration, or fermentation that produces lactic acid (Fox, 2010).

2.2 Historical background

Staphylococci, a group include the major pathogen *Staphylococcus aureus* which it is frequently part of the normal human microflora, found to be widespread in nature and colonizes at the anterior nares of 20-40% of adults.

Staphylococcus aureus has been a plague of mankind since the dawn of history. In fact, an outbreak of staphylococcal skin disease may even be mentioned in the Bible. The book of Exodus, chapter 9, recounts the sixth plague of Egypt, when Moses and Aaron were commanded by God to take two handfuls of soot from a furnace and then scatter the ashes skyward. The Egyptian men and livestock developed festering eruptions known as *shhin*, a term translated as "boils," a festering malady that was extremely difficult to heal. The modern recognition of *S. aureus* dates back to the late 19th century. Sir Alexander Ogston, a Scottish surgeon and early advocate of antisepsis, first described the organism in 1881 as a bacterial cause of "acute suppuration." He named the organism, "*Staphylococcus pyogenes aureus*, taking clues from its microscopic morphology, purulent nature, and tendency to form golden colonies on plated media. Staphylococci were first cultivated in liquid medium by Pasteur in 1880 .The name Staphylococcus was derived from the Greek noun *Staphyle* (a bunch of grapes) and *Coccus* (a grain or berry) For much of the next half-century, this bacterium remained a notable cause of severe morbidity and death

among patients. During World War I, post-influenza staphylococcal pneumonia occurred in young, healthy military personnel producing "dirty salmon pink anchovy sauce" colored sputum, ultimately leading to "cherry-red indigo-blue cyanosis" and rapid progression to death. Skin and soft tissue infections also frequently progressed to sepsis without effective antibiotic therapy to halt their spread. In 1941, and 82% mortality was documented for patients who were treated for *Staphylococcus aureus* septicemia in the pre-antibiotic era. The survival rate in the over-fifty population in their sample was only 2% (Chambers, 2001 and Binh *et al*., 2006).

Staphylococcus aureus is a major cause of healthcare-and community acquired infections. It perhaps the single most common cause of healthcare-associated infection throughout the world. It causes both superficial and deep pyogenic infections and number of toxin mediated illnesses, abscesses, wound infections, pneumonia, osteomylitis, mastitis, meningitis, pericarditis and pulmonary infections (Weigelt, 2007)

2.3 Taxonomy

Staphylococcus aureus is a bacterium which belong to the kingdom bacteria, the phylum Firmicutes, the class Bacilli, its order Bacillales, the family Staphylococcaceae and the genus *Staphylococcus*. This genus *Staphylococcus* has at least 40 species. The three most frequently encountered species of clinical importance are *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* (Jawetz, 2010 and Gotz *et al*., 2006).

2.4 Habitat

Staphylococci are associated with skin, skin glands and mucus membranes of almost all the warm blooded animals. This bacterium is also widely present in the environment. Nearly one-third of the human population supports the colonization of *Staphylococcus aureus* and designated as carriers. Hospitalized patients as well as

medical and paramedical staff show higher incidence of carriage of *Staphylococcus aureus* (Jawetz, 2010).

2.5 Morphology

Staphylococci are non motile, non spore forming, Gram positive cocci which measure around 0.7 to 1.2 micrometers in diameter. After phagocytosis or in old cultures, these may appear as Gram negative. It arranged in irregular clusters that resemble bunches of grapes. This appearance is because of incomplete separation of cells after successive cell divisions which take place in perpendicular planes instead of residual attachment point is usually eccentric to the plane of division, this result in irregular aggregates of cocci. The clusters are usually seen in growth obtained on solid media or in pathological materials. When cultivated in liquid medium, staphylococci usually form short chain. The generation time is about 20 minutes. The cell wall is rich in peptidoglycan which is characterized by unique pentaglycine bridges that link the tetrapeptides attached to the muramic acid residues (Bhatia, 2004).

2.6 Cultural characteristics

The organism is easily cultivated, they prefer aerobic environment but can also grow in the absence of oxygen; range of temperature for growth is 6-44˚C (optimum 37˚C) and the range of PH is 4.2-9.3 (optimum 7). Primary isolation is best done on blood agar medium on which most strains of *Staph. aureus* produce a characteristic golden yellow (aureus) carotenoid pigment. This pigment does not diffuse into the medium. The production of pigment is optimum at 22˚C in the presence of oxygen. Pigment production can be enhanced by cultivating the organisms in Milk agar, 1% glycerol monoacetate agar and Glucose peptone yeast extract agar incubating at 30˚C for 5 days.

Colonies of *S. aureus* are sharply defined, round, convex and measure around 4 mm in diameter. These have a smooth, glistening surface and butyrous consistency. On

nutrient agar it shows smooth, glistening surface and butyrous consistency these look like oily paint colonies. On blood agar medium it appear as smooth, glistening surface and butyrous consistency and surrounded by zone of clear (beta) hemolysis. On MacConkey agar; the colonies are small to medium in size and pink in colour because of the fermentation of lactose. Mannitol salt agar is an indicator and selective medium, it contains manitol, 7.5% NaCl and phenol red in nutrient agar. Staphylococci strains form colonies surrounded by yellow zones due to fermentation of mannitol. NaCl inhibits the growth of other bacteria (Bhatia, 2004).

2.7 Susceptibility to Physical and Chemical Agents

Extremely *Staphylococcus aureus* is hard organism and can survive in adverse environment for very long time. Some strains can even withstand temperature of 60˚C for 30 minutes. As compared to other bacteria these are more resistant to the action of disinfectants but it is readily killed by phenolic and hypochlorite disinfectants at standard in -use concentrations, and by antiseptic preparations such as hexachlorophene, chlorhexidine and povidone-iodine. There is some evidence that multiple-antibiotic-resistant strains of *S. aureus* are slightly less susceptible to some of these agents than ordinary strains (Bhatia, 2004).

2.8 Biochemical Properties

Biochemical tests are often required to identify pathogens including the use of substrates and sugars to identify pathogens by their enzymatic reactions.

There is no consistent pattern of biochemical reactions for *S. aureus*. The single most important test to differentiate *S. aureus* from *S. epidermidis* is the production of coagulase enzyme. Approximately 97% of staphylococci isolated from pathological lesions elaborate this enzyme (Cheesbrough, 2005 and Bhatia, 2004).

2.9 Pathogenicity

Staphylococci are recognized as common causes of osteomyelitis, skin and soft tissue infections and bacteremia in normal hosts. Although there are few recent surveys of the causes of community-associated bacteremia, *S. aureus* is a frequent isolate and one that is also associated with significant morbidity and mortality. Although most *S. aureus* infections are minor episodes of cellulitis or cutaneous abscesses, serious infections associated with severe systemic toxicity and an abrupt death are not infrequent, *S. aureus* causes food poisoning by releasing enterotoxins into food. *S. aureus* causes toxic shock syndrome by release of super antigens into the blood stream. Most experienced physicians remember one or more normal young patients who developed bacteremia and endocarditis from a trivial localized infection or who may have developed staphylococcal pneumonia and died in a few days. Also *S. aureus* is a major cause of hospital acquired (nosocomial) infections of surgical wounds. Rapidly progressive and fetal infection has recently been seen in normal young individuals infected with community-Acquired Methicillin-Resistant *Staphylococcus aureus* (MRSA) infections caused by isolates that contain the Panton-Valentine leukocidin (PVL). *S. aureus* expresses many potential virulence factors; surface proteins that promote colonization of host tissues and factors that probably inhibit phagocytosis (capsule, immunoglobulin binding protein A). Also toxins that damage host tissues and cause disease symptoms. For the majority of diseases caused by this organism, pathogenesis is multifactorial. Thus it is difficult to determine precisely the role of any given factor (Weigelt, 2007).

2.9.1 Virulence Factors

Micro-organisms that successfully invade host tissues increase their numbers by producing a range of factors that enable them to survive the onslaught of innate and specific immunity and which are responsible for the development of clinical disease. *S. aureus* expresses a number of factors that have the potential to interfere with host

defense mechanisms. However, strong evidence for a role in virulence of these factors is lacking (Foster, 2008).

2.9.2 Structural components

The cellular structure of *S. aureus* is complex. Most strains have polysaccharide microcapsules. The cell wall of *S. aureus* is structurally similar to that of group A streptococci; both have a carbohydrate antigen, a protein component and a mucopeptide (Weigelt, 2007).

2.9.2.1 Capsule

The majority of clinical isolates of *Staphylococcus aureus* express a surface polysaccharide. This has been called a microcapsule because it can be visualized only by electron microscope after antibody labeling. The function of the capsule is not clear. It may impede phagocytosis, but in vitro tests this was only demonstrated in the absence of complement (Albrecht *et al.,* 1996).

2.9.2.2 Peptidoglycan and Cytoplasmic Membrane

A complex web of cross-linked peptidoglycan outside the cytoplasmic membrane provides the cell with mechanical strength. It is particularly abundant in Gram-positive bacteria, when it also contains strands of teichoic and lipoteichoic acid (Myint *et al*., 1999).

2.9.2.3 Teichoic Acid

The carbohydrate antigen is a teichoic acid. Antibodies to teichoic acid can be detected in normal human serum, and elevated antibody titers are present in patients with deep-seated Staphylococcal infections. Teichoic acid has no established role in virulence and antibodies to this carbohydrate are not protective (Weigelt, 2007).

2.9.2.4 Protein A

Protein A is a surface protein of *Staphylococcus aureus* which interacts with the Fc component rather than the Fab component of IgG. Protein A may be antiphagocytic, but its role in virulence has not been clearly established (Weigelt, 2007).

2.9.2.5 Leukocidin

Staphylococcus aureus can express a toxin that specifically acts on polymorph nuclear leukocytes (PNLs). Phagocytosis is an important defense against staphylococcal infections so leukocidin should be a virulence factor, which consists of tow leukotoxic proteins that are capable of disrupting lysosomal membranes (Weigelt, 2007).

2.9.3 Enzymes

Staphylococcus aureus can express proteases, alipase, deoxyribonuclease (DNAase) and fatty acid modifying enzyme (FAME). The first three probably provide nutrients for the bacteria and it is unlikely that they have anything but a minor role in pathogenesis. However, the FAME enzyme may be important in abscesses, where it could modify anti-bacterial lipids and prolong bacterial survival. The thermostable DNAase is an important diagnostic test for identification of *S. aureus* (Albrecht *et al.,* 1996).

2.9.3.1 Catalase

Staphylococci produce catalase, which coverts hydrogen peroxide into water and oxygen. The concentration and amount of H_2O_2 present in accelerated hydrogen peroxide skin cleanser overwhelms the bacteria's ability to decompose H_2O_2 into its components part using catalase and the cell dies. Catalase test differentiates the staphylococci, which are positive, from the streptococci, which are negative (Wilkinson, 2010).

2.9.3.2 Coagulase

Staphylococcus aureus has unique ability to clot a variety of mammalian plasmas. Clotting is caused by an extracellular product, coagulase or (free coagulase) enzyme like protein that clots oxalated or citrated plasma. Coagulase binds to prothrombin; together they become enzymatically active and initiate fibrin polymerization. Coagulase may deposit fibrin on the surface of Staphylocci, perhaps altering their ingestion by phagocytic cells or their destruction within such cells. There is no conclusive evidence that coagulase producing strains are more virulent. No loss of virulence is seen in those mutant*s* of *Staphylococcus aureus* which do not produce coagulase. Clumping factor responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, *Staphylococcus aureus* form a clump. Clumping Factor is distinct form coagulase. Since clumping factor induces a strong immunogenic response in the host, it has been the focus on recent vaccine effort (Bhatia, 2004).

2.9.3.4 Staphylokinase

Many strains of *S. aureus* that do not produce β-lysine may produce staphylokinase. It can digest fibrin in the plasma of man. Its activity on a plate of blood agar may result into appearance of tiny spots of clearing at some distance from colonies of *Staph. aureus*; the phenomenon is known as *mullar phenomenon* (Bhatia, 2004).

2.9.3.5 Hyaluronidase

This enzyme is produced by almost all the strains of *S. aureus* albeit in differing quantities. It is postulated that this enzyme hydrolyzes the hyaluronic acid present in the intercellular ground substance of connective tissue, thereby, facilitating spread of infection (Bhatia, 2004).

2.9.3.6 Nuclease

Production of a heat-resistant nuclease by *S. aureus* is a characteristic which is unique to this species in the genus. It is a compact globular protein consisting of single polypeptide chain, the specific role of its production is poorly understood (Bhatia, 2004).

2.9.3.7 Lipases

Act on an array of lipid substrates. The utilization of these substrates is of survival value to the organism and this explains the colonization of this bacterium in the sebaceous areas of greatest activity. Correlation between production of lipase and ability to cause boils has been demonstrated (Jawetz, 2010).

2.9.3.7 Penicillinase

Another important enzyme is penicillinase. Because penicillinase has no role in pathogenicity, Staphylococci that produce pencillinase are no more virulent than non penicillinase-producing strains. Nevertheless, this enzyme is clinically and epidemiologically important, because it hydrolyzes the beta lactam ring of penicillin, thereby, inactivating the molecule. The production of penicillinase is controlled by plasmids, or episomes, which are extrachromosomal DNA molecules that replicate during cell division (Weight, 2007).

2.9.4 Toxins

2.9.4.1 Alpha Toxin

Of even greater interest are the non enzymatic toxins produced by *Staphylococcus aureus*. Alpha toxin is a cytotoxin and the best characterized and most potent membrane-damaging toxin of *S. aureus* by producing pores in cell membranes, thereby, altering their permeability and resulting in cell damage or death. Alpha toxin damages red and white blood cells and activates platelets. They carry high affinity

sites which allow toxin to bind at concentrations that are physiologically relevant (Weigelt, 2007 and Foster, 2008).

2.9.4.2 Beta Toxin

Beta toxin is sphingomyelinase which damages membranes rich in this lipid. The classical test for beta toxin is lysis of sheep erythrocytes. The majority human isolates of *S. aureus* do not express beta toxin. A lysogenic bacteriophage is inserted into the gene that encodes the toxin (Albrecht *et al.*, 1996).

2.9.4.3 Delta toxin

The delta toxin is very small peptide toxin produced by most strains of *Staph. aureus*. It is also produced by *Staphylococcus epidermidis* and *Staphylococcus lugdunensis*. The role of delta toxin in disease is unknown (Albrecht *et al.*, 1996)

2.9.4.4 Gamma Toxin and Leukocidin

The gamma toxin and the leukocidins are tow-component protein toxins that damage membranes of susceptible cells. The proteins are expressed separately but act together to damage membranes. The gamma toxin locus expresses three proteins. The B and C components form leukotoxin with poor hemolytic activity, whereas the A and B components are hemolytic and weakly leukotoxic (Albrecht *et al.*, 1996).

2.9.4.5 Panton-valentine leukocidin (PVL)

This toxin has tow components, it can kill white blood cells of humans and rabbits. The tow components designated as S and F act synergistically on the white blood cell membrane. This toxin is an important virulence factor in community associated methicillin resistant *S. aureus* infections (Bhatia, 2004).

2.9.4.6 Exofoliative Toxins (ET)

These epidermolytic toxins of *Staphylococcus aureus* are 2 distinct proteins of the same molecular weight. Epidermolytic toxin A is a chromosomal gene product and is heat-stable (resists boiling for 20 minutes). Epidermolytic toxin B is plasmid mediated and heat-labile. The epidermolytic toxins yield the generalized desquamation of the staphylococcal scalded skin syndrome (SSSS) by dissolving the mucopolysaccharide matrix of the epidermis. The toxins are superantigens (Bhatia, 2004).

2.9.4.7 Toxic shock syndrome toxin

Most *Staphylococcus aureus* strains from patients with toxic shock syndrome produce a toxin called toxic shock syndrome toxin (TSSS-1), which is the same as enterotoxin F. It binds to MHC class II molecules, yielding T cell stimulation, which promotes the protean manifestations of the toxic shock syndrome. The toxin is associated with fever, shock and multisystem involvement, including a desquamative skin rash. The gene for TSST-1 is found in about 20% isolates, including MRSA (Bhatia, 2004).

2.9.4.8 Enterotoxins

There are multiple (A-E, G-J, K-R U and V) enterotoxins. Approximately 50% of *Staphylococcus aureus* strains can produce one or more of them. Like TSST-1, the enterotoxins are superantigens, are heat-stable and resistant to the action of gut enzymes. Important causes of food poisoning, enterotoxins are produced when *S. aureus* grows in carbohydrate and protein foods. Ingestion of 25 microgram of enterotoxin B results in vomiting and diarrhea. The emetic effect of enterotoxin is probably the result of central nervous system stimulation (vomiting center) after the toxin acts on neural receptors in the gut (Bhatia, 2004).

2.9.5 Clinical Infections

Staphylococcus aureus may infect any organ or tissue of the body. Infections of the skin, soft tissue and bones are the most frequent. These infections may range from a localized abscess to more generalized infections such as cellulitis or empetigo. Staphylococcal osteomyelitis is typically the result of a bacteremia in young children, but in older individuals it is related to an adjacent site of infection. Septic bursitis is another common staphylococcal infection. *Staphylococcus aureus* is also a frequent cause of vascular catheter-associated infection, postoperative wound infection and other infections that are uniquely associated with healthcare (Weigelt, 2007).

2.9.5.1 Skin Diseases

2.9.5.1.1 Wound Infections and Abscesses

Traumatic and surgical wounds may develop localized or spreading infections (Myint *et al.,* 1999). The most prevalent pathogen isolated from skin and soft tissue infections is *Staphylococcus aureus*. The prevalence of *Staphylococcus aureus* as a pathogen in surgical infections has remained constant, whereas the proportion of isolates identified as MRSA has continued to increase.

The host risk factors well established for skin and soft tissue infections are multiple and include increasing age, poor nutritional status, uncontrolled diabetes, impaired immunity, obesity and poor tissue oxygenation at the site of incision. Less important risk factors include nicotine use, prior hospitalization and other infections as a remote site from the site in question. In addition to environmental factors in the operating room and surgical technique, these lesser factors are important risk factors for surgical site infection and other complicated skin and soft tissue infections (Weigelt, 200).

2.9.5.1.2 Impetigo

Impetigo is infection limited to the epidermis, presenting as yellow crusting lesions, most often on the face in young children (Myint *et al.,* 1999).

2.9.5.1.3 Folliculitis

Folliculitis is infection of the hair follicles; it is superficial, localized infections (Myint *et al.*, 1999).

2.9.5.1.4 Carbuncles

Carbuncle is a cluster of infected follicles commonly seen on the neck. Recurrent boils may be associated with carriage of *Staph. aureus* in the nose and other sites, requiring treatment with antiseptics or topical antimicrobials (Myint *et al.,*1999).

2.9.5.1.5 Cellulitis

When the dermis is infected, a red demarcated rash appears. *Staphylococcus aureus* may produce a hyaluronidase that breaks down intercellular junctions, leading to cellulitis. Infection spreading beneath the dermis to involve the subcutaneous fat is cellulitis. This severe condition may occur at any site but commonly involves the legs and presents with a demarcated red lesion often with blisters. The patient is usually systemically unwell and febrile (Myint *et al.,* 1999).

2.9.5.2 Bacteremia

Staphylococcus aureus is found on the skin of most people and can enter the body in wounds; however, many cases are nosocomial and result from surgery or catheter use. The bacteria may disseminate throughout the body. Also patients may develop abscesses involving tissue such as the liver, spleen, or even muscle secondary to bacteremia (Weigelt, 2007 and Fox, 2010).

2.9.5.3 Endocarditis

Endocarditis is an inflammation of the endocardium and usually involves the heart valves. *Staphylococcus aureus*-associated endocarditis can have a high mortality rate. *Staphylococcus aureus* is the most frequent cause of acute bacterial endocarditis. It may be acquired in the hospital or community. Most cases are a consequence of an infection acquired as a result of a healthcare intervention (Weigelt, 2007 and Fox, 2010).

2.9.5.4 Urinary Tract Infections

Complicated urinary tract infections occur in specific clinical settings. Renal abscess can result from hematogenous seeding of the renal cortex (most often due to *S. aureus*) or from ascending infection leading to severe pyelonephritis (most often due to Gram-negative rods) (Fox, 2010).

2.9.5.5 Food Poisoning

S. aureus produces a number of toxins, of which the enterotoxins (A, B, C and D) cause food poisoning. About a third to a half of *S. aureus* strains produce enterotoxins which are heat stable and thus survive cooking (boiling for 30 minutes). They are also resistant to proteolysis by intestinal proteases. Antibiotic treatment is not indicated because the bacteria are not directly involved in causing the symptoms (Fox, 2010).

2.9.5.6 Enterocolitis

The symptoms of enterocolitis are somewhat similar to food poisoning but also include fever. They are also produced by enterotoxin A and leukotoxin. The cause is the treatment of patients with broad spectrum antibiotics that allow *Staph. aureus* to grow in the intestine in preference to the normal bacterial flora. The bacteria can be detected in fecal samples (Fox, 2010).

2.9.5.7 Pneumonia

Aspiration pneumonia can result from entry of oral secretions into the lungs. The bacteria can cause local abscesses and infiltrates. The disease is found in the very young, the very old and patients with pulmonary disease. People with MRSA can get necrotizing pneumonia which has a very high fatality rate. *Staphylococcus aureus* pneumonia was recognized infrequently in the past and was primarily seen as a complication of influenza (Weigelt, 2007 and Fox, 2010).

2.9.5.8 Osteomyelitis

Osteomyelitis is an infection in a bone. This condition happens when a bacterial or fungal infection travels from your bloodstream or other tissues in your body and enters your bones. The infection can also start in the affected bone if it has been exposed to germs by trauma. Bones commonly affected by infection are the leg bones, upper arm bones, spine and pelvis. Osteomyelitis can last several months or less (called an acute form), or several months to years (called a chronic form). The condition is serious and requires aggressive treatment to stop the spread of infection (Myint *et al.,* 1999).

2.9.5.9 Toxic Shock Syndrome

Toxic shock syndrome is caused by infection with strains of *S. aureus* that produces toxic shock syndrome toxin. It may be associated with a wound in which the bacteria multiply rapidly but became particularly prominent to the public in the 1980´s when *S. aureus* infection was found to cause the toxic shock syndrome that was seen after the use of certain tampons. The bacteria were able to divide rapidly within the tampon; they do not disseminate but remain in the vagina. However, the toxin does include fever, macular erythrematous rash, desquamation (all over the body), vomiting and diarrhea. Toxic shock syndrome toxin has the properties of a super antigen, resulting in the production of cytokines, vascular leak and cell toxicity. This

results in hypervolemic shock and death as a result of multi-organ failure. Before the cause of Toxic shock syndrome was discovered, the mortality rate was high but now is around 5% (Fox, 2010).

2.9.5.10 Scalded Skin Syndrome

A minority of *Staphylococcus aureus* strains produces exofoliative toxins (A and B) and either toxin can cause scalded skin syndrome in babies and young children but rarely in adults (Fox, 2010).

2.10 Treatment

Because of the frequency of drug-resistant strains, meaningful staphylococcal isolates should be tested for antimicrobial susceptibility to help in the choice of systemic drugs. Resistance to drugs of the Erythromycin group tends to emerge so rapidly that these drugs should not be used singly for treatment of chronic infection. Drug resistance to penicillins, tetracyclines, aminoglycosides, erythromycins, etc…) determined by plasmids can be transmitted among staphylococci by transduction and perhaps by conjugation. Penicillin G-resistant *S. aureus* strains from clinical infections always produce penicillinase. They constitute >95% of *S. aureus* isolates in communities in the United States. They are often susceptible to β-lactamase-resistant penicillins, cephalosporins or vancomycin. Nafcillin resistance is independent of Beta-lactamase production, and its clinical incidence varies greatly in different countries and at different times (Jawetz *et al.*, 2010).

Infections acquired outside hospitals can usually be treated with penicillinase-resistant *β*-lactams. Hospital acquired infection is often caused by antibiotic resistant strains and can only be treated with vancomycin (Albrecht *et al.,* 1996).

Vancomycin continues to be the drug of choice for treating most MRSA infections caused by multidrug-resistant strains. Clindamycin, cotrimoxazole, fluoroquinolones, or minocycline may be useful when patients do not have life-threatening infections

caused by strains susceptible to these agents. Test the infecting strain for susceptibility to these agents before committing patients to course of therapy (Boyce, 1998). Patients and staff carrying epidemic strains, particularly MRSA, should be isolated. Patients may be given disinfectant baths or treated with a topical antibiotic to eradicate carriage of MRSA   (Albrecht *et al*., 1996).

2.11 Epidemiology and control

Because *Staphylococcus aureus* is a major cause of nosocomial and community-acquired infections, it is necessary to determine the relatedness of isolates collected during the investigation of outbreak. Key to understanding how staphylococcal infections develop is an appreciation of the habits of this organism. Soon after birth; many neonates become colonized in the anterior nares with *Staphylococcus aureus*. The carrier state is well documented to be significantly associated with development of the infections when an injury or skin break occurs. Patients known to be nasally colonized with *Staphylococcus aureus* had a significantly higher risk of developing staphylococcal wound infection after a surgical procedure than someone who is not colonized. Especially in individuals who are nasal carriers. Although the throat is commonly colonized, the importance of this is not well understood. Many individuals who work in hospitals may be nasally colonized. The frequency varies with the extent and type of patient contact (Weigelt, 2007).

Cultures of *Staphylococcus aureus* are classified according to their susceptibility to a set of phages having relatively narrow host range at predetermined dilutions. Phages are chosen so as to make as many epidemiological valid distinctions as possible between the strains. It is, therefore, a method of bacterial classification based on a single class of character. The one thing that this typing system can never do is to show that the tow isolates are "the same ". What it can establish with varying degree of success is that the isolates are "different ". Its use in the field investigations is to narrow down the field of enquiry by the exclusion of alternative sources of infection.

Most strains of *S. aureus* are lysogenic and they carry phages to which they themselves are immune but which will lyse some of the other members of the species. Susceptibility of *Staphylococcus aureus* strains to the various temperate phages provides the basis for phage typing system. The phage patterns of different strains fall into four broad groups (I, II, III, V).

Basic strategies for preventing transmission of MRSA in the community uses similar practices as those used in the hospital setting include: hand hygiene ; washing, dialy showers, use of antibacterial soap, covering any draining lesions, isolation of infected patients needing hospitalization and avoidance of sharing personal items such as razors or towels and ensuring that communal bathing areas are clean. Isolation may require withholding athletes with draining wounds from competition, infected patients and not allowing healthcare workers with open wounds to return to work until they have healed. Clean potentially contaminated surfaces carefully with a disinfectant or a bleach-water solution (1:100 dilution of sodium hypochlorite, which is approximately ¼ cup of 5.25% household chlorine bleach to 1 gallon of water) after caring for the wound. Students who are infected with MRSA should follow the healthcare provider's treatment plan, including completion of any antibiotics prescribed.

Complex situations should be assessed on a case-by-case basis in conjunction with the local or state health department and pediatric infection control specialists (Edward *et al.,* 2005 and Bhatia, 2004).

2.12 Staphylococcus aureus nasal carriage

Staphylococcus aureus has long been recognized as an important pathogen in human disease. Staphylococcal infections occur regularly in hospitalized patients and have severe consequences, despite antibiotic therapy. Due to an increasing number of infections caused by Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains, which are now most often multi-resistant, therapy has become problematic. Therefore, prevention of staphylococcal infections is now more important than ever.

Carriage of *S. aureus* in the nose appears to play a key role in the epidemiology and pathogenesis of infection. The ecological niches of *S. aureus* strains are the anterior nares. Elegant studies have shown that the nares are the most consistent area from which this organism can be isolated. Moreover, when the nares are treated topically to eliminate nasal carriage, in most cases the organism also disappears from other areas of the body.

Over time, three patterns of carriage can be distinguished. Approximately 20% of individuals almost always carry one type of strain and are called persistent carriers. A large proportion of the population (60%) harbors *S. aureus* intermittently, and the strains change with varying frequency. Such persons are called intermittent carriers. Finally, a minority of people (20%) almost never carry *S. aureus* and are called non carriers. Persistent carriage is more common in children than in adults, and many people change their pattern of carriage between the age of 10 and 20 years. The reasons for these differences in colonization patterns are unknown. Persistent carriage seems to have a protective effect on the acquisition of other strains, at least during hospitalization. This barrier to colonization is reduced when carriers are treated with antibiotics. These findings suggest that the acquisition and transmission of antibiotic-resistant *S. aureus* in the hospital mainly concern intermittent carriers and persistent carriers treated with antibiotics. The prevalence and incidence of *S. aureus* nasal carriage vary according to the population studied. In the general population, a mean carriage rate of 37.2% was found. However, the range of carriage rates reported is large. This may be due partly to differences in the quality of the sampling and of the culture techniques used in these studies. Also, the studies were reported between 1934 and 1994, and changes in *S. aureus* nasal carriage may have occurred over the years. The older studies tended to find higher carriage rates. The rates in the general population are comparable to those found in health care workers and in patients on admission and during hospitalization. Some studies, however, reported increased carriage rates when patients were hospitalized. Subgroups of patients with significantly increased carriage rates include those with insulin-dependent diabetes

mellitus, those on hemodialysis, those on continuous ambulatory peritoneal dialysis (CAPD), intravenous drug addicts, patients with *S. aureus* skin infections, and those with human immunodeficiency virus (HIV) infection or AIDS. In the first four groups, the common factor seems to be repeated or long-term puncture of the skin by needles and/or intravascular catheters. To confirm this association, higher carriage rates were also found among relatively healthy patients receiving repeated injections for allergies. It is predictable that high nasal carriage rates are found in patients with *S. aureus* skin infections since nasal cultures were taken at the time the *S. aureus* infection was present. However, the reason for the higher carriage rates in HIV-positive patients is unclear. The studies on HIV have excluded HIV-positive intravenous drug addicts, since their carriage rate would be expected to be high even in the absence of HIV infection. Furthermore, the stage of HIV disease did not seem to influence the carriage rate. To control for hospitalization as a risk factor, (Weinke *et al.,* 1992) selected patients with other types of chronic disease as controls; nevertheless, the carriage rate in HIV-positive patients was higher. The authors proposed that immunological defects were the basis for the higher carriage rate in HIV patients. However, the exact nature of these defects remains to be elucidated. In addition to the groups mentioned above, there have been some anecdotal reports on other groups with high *S. aureus* carriage rates. (Decker *et al*, 1986) found a carriage rate of 65.6% in river-rafting guides, and the *S. aureus* infection rates were also high in this group. Maceration of the skin caused by prolonged contact with water, together with repeated small skin injuries, were the proposed reasons. Finally, (Gittelman *et al,* 1991) found high carriage rates in patients with rhinosinusitis (Kluytmans *et al.,* 1997).

2.12.1 Risks of *Staphylococcus aureus* nasal carriage

Most studies regarding the risks of acquiring *S. aureus* infections in the community concern skin and soft tissue infections. Several, mostly older, studies investigated the relation between *S. aureus* nasal carriage and skin infections, including furunculosis, impetigo and stye. On average, 80% (range 42–100%) of those with skin lesions were *S. aureus* nasal carriers, and 65% (range 29–88%) had the same phage type in the nose and lesion. In one large prospective population-based study among elderly people there was no relation between persistent *S. aureus* nasal carriage and all-cause mortality, a surrogate end-point for serious staphylococcal disease (Nouwen, 2004).

Earlier retrospective cohort or case-control studies have demonstrated increasing age, male sex, alcoholism, lung disease, cancer, diabetes mellitus, end stage renal failure, and dialysis to be risk factors for community-acquired *S. aureus* infections necessitating hospital admission (Laupland *et al.,* 2004).

These factors have also been identified earlier as determinants of *S. aureus* nasal carriage in case-control or cross-sectional studies (Kluytmans *et al.,* 1997).

The spectrum of community *S. aureus* disease is rapidly changing with the advent and spread of community-onset MRSA strains. Overall MRSA carriage rates in the community are still low, but seem to be rising rapidly in certain parts of the world. In the only prospective study done so far on nasal carriage of community-onset MRSA and risk of infections in soldiers, Ellis and co workers found a relative risk of for nasal MRSA carriers to acquire a MRSA infection (cellulitis, abscesses) in the community. In a retrospective study concerning community-onset MRSA skin infections among professional football players, Kazakova and colleagues (Kazakova *et al*., 2005) did not find any MRSA in nasal swabs or environmental cultures, although 42% were nasal carriers of MSSA strains. Apart from these highly selected populations, it remains questionable whether the results from these studies can be extrapolated to the general population. We need more community-based studies to better understand the ecology, pathophysiology, and epidemiology of *Staphylococcus*

aureus nasal carriage and infections in the community and to develop and target preventive measures.

Children and their guardians were specifically enquired about risk factors for increased *Staphylococcus aureus* and CA-MRSA carriage. These included overcrowding, visit to local doctor, recent hospital check up or hospitalization, recent visit to hospital and skin infection (kluytmans *et al.,* 1997).

2.12.2 Elimination Strategies of *Staphylococcus aureus* Nasal Carriage

It is conceivable that in populations in which *S. aureus* nasal carriage is identified as a risk factor for infection, elimination of carriage would reduce the infection rate. Three approaches to the elimination of carriage are available. The first is the local application of antibiotics or disinfectants. Most often used are nasal ointments or sprays, sometimes combined with the application of disinfecting agents to the skin. In general, the results have been disappointing. Both a low efficacy and a rapid emergence of resistance to the agents used were observed.

Results of such studies have been reviewed by others. Recently, mupirocin, a new antibiotic, has become available for topical use. This agent has been shown to have excellent efficacy for the elimination of   *S. aureus* carriage and therefore has offered a new opportunity to eliminate *S. aureus* nasal carriage. Mupirocin is well tolerated, and when it was used for short courses (application to the nose twice daily for 5 days, as recommended by the manufacturer), development of resistance was not reported.

A second approach to eliminating nasal carriage is administration of systemic antibiotics. The results have been disappointing for most agents. To date, only rifampin has proven to be an effective agent, but side effects and the rapid emergence of resistant strains have limited its use for this purpose.

The third strategy is bacterial interference, i.e., active colonization with a strain of *S. aureus* (type 502A) which is considered to possess minimal pathogenic properties but

is able to prevent colonization by more virulent strains, presumably by competition for the binding sites in the nose. However, the exact mechanism for this effect has never been elucidated.

Interference was used successfully in nurseries during outbreaks of *S. aureus* infections in the 1960s and for treatment of patients with recurrent furunculosis. However, this approach was occasionally complicated by serious infections due to *S. aureus* 502A, and even a fatal infection has been reported. Although the report documenting a fatal outcome concluded that, "The benefits of *S. aureus* 502A programs far outweigh their hazards," this strategy was not pursued further at that time.

In conclusion, most strategies to eliminate the carriage of *S. aureus* have been disappointing. Mupirocin has offered a new opportunity for this purpose and is considered by far the most effective agent available (kluytmans *et al.,*1997).

2.13 Methicillin Resistant *Staphylococcus aureus* (MRSA)

2.13.1 Historical Perspective

MRSA is a bacterium responsible for difficult to treat infection in human. It may also referred to multidrug-Resistant or oxacillin resistant *S. aureus* (ORSA). MRSA is by definition a strain of *S. aureus* that is resistant to large group of antibiotics called β-lactams, which include penicillin's and cephalosporin´s (Bilal and Gedebou, 2000).

The introduction of penicillin G in 1941 was revolutionary, and mortality rates due to staphylococcal infections dropped precipitously. However, non susceptible strains of *S. aureus* were described almost immediately, and resistance to chloramphenicol, erythromycin, and the tetracyclines also emerged within the next decade. Within 5years, approximately 50% of *S. aureus* isolates expressed resistance to penicillin via production of the β-lactamase enzyme. Vancomycin, a glycopeptide antibiotic, was introduced in 1956, and methicillin, the first semisynthetic antistaphylococcal penicillin, was introduced in 1961, both in an attempt to combat penicillin-resistant *S.*

aureus (Table1). However, the availability of these agents did not stem the tide of resistance.

The first case of methicillin resistant *Staphylococcus aureus* was described in the United Kingdom in 1961, and MRSA was widespread in Europe by the 1970s and in the U.S. by the late 1980s. The decade of the 1990s saw nosocomial MRSA rates almost double from approximately 30% in 1990 to nearly 57% in 2000. Currently, nosocomial MRSA rates approach 60% or more in many areas of the country. The increasing prevalence of MRSA among *S. aureus* strains resulted in a significant increase in the utilization of vancomycin. By the mid 1990s, *S. aureus* strains less than susceptible to vancomycin began to emerge (Figure 1). In 1997, the first *S. aureus* strains possessing reduced susceptibility to vancomycin, known as VISA (vancomycin intermediate *Staphylococcus aureus*), were documented in Michigan and New Jersey in patients on peritoneal dialysis who were administered vancomycin for prolonged duration. The first case of vancomycin resistant *Staphylococcus aureus* (VRSA) was subsequently documented in Michigan in 2002 in a patient administered vancomycin for almost 6 weeks. Subsequent studies have documented progressive overall decreasing vancomycin susceptibility among clinical isolates of *Staphylococcus aureus* (i.e., "susceptibility creep") as well as the emergence of heteroresistant strains *S. aureus* strains. These strains form subpopulations of vancomycin resistant daughter cells in a larger population of more susceptible *S. aureus* strains which may be selected out in the presence of vancomycin therapy. MRSA has also become a prominent pathogen outside the healthcare setting. In the late 1980s, reports surfaced of MRSA cases occurring in the community in patients without exposure to hospitals or nursing homes. These cases affected younger individuals, ethnic minorities, and often involved severe skin or soft tissue infections. Outbreaks were associated with certain higher risk groups, including individuals who used IV drugs, participants in close contact sports, and residents living together in crowded conditions, such as inmates, military recruits, and disabled individuals in group homes. In the late1990s, four pedaitric deaths attributable to "community

acquired MRSA" (CA-MRSA) was documented involving previously-healthy children. CA-MRSA infections differ from healthcare-associated MRSA infections in a number of ways. CA-MRSA infections are predominantly skin and soft tissue infections, are often susceptible to other non-β-lactam antimicrobial drugs, and carry a type IV or V staphylococcal cassette chromosome (SCC) with the *mec*A gene. In contrast, healthcare-associated MRSA infections are found at multiple body sites, are usually multidrug resistant, and carry the SCC*mecc* types I, II and III. In the United States, 2 major clones of CA-MRSA have been identified by pulsed-field gel electrophoresis (PFGE) and designated USA300 and USA400 by the CDC. Toxin expression of the CA-MRSA strains carry the intracellular toxin Panton-Valentine leukocidin (PVL), which is known for pore formation on polymorphonuclear cells of the host. In addition, the USA300 clone appears to be emerging as the predominant strain of MRSA nationwide in both community and hospital-acquired settings, with outbreaks of skin and soft tissue disease, community and hospital-acquired pneumonia and bacteremic syndromes. CA-MRSA rates in some areas of the country now exceed 75% of the strains, especially in the pediatric population. Thus, trends in the second half of the current decade include the increasing role of MRSA as a community-acquired pathogen, the re-emergence of surgical debridement as a therapeutic modality in managing *Staphylococcus aureus* skin and soft tissue infection, and the waning use of vancomycin, as other gram positive antibiotic agents take a more prominent role in the treatment of MRSA skin infections, pneumonia, bacteremia, and endocarditis (Oehler, 2007).

Table 1: Time required for prevalence rates of resistance to reach 25% in hospitals.

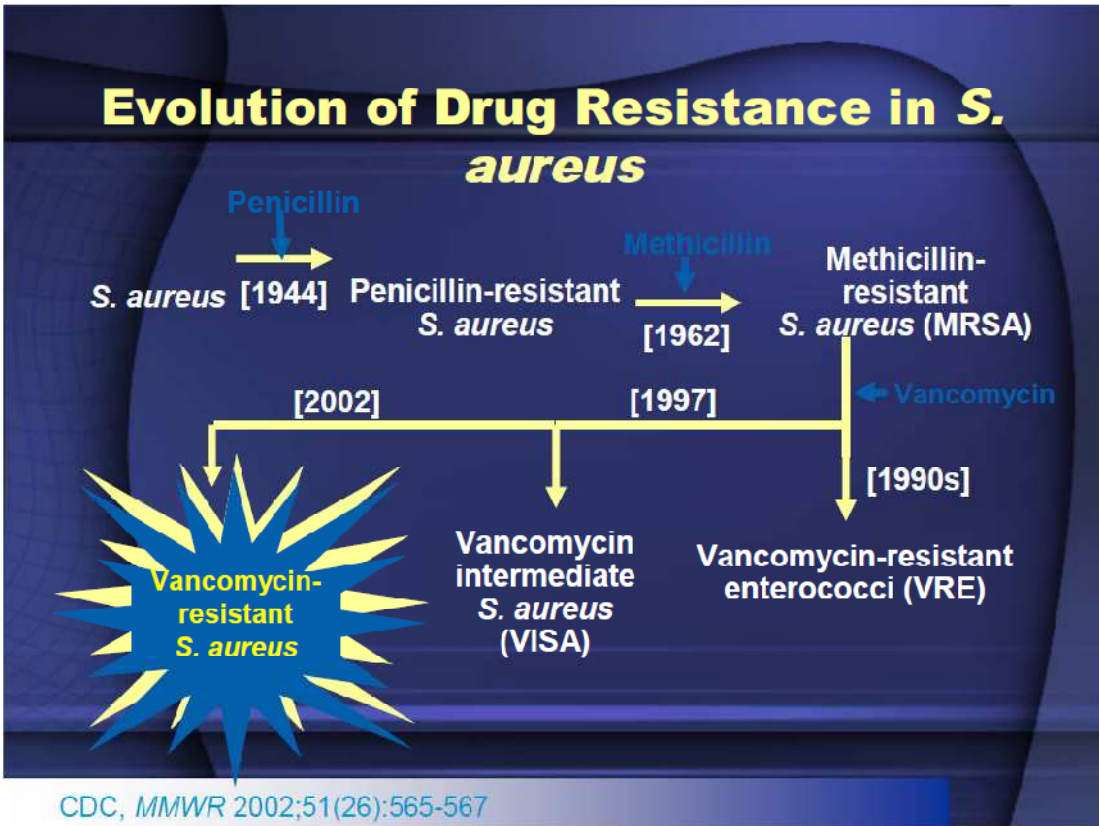| Drug	Year Drug Introduced	Years to report of resistance	Years until 25% rate in hospitals	Years until 25% rate in the community
Penicillin	1941	1-2	6	15-20
Vancomycin	1956	40	?	?
Methicillin	1961	<1	25-30	40-50 (projected)

Figure 1: Chronologic evolution of resistant *Staphylococcus aureus*

2.13.2 Detection of MRSA

Detection of oxacillin resistance (methicillin resistant) strain of *Staphylococcus aureus* (MRSA) have become a significant problem in the hospital setting and more recently in the community setting because of their increasing frequency as the cause of serious invasive infections leading to sepsis, endocarditis and other life threatening illnesses. The hospital-acquired strains are usually multiresistant, whereas the community-acquired strains flourish even in the presence of β-lactam antibiotic because the penicillin binding protein (PBP) of the organism have been altered because of acquisition of *mecA* gene. The beta lactam antibiotics lack the sufficient affinity for the altered PBP called PBP2a. They are resistant to all β-lactams antibiotics, including the cephalosporins and carbapenems. These organisms must be challenged with non-beta-lactam agents such as the glycopeptides vancomycin, which act to interfere with cell wall synthesis in a different manner. Tow other mechanisms may be responsible for MRSA. One possible method proposed is the overproduction of beta lactamase. With enough enzymes, even the penicillinase resistant penicillins can be destroyed**.** Another potential means is because of strains that do not contain the *mecA* gene. They can have modified PBP genes that do not bind as well with the beta-lactams or the strains have the ability to overproduce PBPs. Both mechanisms are rare but may account for borderline or low level methicillin resistance. Resistance can be difficult to detect. All the organisms tested may possess the gene for resistance, but only a few may display it when tested. This is known as heteroresistance. A small volume of the inoculum is used for testing, and so the number of organisms exhibiting resistance is even smaller. These organisms tend to grow more slowly and better at temperatures equal or less than 35°C. They can potentially be overgrown when tested on rapid automated commercial systems. Currently agar and broth based methods and nucleic acid Amplification tests can detect MRSA (Karen *et al.,* 2011).

2.13.2.1 Oxacillin Method

Oxacillin resistance is best determined by broth dilution methods or on agar screen. Automated commercial systems must be validated to insure the detection of MRSA. The most commonly used agar screen involves the use of Mueller hinton agar with 4% NaCl and 6 microgram of oxacillin per milliliter. The organism inoculum is standardized to match the turbidity of 0.5 McFarland standard. Using a sterile swab, spot inoculate the agar with the suspension. The agar is incubated for a full 24 hours at 33-35°C in ambient air. It can be held up to 48 hours. Growth of more than one colony on the agar medium is as a sign of methicillin resistance, whereas no growth is an indication of susceptibility to methicillin (Karen *et al.,* 2011).

2.13.2.2 Cefoxitin Method

Cefoxitin can be used to predict resistance because of the presence of the *mecA gene* in *S. aureus*, even with heteroresistance. Cefoxitin is able to strongly induce the expression of *mecA* gene much better than the beta-lactams.

It can be performed using a 30 micro gram cefoxitin disk for the disk diffusion test or done using a broth dilution method. Interpretation of the results differs for *S. aureus* and coagulase-negative staphylococci. Refer to the CLSI guidelines for details for interpretation. Cefoxitin can be used alone to accurately determine oxacillin resistance or as a backup test if oxacillin results show borderlines resistance (Karen *et al.,* 2011).

2.13.2.3 Latex Agglutination Test

This method detects MRSA, which possess the *mecA*, but not those that appear as oxacillin resistant because of hyperproduction of β-lactamase or other modified PBPs. The basis of the test is monoclonal antibody against the product of the *mecA* gene PBP2a. A heavy inoculum is required. The PBP2a is extracted with the use of reagents and heat. The test can be used for testing coagulase-negative staphylococci.

They must first be induced to produce PBP2a by performing disk diffusion with oxacillin prior testing. Although it has been shown to be a good test, all the required manipulation delays results for the patient (Karen *et al.,* 2011).

2.13.2.4 Chromogenic Agar

Direct specimens, often nasal and perianal swabs, can be inoculated onto a specialized agar. Chromogenic agar is selective and differential for MRSA. The addition of an antibiotic such as oxacillin or cefoxitin selects for methicillin resistant organisms. A chromogenic or color changing substrate specific for *S. aureus*, is also incorporated MRSA then creates pigmented such as mauve or denim blue, colonies that are easily differentiated from other organisms that may grow. The agar plates are read at 24 hours and again at 48 hours if no suspicious colonies are present. These agars have been shown to be cost effectively screen surveillance specimens for MRSA from hospitalized patients. Susceptibility testing is usually not performed on surveillance cultures. The key information is the presence of MRSA. Patients harboring MRSA must remain in isolation. Those with negative surveillance cultures can be removed from isolation and therapy save the facility money(Karen *et al.,* 2011).

2.13.2.5 Nucleic acid detection

Another method for detecting MRSA is to identify the presence of the gene responsible for methicillin resistance, the *mecA* gene. This can be accomplished through the use of molecular diagnosis on colonies or directly on specimens. Molecular method such as PCR, detect the staphylococcal cassette chromosome (SCC) .This mobile piece of DNA carries the *mecA* gene. Real- time PCR can be used to amplify the gene encoding resistance to increase sensitivity and specificity.Although molecular methods tend to be expensive they can provide results from direct specimens much faster than traditional testing using colonies (Karen *et al.,* 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

3.1.1 Type of Study

Descriptive cross-sectional study.

3.1.2 Study Location

This study was done in tow primary schools in Omdurman City.

3.1.3 Study Duration

The study was done during period from February to June 2014.

3.1.4 Study Population

6-10 years old primary school children.

3.1.5 Sample Size

The total number of samples was 100 samples**.**

3.1.6 Ethical Clearance

This study was approved by the National Ethical Committee, Fedral Ministry of Health. Consent was obtained from school manger before enrolling children in the study.

3.1.7 Sampling Technique

Based on non-probability simple random technique.

3.1.8 Data Collection

Data were collected by using direct interviewing questionnaire (see appendix IV).

3.2 Methods

3.2.1 Collection of specimens

Cross-sectional descriptive study was conducted among children aatending primary schools, the specimens were collected from anterior nares by using sterile cotton swabs, Collection was by inserting the swab and gently rotating it three times Samples were collected randomly from 100 healthy primary school children.Collected specimens were labeled by numbers.

All the volunteers were not on any antibiotic treatment and had not been hospitalized in the last one year at the period of sampling. All samples were processed within 2 hours of collection time.

All procedures were carried out in Sudan University of Science and Technology / Microbiology Lab.

3.2.2 Sterilization

Sterilization is used when the inactivation of all micro-organisms is an absolute requirement. This is achieved by physical, chemical or mechanical means. Dry or moist heat are the most commonly used methods in hospital and laboratories.

3.2.2.1 Dry heat

Dry heat is only suitable for items able to withstand temperatures of at Least 160 ˚C is used to sterilize glassware and metal instruments.

Complete combustion in high-temperature incineration is used for the disposal of human tissues and contaminated waste: heat in flame, hot air ovens at 160-180 ˚C for 1 hour and incineration at >1000˚C.

3.2.2.2 Moist heat

Moist heat sterilization uses lower temperatures than dry heat and can better penetrate porous loads, common used and suitable for culture media and reagents. The most effective and commonly used method is autoclaving. Which are similar to domestic pressure cookers, Operating on the principal that water under pressure boils at a higher temperature (15 psi the steam forms at 121˚C) this sufficient to kill all microorganisms, including spores. Boiling, tyndallsation and pasteurization are other types of moist heat sterilization.

3.2.2.3 Control of sterilization

In dry and moist heat sterilization, it is critical that adequate temperature and exposure times are attained. This will vary with the nature and size of the load, browne´s tubes and autoclave tape contain a chemical that changes colour when exposed to various temperatures and paper strips impregnated with heat-resistant *Bacillus stearothermophilis* spores can be placed inside autoclave loads: spore survival indicates a problem with the autoclave process.

3.2.3 Phenotypic characterization of the Isolates

3.2.3.1 Culture of Specimens

Each nasal swab was inoculated on Manitol Salt Agar (MSA) and incubated aerobically at 37˚C for 24h. A control strain, *S. aureus* NCIBB 8588 was also included.The samples were streaked by using sterile wire loop after incubation. Isolates show significant growth were identified using standard microbiological methods which included colonial morphology, Gram's stain reaction and biochemical tests.

3.2.3.2 Colonial Characteristics

The first step for bacterial identification is the studying the colonial characteristics which depend on size, color, edge, side, type of hemolysis and sugar fermentation. *S.aureus* appears as golden yellow colonies on blood agar (Color Plate 1).

3.2.3.3 Gram's Stain

Most bacteria can be differentiated by their Gram reaction due to differences in their cell wall structure. Those organisms are called Gram positive which after being stained dark purple with crystal violet are not decolorized by acetone or ethanol.

Organisms called Gram negative which after being stained with crystal violet lose their color when treated with acetone or ethanol and stain red with neutral red, safranin, or other red counter stain. The iodine solution used in the technique acts as mordant for the crystal violet.

Smears were prepared by adding small drop of normal saline in clean dry slide, by using flame-sterilized wire loop; one colony was taken and emulsified in normal saline, the smear was fixed by gentle heat after it dry, then it was covered with crystal violet as basic stain for 1 minute, the lugol´s iodine as mordant was added for 1 minute after crystal violet was washed, 70% alcohol as decolorizer was added for five seconds after iodine was washed, then the safranin was added as counter stain for 2 minutes (Appendix III) (Cheesbrough, 2008).

S.aureus appears as Gram-positive cocci arranged in clusters. See (Color plate 3).

3.2.4 Biochemical Tests

Biochemical tests are often required to identify pathogens including the use of substrates and sugars to identify pathogens by their enzymatic and fermentation reactions (Cheesbrough, 2012). Biochemical tests of *S.aureus* include:

3.2.4.1 Catalase Test

Catalase enzyme act as a catalyst in the breakdown of hydrogen peroxide to oxygen and water (Cheesbrough, 2012).

From 24 hours isolate, by using sterile wooden stick; small colony was removed and immersed in 0.5 ml hydrogen peroxide solution (Appendix III) contained in sterile

test tube. Formation of active bubbling were observed and indicate positive result (Color plate 4).

3.2.4.2 Coagulase Test

This test divided into slide and tube methods. The first one was done by placing a drop of distilled water on each end of slide. A colony of the tested organism was emulsified in each of the drops to make thick suspensions. A loopful of plasma was added to one of the suspensions and mixed gently. Clumping of the organisms within 10 seconds was observed (Color plate 5). The second method can be done by taking three small test tubes; Test organism (18-24 hours broth culture), positive control and negative control (sterile broth). Pipette 0.2 ml of plasma into each tube and 0.8 ml of the *Staphylococcus aureus* culture to the positive tube and 0.8 ml of sterile broth to the negative tube. After mixing gently, the three tubes were incubated at 35-37°C. Clotting after 1 hour, 3 hours or overnight is observed.

3.2.4.3 Deoxyribonuclease (DNAase) Test

This test is used to differentiate *S. aureus* which produces the enzyme DNAase from other staphylococci which do not produce DNAase. It is particularly useful if plasma is not available to perform a coagulase test or when the results of a coagulase test are difficult to interpret (Cheesbrough, 2012).

The DNAase enzyme hydrolyzes deoxyribonucleic acid , organism is spot inoculated in DNAase medium by using sterile wire loop, incubated at 37°C for 24 hours, after incubation time the plate is covered by 1 mol/L hydrochloric acid solution with removal of the excess acid, clearing zone around colonies were observed (Colored plate 6).

3.2.4.4 Manitol Salt Agar (MSA)

The pure colonies were picked using a sterile inoculating loop and subcultured onto the surface of selective agar, mannitol salt phenol red agar (Oxoid, United Kingdom).

Then, the plates were incubated at 37°C for 24 hours. The changes in color of the medium from pink to yellow indicate manitol fermentation (Colored plate 2).

3.2.5 Antibiotic Susceptibility Testing

The antibiotic susceptibility pattern of all the *S. aureus* isolates was determined against four different antibiotics: Penicillin G, oxacillin, erythromycin and vancomycin (Appendix III). The sensitivity of different isolates was studied using the standard disc diffusion method (Kirby Bauer Sensitivity testing technique) following the Clinical Laboratory Standards Institute Guidelines (CLSI, 2011).

3.2.5.1 Mueller Hinton Agar

The medium was prepared and sterilized as instructed by the manufacturer (HIMEDIA, India). The PH of the medium was adjusted at 7.2-7.4. About 25 ml was poured on each plate (Cheesbrough, 2012).

3.2.5.2 Turbidity standard equivalent to McFarland 0.5

This was a barium sulphate standard against which the turbidity of the test and control inoculums could be compared. When matched with the standard, the inoculums should give confluent or almost confluent growth. The standard was shaken immediately before use (Cheesbrough, 2012).

3.2.5.3 Preparation of Inoculum and Inoculation

The inoculums were prepared by emulsifying 3-5 colonies of the tested organism in about 3-5 ml of sterile normal saline or nutrient broth. In order to prevent further growth the diluted and standardization inoculums should not be allowed to stand longer than 15-20 minutes before inoculation on the plates after make its turbidity match with the turbidity standard. A sterile cotton swab was dipped into the suitably diluted culture or suspension and rotated; the swab was pressed a gains the side of the tube to remove excess fluid and streaked across the medium in three directions and rotating the plate approximately 60˚ to insure even distribution (Cheesbrough, 2012).

3.2.5.4 Application of Sensitivity Discs

The Kirby-Bauer disc diffusion technique was used. A paper disc saturated with a known amount of the antibiotic was used, not more than 6-8 discs should be tested on a single plate. The petri dishes were dried in the incubator for no longer than 15 minutes, using a sterile forceps the antibiotic discs were carefully placed on the inoculated plates, each disc was highly pressed down to ensure its contact with the agar. After overnight aerobic incubation at 37°C, the culture was examined for zones of inhibition of bacterial growth around the respective discs.

3.2.5.6 Interpretation of Zone Sizes

The interpretative chart was used and interpreted the zones sizes of each antibiotic. The organism is reported as resistant, intermediate/moderately sensitive, sensitive (susceptible). See (Table 2).

Table 2. Interpretation of inhibition zone diameter

Antibiotic	Symbol	Disk potency	Resistant	Intermediate	Sensitive
Penicillin G	P	10 units	20 or less	21-28	29 or more
Methicillin	Me	5 mcg	9 or less	10-13	14 or more
Erythromycin	E	15 mcg	13 or less	14-22	23 or More
Vancomycin	V	30 mcg	9 or less	10-11	12 or more

3.2.6 Statistical Analysis

Statistical data entry and analysis were performed using SPSS version 16 (SPSS Inc., Chicago, III, USA). Chi-Square tests used to identify risk factors of *Staphylococcus aureus* nasal carriage. All *P* values were 2-tailed and $P < 0.05$ was considered as significant.

CHAPTER FOUR

RESULTS

4.1 Epidemiological Findings

4.1.1 Samples Distribution

A total of 100 (*n* =100) nasal swabs were obtained from school children. The children are attending primary schools (Nour Algofran and Gobaa Primary School) which located in Omdurman city. Overall nasal carriage of *S. aureus* in this study population were 43% and out of 43 isolates, 16% were MRSA.
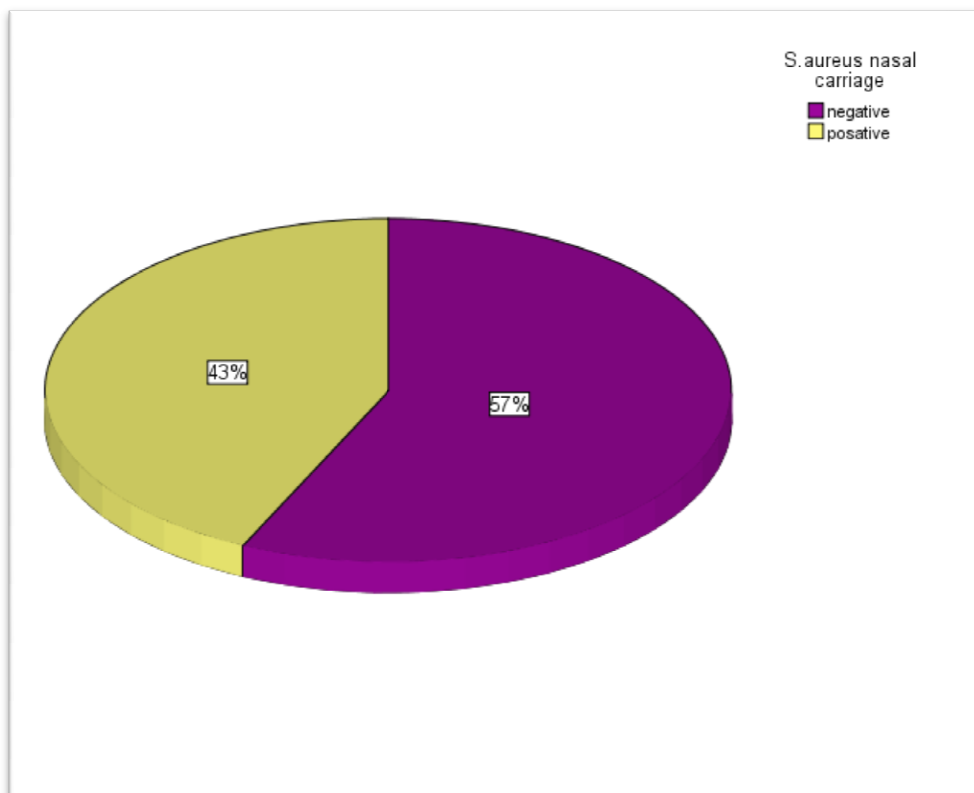

Figure 2. Frequencies of *S. aureus* nasal carriage

4.1.2 Gender

The distribution of gender was as follows: 76(76%) were boys and 24 (24%) were girls (Figure 3). From 43 isolates of *S. aureus* 31 (72%) were boys and 12 (27%) isolates were positive for girls (Figure 4).

Gender was not significantly associated with *Staphylococcus aureus* nasal carriage (*P* value = 0.427).
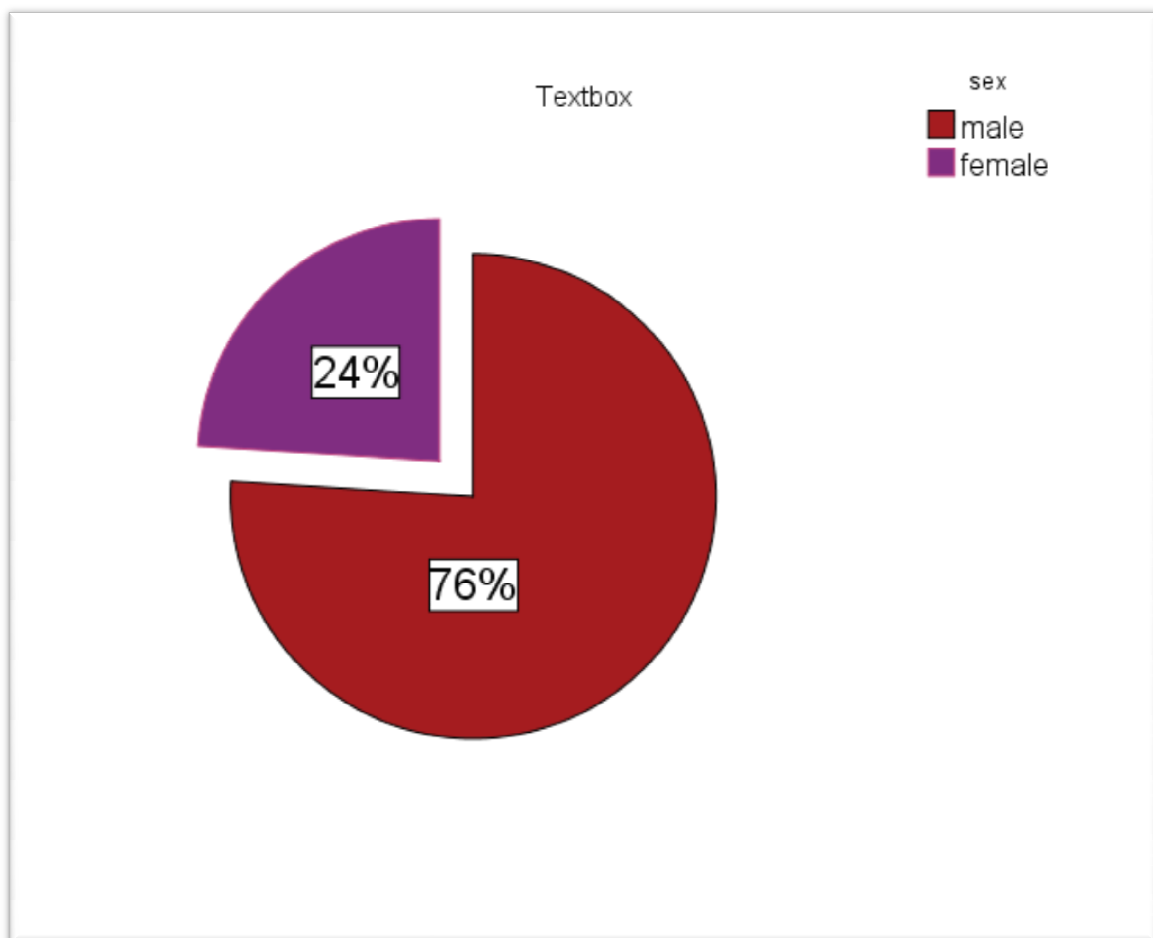

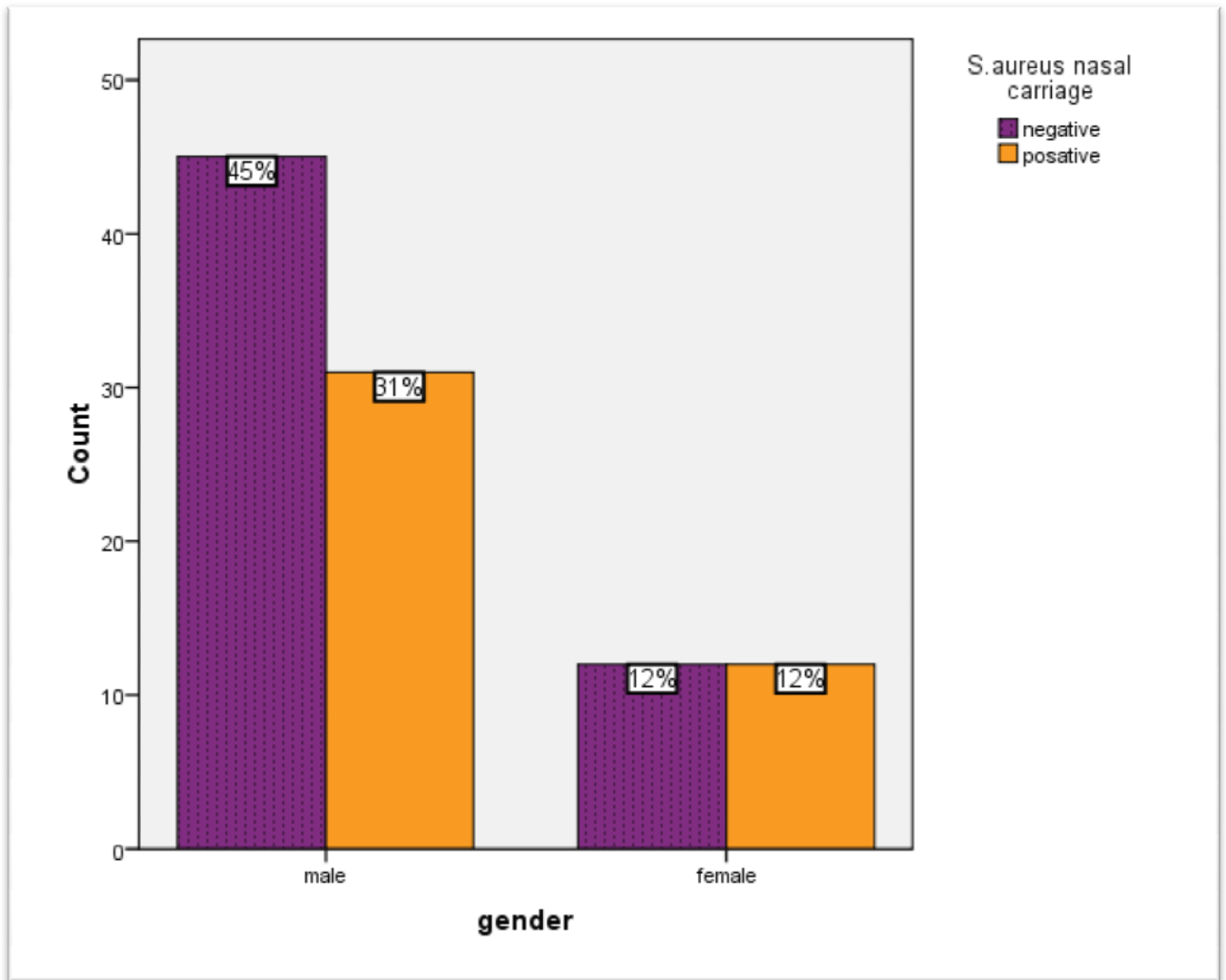**Figure 3. Distribution of samples according to gender**

Figure 4. Distribution of *S. aureus* Isolates among enrolled children.

4.1.3 Age

Children included in this study within age from 6 _10 years (Table 3). 35% of *S. aureus* nasal carriage at age 9 year (Figure 5) but there is no significant association between age and *Staphylococcus aureus* nasal carriage (*p*=0.884).

Table 3. Distribution of samples according to age / year

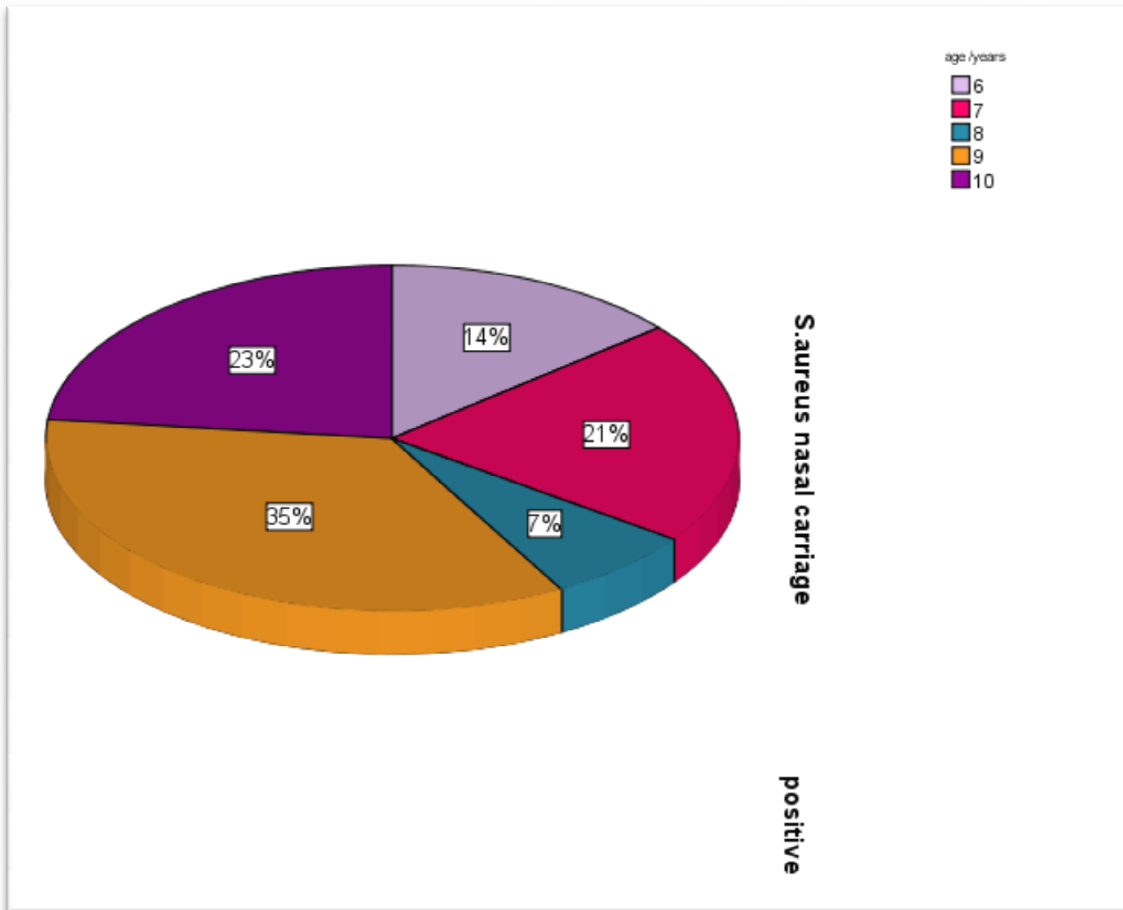| Age / year	Number of enrolled children
6	19
7	17
8	12
9	19
10	33
Total	100

Figure 5. Distribution of *Staphylococcus aureus* nasal carriage according to age

4.1.4 Household Number

5% of nasal carriage were from family number ≤ 4 while 38% of them were from >4 family number (Figure 6). No significant association between nasal carriage and household member (P value = 0.496).
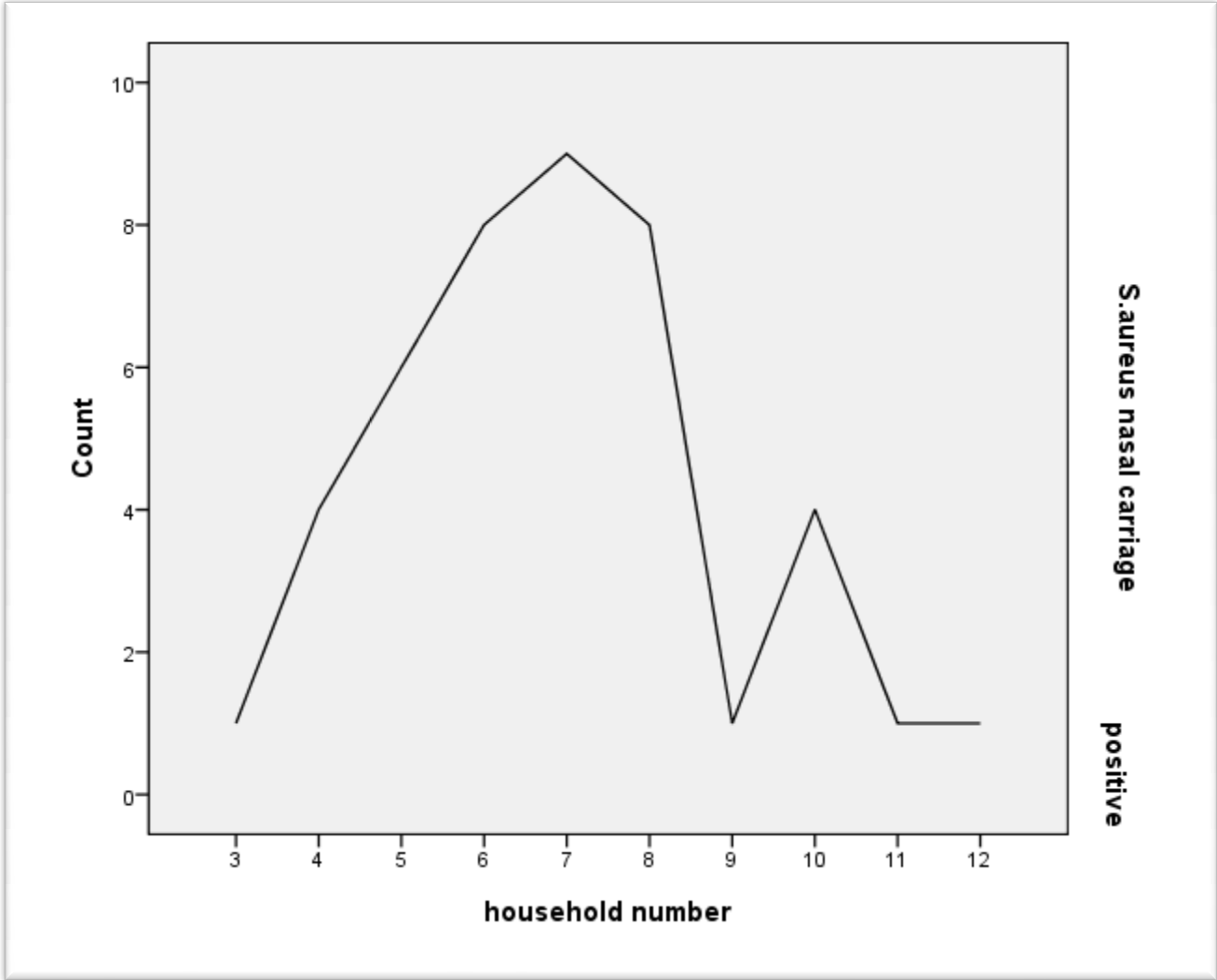

Figure 6. *S.aureus* **nasal carriage according to household number.**

4.1.5 Other Risk Factors

In addition to Gender, Age and Household number; recent respiratory tract infections are included as a risk factor effect on *S.aureus* nasal carriage. Insignificant association between them were observed *(p = 0.104)*.

Table 4. Potential Risk Factors for *S. aureus* nasal carriage among school children.

Factor		Positive No.(%)	Negative No.(%)	*p*-value
Gender	boy	31(31%)	45(45%)	
				0.483
	Girl	12(12%)	12(12%)	
Recent respiratory tract infections	Yes	7(7%)	18(18%)	0.104
			39(39%)	
		36(36%)		
	No			
Household number	≤4	5(5%)	4(4%)	0.496
	>4	38(38%)	52(52%)	

4.2 phenotypic Characteristics

4.2.1Gram´s Colonial Morphology and Biochemical Characteristics

The results of colonial morphology by Gram´s stain and biochemical characteristics of the isolates were shown in (Colored plate 3, 4, 5, 6 Appendix I).

4.2.2 Bacteriological Findings

43/100 (43%) of bacterial isolates were *Staphylococcus aureus*. Which were divided into MSSA 36/43 (83.7%) and MRSA 7/43 (16.3%) (Figure7).

4.2.3 Drug Susceptibility Testing (DST)

The sensitivity testing was done against four different antibiotics (penicillin G, methicillin, erythromycin, vancomycin). The result was as follows: 100/100 (100%) resistant to Penicillin G, 7/43 (16.3%) were resistant to methicillin, 7/43 (16.3) were resistant to erythromycin and no resistance was observed against vancomycin antibiotics (Table 5).
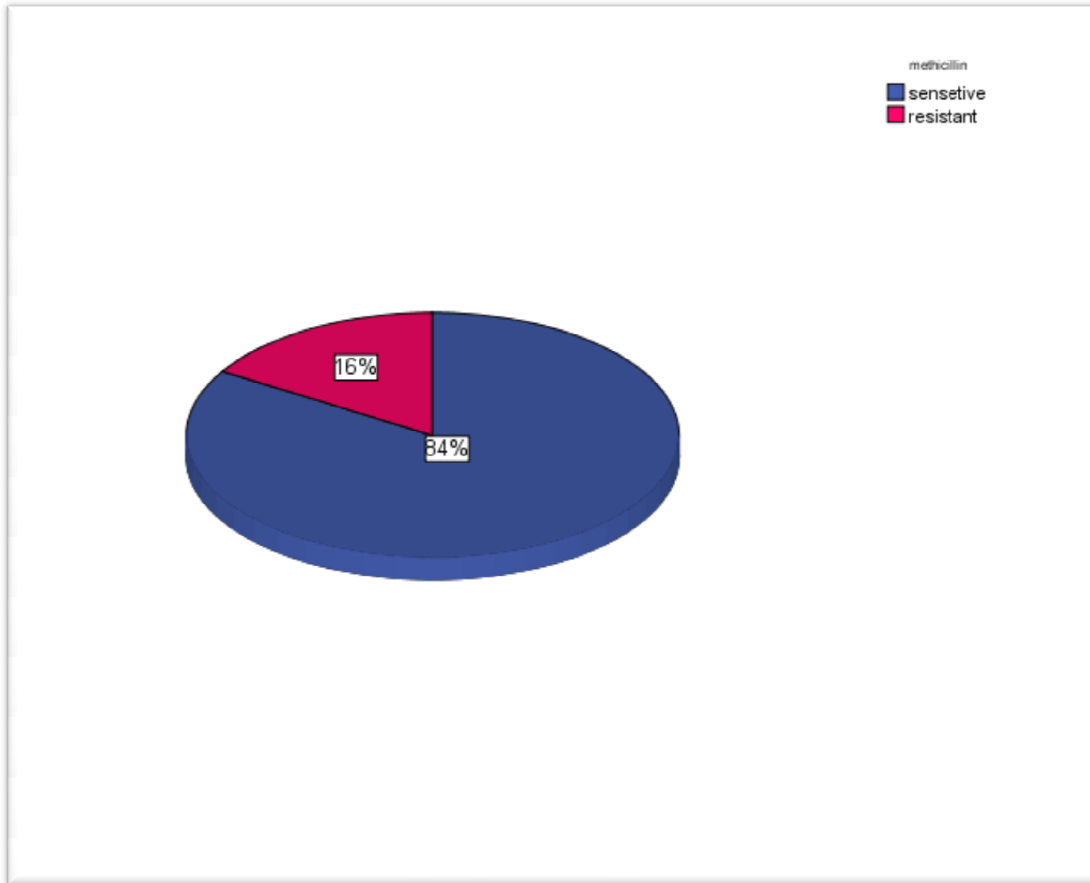
Figure 7. Frequencies of MRSA versus MSSA among isolated
Staphylococcus aureus

Table 5. Drug susceptibility patterns of *Staphylococcus aureus* isolates and MRSA.

Antibiotic	Sensitive	Resistant	%of resistance
Penicillin G	0	43	100%
Methicillin	36	7	16.3%
Erythromycin	36	7	16.3%
Vancomycin	43	0	0%

CHAPTER FIVE

5.1Discussion

The anterior nares have been shown to be the main reservoir of *S. aureus* in adults and children (Hussain *et al.,* 2001). Nasal carriage of *S. aureus* has been demonstrated to be a significant risk factor for nosocomial and community-acquired infection in a variety of populations (Williams, 1963). Self-inoculation is thought to occur when organisms from the nose colonize other areas of the skin, leading to Infection through skin lesions (Suggs *et al*., 1998). Multiple studies of infected patients demonstrate the same MRSA strain in both the nose and the infection site, and eradication of nasal carriage with topical antimicrobials in most cases eliminates the organism from other body sites. Studies have found that nasal colonization with MRSA poses an increased risk of infection over that seen with MSSA carriage (Kluytmans *et al*., 1997).

 Additionally many cases have been documented of healthy carriers transmitting staphylococci to others with resulting infection (Williams 1963). Given the relationship between nasal carriage and infection risk, knowledge of the prevalence of MRSA nasal carriage in a community provides a sense of the probability of contracting an MRSA infection in that community.

In this study, 100 nasal swabs were collected from anterior nares of primary school children included in this study. The children were from two primary schools (Nour Algofran and Gobaa primary school) which located in Omdurman city, Khartoum state, Sudan. Overall nasal carriage of *S. aureus* in this study population was 43%.This finding is near to (Chaterjee *et al., 2*009) who found 56% in community of india and (Halabhab *et al*., 2010) who found 57% in Lebanon. Our finding differ from (Joshi *et al*., 2008) who found 11.8% nasal carriage in Kathmandu Valley, nepal.

Such differences may due to variations in sample size, socioeconomic status and community nature of enrolled children.

Gender distribution in this study was as follow; 76% boy and 24% girl. Though the higher proportions of boys (31%) of the subjects were found to be the carriers of *S. aureus* than girls (12%) but there is No significant association between gender and nasal carriage *(p* value = 0.484). Similar findings were reported by (Joshi *et al*., 2008) and (Joshi *et al.,* 2003) in Nepal community.

5% of nasal carriage was from household number ≤ 4while 38% of them was from >4 household number. Althugh household crowding were reported as a risk factor for *S. aureus* nasal carriage, there was no significant statistical association between nasal carriage and family number (*P* value = 0.496). This may be due to small sample size. Children included in this study at age group from 6 _10 years. 35% of *S. aureus* nasal carriage at age 9 year but there is no significant association between age and *Staphylococcus aureus* nasal carriage (*p=*0.884).

Recent respiratory Tract infections do not appear as risk factor for *S. aureus* nasal carriage (*P* value = 0.104). Similar result was obtained by (Joshi., 2008) and (Muge Oguzkaya-Arten *et al.,* 2008).

Sensitivity testing against 4 antimicrobials (Penicillin G, oxacillin, erythromycin, vancomycin) was done by standard disk diffusion method according to CLSI recommendations.

The isolates show 100% resistance to Penicillin G and that is similar to result obtained by (Heifa Elbashier, 2012) in sudan. The prevalence of MRSA among *Staphylococcus aureus* isolates was 7 (16%). This finding is near to what was obtained from Indian school by (Ramana, 2009) who was found 19% and lower than (Lu et al., 2005) study which show 3.3% MRSA.

These variable results in the prevalence of MRSA were reflections of the local endemicity, sanitary standard, environmental conditions, timing and seasonal differences in the design of the work and personal hygiene.

7 isolates (16%) show resistance to erythromycin. This result is also obtained by (Muge *et al.,* 2008). No resistance against Vancomycin (100% susceptibility) was showed by *S. aureus* isolates, (Joshi *et al*., 2008) also report this result.

These indicate the frequency of antimicrobial resistance pattern among *S. aureus* carriers with no exposure to the hospital systems.

5.2 Conclusion

The rate of nasal colonization of *S. aureus* in school children who were between 6 to10 years old in a community in Omdurman City was 43%. Factors studied did not show significant association with nasal colonization. There was a high rate of CA-MRSA nasal carriage in the study population and all isolates were sensitive to vancomycin.

The findings of this study showed that the carriage of MRSA exists among young healthy school children who lack traditional risk factors for MRSA. Our knowledge of community-acquired MRSA epidemiology is incomplete, which adds to the challenge of controlling infection by community-acquired MRSA, so, continuing surveillance is needed to more accurately assess the prevalence, geographic distribution and epidemiology of community-acquired infection and to develop strategies that will improve therapy and control the spread.

5.3 Recommendations

In clinical practice, this study is useful for the choice of treatment for community acquired *S. aureus* infections. It shows a high rate of MRSA in the study community with variable susceptibility to standard anti-staphylococcal antibiotics. The varying rates of CA-MRSA carriage among different reports emphasize the need for local surveillance studies to guide the clinician in appropriate antibiotic choices in treating *S. aureus* infections.

1. The findings in this study may be applied only to children within the same age group and with the same socioeconomic status.

2. Future research should include communities of different socioeconomic classes.

3. Local surveillance studies are essential in the control of CA-MRSA and in guiding local antibiotic policies for staphylococcal infections.

4. A larger study population involving several communities is needed to identify CA-MRSA rates, antibiotic resistance patterns and the epidemiologic risk factors associated with nasal colonization in the local setting.

5. All sensitivity testing in this study was done by using disc diffusion method, so further study is needed by using other sensitivity methods against more antibiotics.

6. Molecular techniques are needed for detection genes of resistance.

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Appendix I

Colored plates
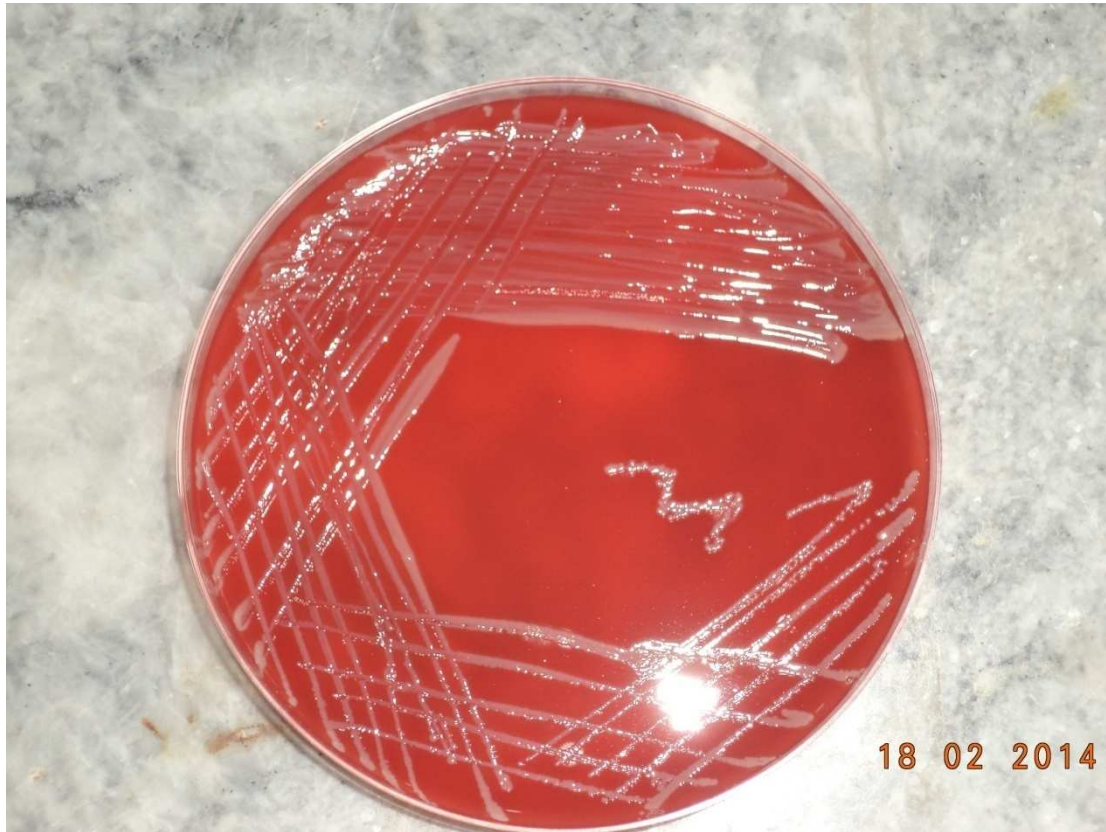
Color plate 1. Overnight growth of *Staphylococcus aureus* on Blood Agar medium.
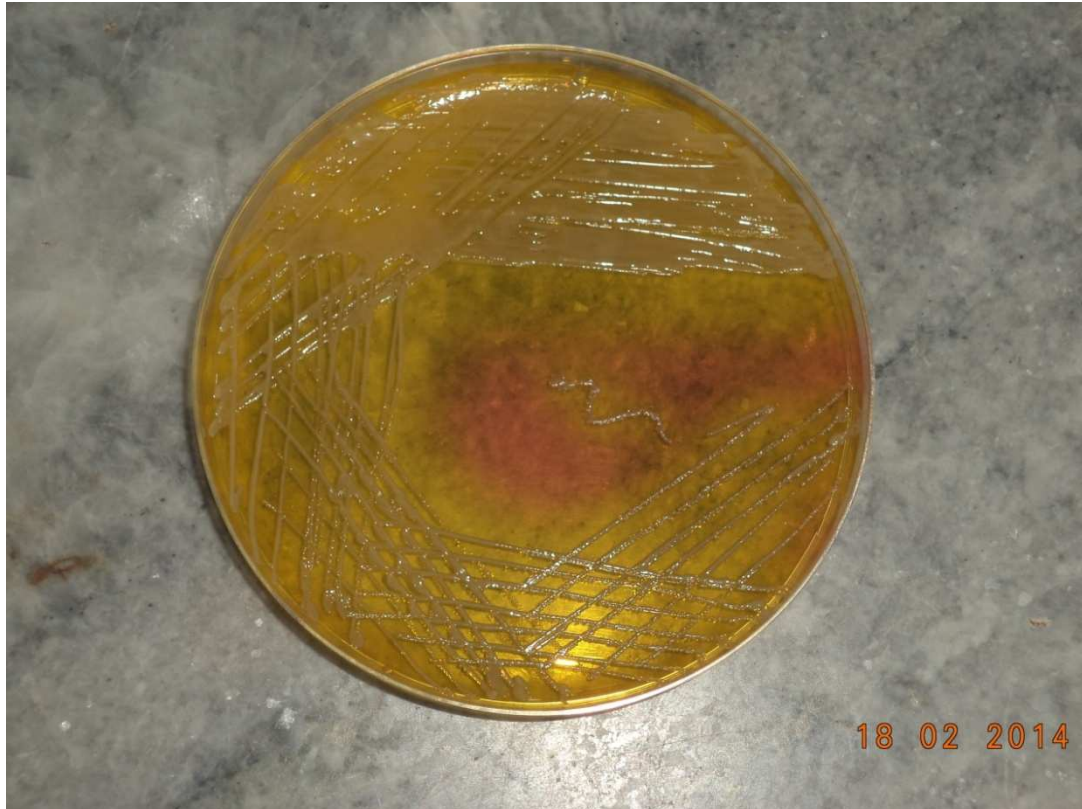
Color plate 2. *Staphylococcus aureus* on Manitol Salt Agar (MSA) appears as yellow (manitol fermener) colonies.
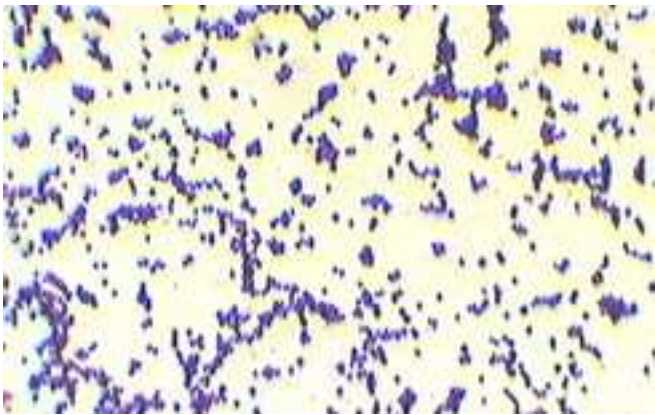
Color plate 3. Gram's stain of *Staphylococcus aureus* under microscope with X100 objectives .
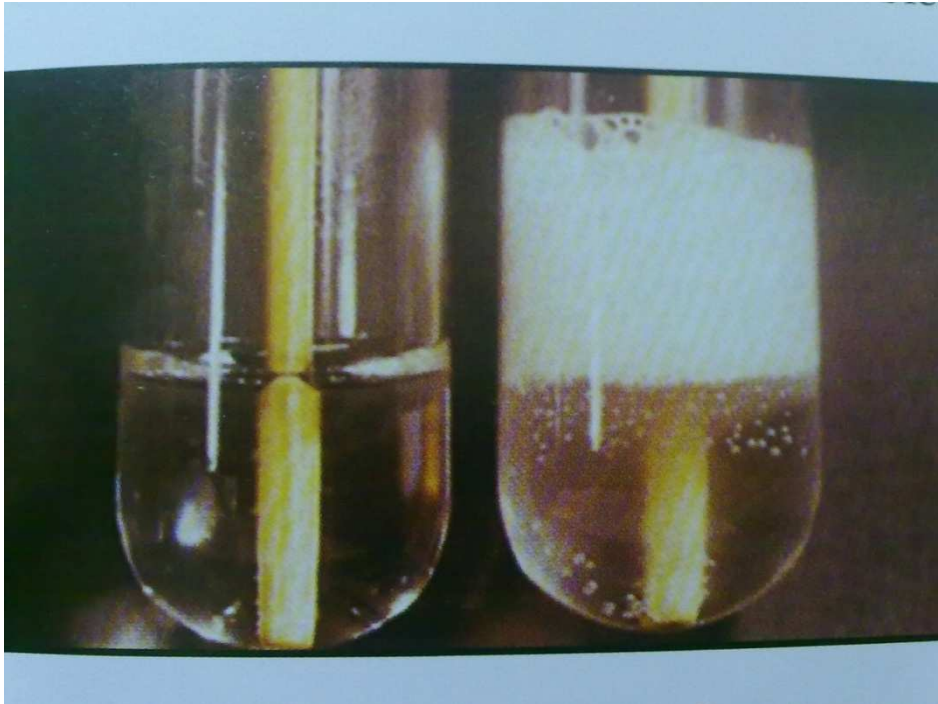
Color plate 4. Catalase test; tube at left show negative result, right tube with active bubbling indicate positive reaction.
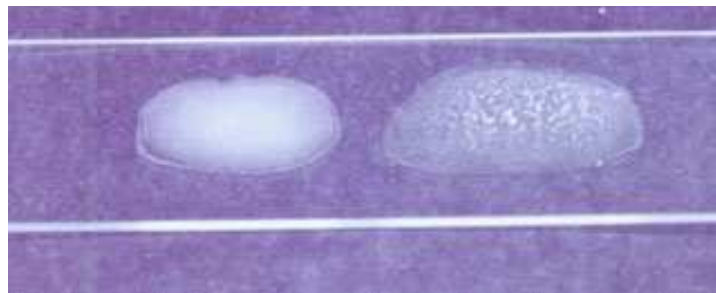
Color plate 5. Slide coagulase test; Left side show negative reaction, right show positive reaction.

color plate 6. DNAase test; *S.aureus* show positive result with clear zone around colonies (right), Left is negative (control).
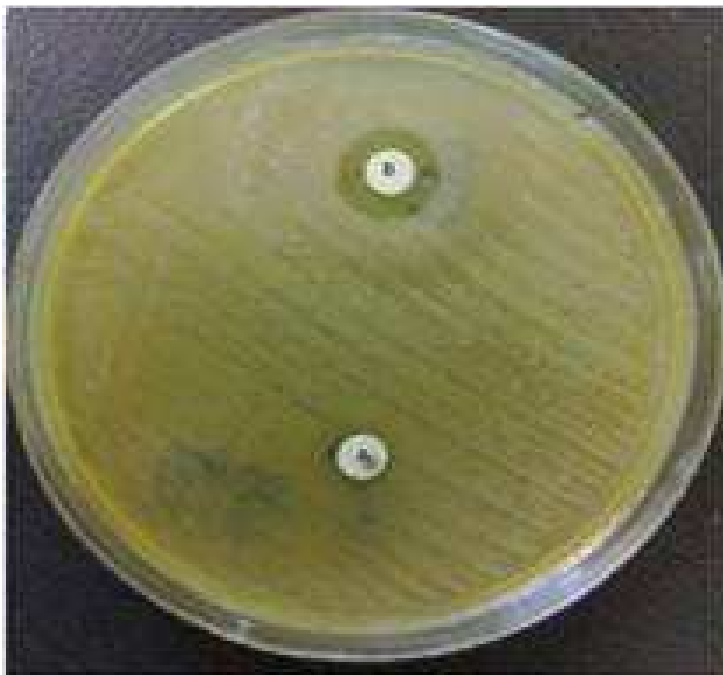
Color plate 7. *S.aureus* on Mueller-Hinton Agar appears sensitive to Methicillin.

Appendix II

Preparation of Media

1. Blood agar

Nutrient agar was used as basal media and was prepared according to instruction of manufacturer, after sterilized by autoclaving at 15Ib pressure (121˚C) for 15 minutes, the media was cooled to 50-55˚C, then 5 ml of blood (for each 100 ml) was added to the basal media and 20-25 ml of molten preparation were poured into sterile disposable 90 mm in diameter petri dishes (Mackie and McCartney, 1996).

2. Manitol Salt Agar Media

2.1 Formula of Manitol Salt Agar Media (PH 7.3) (Oxoid,United kingdom) (gm/L)

Lab-lemco powder……………………………………..1.00 g

Peptone………………………………………………..10.00 g

Mannitol…………………………………………………10.00 g

Sodium chloride…………………………………………75.00 g

Phenol red……………………………………………..0.025g

Agar……………………………………………………15.00g

2.2 Preparation

Media was prepared according to instruction of manufacture as follow: 11.1 grams were suspended in 100 ml distilled water. The preparation sterilized by autoclaving at 15Ibs pressure (121˚C) for 15 minutes. After cooling to 50-55 ˚C, 20-25 ml of molten

preparation were poured into sterile disposable 90 mm in diameter Petri dishes. Date the medium and give it a batch number (Mackie and McCartney, 1996).

3. DNAase Agar

3.1 Formula of DNAase Agar (oxoid, United Kingdom) (gm/L) (PH 7.3).

Trypose ………………………………………….20.00 g

Deoxyribonucleic acid (DNA) ……………………….2.00g

Sodium chloride………………………………………..5.00 g

Distilled water………………………………….....1000 ml

3.2 Preperation

Suspended 39 grams in 1 litre of distilled water mix well and sterile by autoclaving at 12-15 ibs pressure (118-121) for 15 minutes.

4. Peptone Water

4.1Formula of peptone water (Oxoid,United kingdom) (gm/L)

(PH 7.2)

Peptic digest of animal tissue _____ 10.00g

Sodium chloride_____ 5.00g

4.2 Preperation

15g of powder dissolved in 1liter of distal water, sterilized by autoclave at 121˚ C for 15 minutes then it was cooled and powered in sterile tube.

5. Mueller-Hinton Agar

5.1 Formula of Mueller-hinton Agar (HIMEDIA,India) (gm/L)

(PH 7.4)

Beef, infusion ………………………………………….300.0g

Cas amino acids………………………………………17.5 g

Starch…………………………………………………..1.5g

Agar …………………………………………………….17.0g

Distilled water……………………………………….1000ml

5.2 Preparation

38.0 g of media was suspended in 100 ml distilled water. Sterilized by autoclaving at 15Ib pressure (121˚C) and poured in sterile petri dishes.

Appendix III

Reagents and Stains

1. Gram's Stain

Most bacteria can be differentiated by their Gram reaction due to differences in the cell wall structure into Gram positive which after being stained dark purple with crystal violet are not decolorized by acetone or ethanol and Gram negative which after being stained with crystal violet lose their color when treated with acetone or ethanol and stain red with safranin.

1.1 Requirements

1.1.1 Crystal violet Gram stain (HiMedia)

To make 1 liter:

Crystal violet……………………………………….20 g

Ammonium oxalate………………………………9 g

Ethanol or methanol, absolute……………….95 g

Distilled water……………………………………… to 1 liter

1.1.2. lugol's iodine (HiMedia)

To make 1 liter:

Potassium iodide……………………………………………20 g

Iodine………………………………………………………………..10 g

Distilled water…………………………………………………….. to 10 liter

1.1.3. 70% alcohol

Absolute alcohol……………………………………..70 ml

Distilled water………………………………………….30 ml

1.1.4 Safranin (HiMedia)

1.2 Method of Preparation

- The dried smear was fixed by heat.
- The fixed smear was covered with crystal violet for 30-60 minutes.
- The stain was washed off with clean water.
- All water was tipped and the smear covered with lugol´s iodine for 30-60 minutes.
- The stain was washed off with clean water.
- 70% alcohol was rapidly applied for 10-20 seconds for decolourization and then washed rapidly with clean water.
- The smear then covered with safranin stain for 2 minutes.
- The stain was washed off with clean water, back of slide was cleaned.
- After air-dry, smear was examined microscopically by using X 100 lens.

1.3 Results

Staphylococcus aureus appear as (grape like cluster) Gram positive cocci.

2. Catalase

2.1 Requirements

2.1.1 Hydrogen peroxide (3% H_2O_2)

H_2O_2...……………………………3ml

D.W………………………………97 ml

2.2 Method

- 2-3 ml of hydrogen peroxide was poured into sterile test tube
- Several colonies was removed by using sterile wooden stick and immersed in the hydrogen peroxide solution.
- look for Immediate bubbling

2.3 Result

Active bubbling ……………..Positive catalase test

No bubbles ………………….Negative catalase test

3. Coagulase Test

3.1 Requirements

Undiluted human plasma

3.2 Method

- place a drop of distilled water on a slide.
- Emulsify a colony of the test to make thick suspension.

- Add a loopful of plasma to one of the suspension and mix gently. Look for clumping of the organisms within 10 seconds.

3.3 Result

Clumping within 10 sec………….positive coagulase test

No clumping within 10 seconds………………Negative test

4. DNAase Test

4.1 Requirements

4.1.1DNA agar (appendix 1)

4.1.2 Hydrochloric acid (1mol/L)

To make 100 ml:

Hydrochloric acid concenterated …………………………..8.6 ml

Distilled water …………………………………………….to 100 ml

4.2 Methods

- Divide a DNA agar plate into the required number of strips by making the underside of the plate.
- Using a sterile loop, spot-inoculate the test and control organisms. Make sure each test area is labeled clearly.
- Inocubate the plate at 35-37˚c overnight.
- Cover the surface of the plate with 1 mol/L hydrochloric acid solution. Tip off the excess acid.

- Look for clearing around the colonies within 5 minutes after adding the acid.

4.3 Results

Clearing zone around the colonies ………….Positive DNAase

No clearing zone around the colonies ……….Negative DNAase

5. Preparation of Turbidity Standard

- 1% v/v solution of sulpharic acid was prepared by adding 1 ml of concentrated sulfuric acid to 99 ml of water. Mix well.

- 1.17% w/v solution of barium chloride was prepared by dissolving of 2.35g of dehydrated barium chloride ($BaCl_2.2H_2O$) in 200 ml of distilled water.

- To make the turbidity standard 0.5 ml of barium chloride solution was added to 99.4 ml of the sulpharic acid solution. Mix well.

- A small volume of the turbid solution was transferred to screw-caped bottle of the same types as used for preparing the test and control inoculate (Mackie and MaCareny, 1996).

Table 6. Antibiotics Discs used for disk Diffusion Test.

number	Antibiotic	Potency	Symbol	Source
1	**Penicillin G**	10 units	**P**	HiMedia
2	**Methicillin**	5 mcg	**ME**	HiMedia
3	**Erythromycin**	15 mcg	**E**	Axion
4	**Vancomycin**	30 mcg	**V**	HiMedia

Appendix IV

A questionnaire

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Medical Labratory Science

Microbiology Department

2013_2014

Title:

Nasal Carriage of Methicillin Resistant *Staphylococcus aureus* (MRSA) A mong Healthy Primary School Children.

A questionnaire

Name: ....

Participant code: ………………………………………………

Age: ....

Sex: ....

Household number: ....

 Recent respiratory infections: present   Absent

Sample**:** nasal swab

Results:

Isolated Bacteria: ……………………………..

Sensitivity Testing:

 Organism is sensitive to…………………………………..and resistant to………………………………………….. .