CHAPTER-I

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) is one of the versatile nosocomial pathogens worldwide (Rehm *et al.,* 2008) causing skin infections to life threatening systemic illness including pneumonia, osteomyelitis endocarditis (Mallick and Basak, 2010). Approximately, 25.0% of all nosocomial infections are caused by *S. aureus*, affecting both surgical and non surgical patients and leading hospital stay, antibiotics use costs and mortality (Mintjes-de Groot *et al.*, 2000). *S. aureus* can infect almost all tissue and organs (Deleo *et al.*, 2010). They can cause food poisoning by releasing enterotoxins into foods to potentially life threatening infections such as septicemia and toxic shock syndrome by release of superantigens into blood stream.

S. aureus is a gram positive cluster forming cocci, which is about 1µm in diameter. It is catalase and coagulase positive, Novobiocin sensitive and is fermentative organism. It grows rapidly under aerobic or anaerobic conditions and is positive towards Voges Proskauer (VP) test (Greenwood, 2004).

Originally, all *S. aureus* were naturally susceptible to almost every antibiotics developed so far, it frequently gains resistance by gene mutations and horizontal gene transfer. But after first discovery of Penicillin and soon after penicillin was put in clinical use in 1940s, penicillin resistance (PRSA) developed in1948s. Multidrug resistant (MDR) strains of staphylococci have been reported with increasing frequency worldwide, including isolates that are resistant to methicillin, lincosamides, macrolides, aminoglycosides, fluoroquinoles or combinations of these antibiotics (Lowly, 1998; Moellering, 1998). *S. aureus* had acquired the ability to inactivate the β- lactam ring of penicillin with the help of plasmid- encoded β- lactamase enzyme (de Lancastrre *et al.*, 2007). Currently more than 95.0% of *S. aureus* are resistant to penicillin (Westwell and Williams, 1996). *S. aureus* resistant to Oxacillin, Methicillin and a few others related antibiotics are all known under the generic term methicillin resistant *S. aureus* or MRSA. The emergence of MRSA typically coincides with that of penicillin-resistance *S. aureus* (PRSA) (Chambers, 2001)

MRSA is a specific strain of the *S .aureus* that has developed antibiotic resistance, first to penicillin since 1948s, and later to methicillin and related anti staphylococcal

drugs (such as flucloxacillin, nafcillin and oxacillin). It may also be called oxacillin resistant *S. aureus* (ORSA). Non methicillin resistant *S. aureus* is termed as methicillin susceptible *S. aureus* (MSSA) to make the distinction. Methicillin-resistance in *S. aureus* is genetically mediated by eight different Staphylococcal cassette chromosome *(SCCmec*), a mobile genetic element encoding for an altered penicillin-binding protein (PBP2a, *mecA*) with decreased affinity to beta-lactams (de Lencastre *et al.*, 2007; Otter *et al.*, 2010; Gordo *et al.*, 2008).

MRSA is a global public health problem, associated with considerable morbidity and mortality, causing both hospital and community-acquired infections (Ray *et al.*, 2011). In the past MRSA was considered as nosocomial pathogen (HA-MRSA), however about three decades after emergence of HA-MRSA, infections due to MRSA were reported in people without any previous health-care contact (CA-MRSA) in 1990s. The community acquired MRSA (CA-MRSA) strains evolved either from the hospital strains and underwent genetic changes or were the result of *mecA* gene transfer to formerly susceptible subsets in the community (Chambers, 2001).

S. aureus is a normal flora of nasal cavity, about 20% of the population is always colonized with *S. aureus*, 60% are intermittent carriers and 20% never carry the organism (Paecock *et al.*, 2001; Von Eiff *et al.*, 2001). About 25% health personnel are stable carriers of *S. aureus*. Worldwide 2.7% are thought to be MRSA carrier. Nasal carriage rates of MRSA have been reported to be 0.8 to 3.0% among adults in the community elsewhere in the world (Abudu *et al.*, 2001; Grundman, 2002; Jernigan *et al.*, 2003). Hospital personnel tend to have higher colonization rates than general population (Frank *et al,* 1990). Among health care workers in hospital setting, it ranges from 6.0 to 17.8% (Mulqueen *et al.*, 2007; Eveillard *et al.*, 2004; Cesur *et al.*, 2004). Health care personnel may become transient or persistent MRSA carriers while working in the hospitals in which MRSA is endemic (Mitsuda *et al.,* 1999).

Limited options are available for the therapeutic management of MRSA as they are resistant to many antibiotics developed so far. The CA-MRSA associated skin and soft tissue infections are usually treated with oral antibiotics including doxycycline, clindamycin, trimethoprim-sulphamethoxazole (TMP-SMX), rifampicin and fusidic acid. Severe CA-MRSA and HA-MRSA infections necessitate intravenous vancomycin therapy (Ray *et al.,* 2011).

Many studies done in Nepal about *S. aureus* and their antibiotic susceptibility pattern suggest the gradual emergence of MRSA in hospitals. The prevalence of MRSA in Nepal ranges from 11.7% to 54.9% (Lamichanne *et al.,* 1999; Pokharel *et al*., 1993; Rajbhandari *et al*., 2002; Thapa 2004; Kumari *et al.,* 2008; Sanjana *et al.,* 2010). Many studies have also shown the emergence of MDR *S. aureus* in hospitals (Anupurba *et al*., 2003; Shah *et al*., 2002). These studies clearly indicate about the appropriate steps to be taken to reduce MRSA and MDR strains in hospitals settings to minimize nosocomial infections.

Although the transmission of MRSA infections may be limited by universal infection-control measures, patient education, screening and decolonization of asymptomatic MRSA carriers, there is still higher prevalence of MRSA among health-care and community settings.

This study primarily focuses on the distribution and prevalence of *S. aureus* and MRSA in different clinical samples collected in microbiology laboratory of Shree Birendra hospital. This study also evaluates the present scenario of nasal carriage of *S. aureus* in medical personnel and patient visitors and correlates carrier rate among these two populations. As antibiotic tests are mandatory for all the isolates, antibiotics susceptibility tests are very useful in making appropriate antimicrobial profile to treat staphylococcal infections. The susceptibility test also helps in understanding the present pattern of *S. aureus* towards the tested antibiotics. This study explores susceptibility pattern of the isolated MRSA strains towards Vancomycin, which is the only drug of choice for treatment with MRSA infections. Similarly nasal screening for *S. aureus* and MRSA strains among health staffs and visitors helps to know their carrier state and their role in transmission of *S. aureus* to patients during patient-care.

CHAPTER-II

2. OBJECTIVES

2.1 General objective

To determine the prevalence of Methicillin-resistant *S. aureus* (MRSA) in clinical samples and to assess the nasal carrier state of MRSA among healthy carriers in hospital setting.

2.2 Specific objectives

i. To describe the distribution pattern of *S. aureus* in different clinical samples and healthy carriers.
ii. To assess the antibiotic susceptibility pattern of clinical isolates and nasal isolates from healthy carriers.
iii. To identify and describe Methicillin-resistant *S. aureus* (MRSA) among clinical isolates and nasal isolates.
iv. To assess the antibiotic susceptibility pattern of MRSA strains.

CHAPTER-III

3. LITERTURE REVIEW

3.1 *Staphylococcus*

Staphylococcus (plural *Staphylococci)* is a gram positive cluster forming, non-motile coccus measuring 0.5-0.7 in diameter, which belongs to staphylococcaceae family (Holt *et al*., 1994). Staphylococci are truly facultative anaerobic and catalase positive organisms. They can normally grow in basic media like nutrient agar and nutrient broth. Some fastidious strains require various amino acids and other growth factors as supplements (Anantanarayan and Paniker, 2000).

Staphylococci are relatively resistant to heat and drying and thus persist for long periods on fomites, which can then serve as sources of infection. They are also tolerant to high salt concentration and inhabit humans' skin and mucosa. Some of the species are normal flora of humans. Species of staphylococci found on human skin include *S. epidermidis, S. haemolyticus, and S. hominis, S. warneri, S. capitis, S. lugdunesis, S. cohnii, S. simulans and S. xylosus* (Colle *et al.,* 1996). The organisms can also isolated from a variety of environmental sources e.g. fomites, sewage, soil, water, air, and animal products such as chesses, egg, meat and milk (Easmon and Goodfellow, 1990). The main species considered clinically important include *S. aureus, S. epidemidis and S. saprophyticus*.

3.2 Characteristics of *S. aureus*

S. aureus is a spherical gram positive cocci arranged in irregular grape like clusters. Cluster formation is due to successive cell division in asymmetric three planes. The organism has a diameter of 1μm in an average and liquid culture shows the arrangement of cocci in single, pairs, tetrads, or short chains of three or four cells. A few strains have capacity to produce capsules in young cultures (Chakraborty, 2004). The organism is non- motile, non-sporulating, and is facultative anaerobe showing better growth at aerobic conditions.

3.2.1 Cultural Characteristics

S. aureus can grow easily on basic media like nutrient agar within the temperature range of 12-44℃. The optimum temperature and PH for growth is 37℃ and 7.5 respectively. It can be cultured on supplemented and enriched media like mannitol

salt agar (MSA), blood agar and most other media (Collins and Lyne, 1983). The growth characteristics of *S. aureus* on different media can be summarized below.

Nutrient agar

Most strains produce golden yellow colonies while a few strains are non- pigment producers when incubated aerobically at 22℃ (Chakraborty, 2004). Pigment production can be enhanced when 1% glycerol monoacetate is incorporated into the medium (Jacobes *et al.*, 1964) or by culture for 5 days at 30℃ on glucose peptone yeast extract agar (Baired-Parker, 1997).

Blood agar

Organism when grown on 5% sheep blood agar shows medium to large, smooth, slightly raised and translucent colonies. Most colonies are clearly yellow pigmented and most of them show beta-haemolysis (Forbes *et al.*, 2002). Haemolysis is enhanced when incubated in an atmosphere of 20% Carbon dioxide. Haemolysis is weak or absent on horse blood agar (Collee *et al.*, 1999).

MacConkey and CLED agar

On MacConkey or CLED agar, they produce very smaller colonies (0.1-01.5 mm) and give the appropriate colour of the incorporated indicator based on lactose fermentation. Pink colonies are produced on MacConkey agar (Baired-Parker, 1997).

Mannitol salt agar (MSA)

S. aureus is able to grow on media containing 7-10% sodium chloride (Chessbrough, 2002).Most bacteria are inhibited on MSA while *S. aureus* is tolerant to sodium chloride incorporated into the media and forms 1mm diameter golden yellow colonies surrounded by yellow media due to acid production from mannitol fermentation (Collee *et al.*, 1996).

3.2.2 Biochemical characteristics

S. aureus is catalase positive and coagulase positive. It ferments a number of sugars namely Glucose, lactose, sucrose, maltose, lactose, and mannitol with the production of acid but no gas. The organism hydrolyzes urea, reduces nitrates to nitrites, liquefy gelatin and is MR/VP positive but indole negative. Urease and esterase production and lactose fermentation are variable characters useful in the differentiation of

methicillin resistant strains (Coia *et al.,* 1996). *S. aureus* also produces deoxyribonuclease (DNase) and thermonuclease (TNase). Though coagulase test is of diagnostic value in detecting *S. aureus*, some other rare strains also give positive coagulase test such as *S. intermedius, S. schleiferi subsp. coagulans and S. hyicus* (Forbes *et al*., 2002).

3.3 Virulence factors and pathogenesis

S. aureus has the capacity to produce a wide array of virulence factors, causes various pyogenic infections, food poisoning, and toxic shock syndrome. *S. aureus* posses a large number of cells associated and extracellular factors, which overcome the body's defense and invade, survive and colonize the tissue. The organism posses such virulence factors which oppose destruction by the component of innate immunity i.e. complement and phagocyctosis.

3.3.1. Cell wall associated factors

i. Peptidoglycan

It gives rigidity to the cell and represents 50% of cell wall weight. It has endotoxin like property and septic shock may result from severe infection. The peptidoglycan can stimulate macrophages to produce cytokines and can activate the complement and coagulation cascade.

ii.Teichoic acid

It confers antigenicity and behaves as surface receptors for Staphylococcal bacteriophage. It mediate adherence of staphylococci to mucosal cells.

3.3.2. Cell surface proteins

i. Protein A

This cell wall protein is an important virulence factor because it binds to the Fc portion of IgG at the complement site, thereby preventing the activation of complement. As a result C3b production is hampered and the opsonization and phagocyctosis of the organism is greatly reduced. About more than 95% of *S. aureus* isolates produce protein A (Maranan *et al.*, 1997; Kloos and Lamb, 1991). Coagulase negative staphylococci do not produce protein A.

ii. Clumping factor

It is a surface associated protein also known as bound coagulase, which reacts with fibrinogen.

iii. Fibrinonectin-binding protein (FBP)

It promotes binding to mucosal cells and tissue matrices.

3.3.3. Microcapsule

Most strains of *S. aureus* are associated with a small amount of polysaccharide capsule (microcapsule) that has antiphagocytic property. There are 11 serotypes of *S. aureus* based on antigenicity of the capsular polysaccharide (Levinson and Jawetz, 2003).

3.3.4. Exoproteins

Cytolytic exotoxins

α, β, γ and δ attack mammalian membranes (including red blood cells) and are often called haemolysins. Alfa-toxins is chromosomally encoded which polymerizes into tubes and forms holes in the membrane causing loss of important molecules and eventually osmotic lysis of the cell.

3.3.5. Super-antigen exotoxins

These toxins have an affinity for the T-cell receptor-MHC class II antigen complex. They stimulate maximum number of T-lymphocyte, which can cause toxic shock by release of high amount of T cell cytokines such as interleukin-2 (IL-2), interferon-γ (INF-γ) and tumor necrosis factor-α (TNF-α).

i.Enterotoxins

Enterotoxins types A-E, G, H, I and J are commonly produced by 65% of strains of *S. aureus* (Greenwood, 2003). When ingested as preformed toxins in contaminated foods, they stimulate the vomiting centre in the brain by binding the neural receptors in the upper GI tract. These toxic proteins are heat stable and withstand up to 100℃ for several minutes.

ii. Toxic shock syndrome toxin (TSST)

TSST is a super antigen and causes toxic shock by stimulating the release of large amounts of IL-1, IL-2 and tumor necrosis factor (TNF). Approximately 5.25% of *S. aureus* isolates carry the gene for TSST (Levinson and Jawetz, 2003). Toxic shock occurs especially in tampon using women or in individuals with wound infections who do not have antibody against TSST.

iii. Exofoliatin (Exfoliative toxin)

It causes scalded skin syndrome mainly in young children. After localized infection, the strain produces diffusible exofoliative toxin that exerts distant effects. After the development of painful rash, the epidermis slough off and the skin surface resembles scalding.

3.3.6. Extracellular enzymes

The pathogenicity of *S. aureus* infections is supplemented by its ability to produce wide variety of tissue damaging enzymes.

i. Hyaluronidase

This enzyme breaks down the connective tissue of the host by hydrolyzing the hyaluronic acid, which helps the organism to spread from the localized part to surrounding tissues. This enzyme is also called spreading factor.

ii. Lipase

This enzyme degrades lipid of the skins and tissues and helps in its spread. Lipase degradation facilitates *S. aureus* to colonize the sebaceous glands.

iii. Staphylokinase

It is also called fibrinolysin, which lyses fibrin by activating plasminogen. It forms a complex and causes dissolution of fibrin clots by proteolytic activity.

iv. Deoxyribonuclease

This enzyme degrades host's DNA.

v. Coagulase

It is an enzyme, which causes plasma to clot by activating prothrombin, which in turn converts fibrinogen to fibrin. It is of two types; free coagulase and bound coagulase. About 97% of S aureus produce both forms of coagulase (Maranan *et al.*, 1997; Langone, 1982).

vi. Phosphatase

This enzyme breaks down phospholipid of the host cell.

3.4 Human diseases caused by staphylococcal infections

The important clinical manifestation caused by *S. aureus* can be divided into two groups: Pyogenic or inflammatory and toxin mediated infections. *S. aureus* is a major cause of skin and soft tissue, bone, joint, lung, heart, and brain and kidney infections.

3.4.1. Localized skin infections:

Skin infections caused by *S. aureus* are generally believed to follow colonization of the skin or nares of the host. The most common *S. aureus* skin infections are small, superficial abscesses involving hair follicles (folliculitis on sweat or sebaceous glands. for example, the common sty is caused by infection of the follicle of the eyelash. the infection of follicles can penetrate deep into the subcutaneous tissue to become furuncle. these may bore through to produce multiple contiguous painful lesions communicating under the skin called carbuncle. carbuncles are larger, deeper, multiloculated skin infections that can lead to bacteremia and require antibiotic therapy and debridement.

a. Impetigo

This contagious infection usually occurs on the face, especially around the mouth. Small vesicles lead to pustules, which crust over to become honey-coloured, wet and flaky.

b. Cellulites

This is a deeper infection of the cells. The tissue becomes hot, red, shiny and swollen.

c. Wound infections

Any skin wound can be infected with *Staphylococcus aureus*, resulting in an abscess, cellulites or both. When a sutured post-surgical wound becomes infected, it must be reported and treated.

d. Abscesses

These can occur in any organ when the organism circulates in the bloodstream. These abscesses are often called metastatic abscesses because they occur by the spread of bacteria from the original site.

3.4.2. Diseases due to organ invasion

a. Pneumonia

S. aureus is a rare but severe cause of community acquired bacterial pneumonia. Pneumonia is more common in hospitalized patients (Strohl *et al.,* 2002). Pneumonia is more commonly seen in postoperative patients or following viral respiratory infection especially by influenza virus. The violent, destructive, necrotizing pneumonia frequently causes effusions and emphysema. In community, pneumonia cases primarily occurred in children, but older age groups may be affected (Francis *et al.,* 2005)

b. Osteomyelitis

This bone infection usually occurs in boys under 12 years of age. The infection spreads to the bone hematogenously, presenting locally with warm, swollen tissue over the bone and with systematic fever and shakes (Fridkin *et al.,* 2005).

c. Septic arthritis

Invasion of the synovial membrane by *S. aureus* results in a closed infection of the joint cavity. Septic arthritis should be treated immediately because collected pus can rapidly cause irreparable cartilage damage. Therapy requires drainage of the joint and antimicrobial therapy (Fridkin *et al.,* 2005).

d. Acute Endocarditis

It is generally associated with intravenous drug abuse and is caused by injection of contaminated preparation or by needles that are contaminated with *S. aureus*. It causes

destructive infection of heart valves with the sudden onset of high fever, chills and myalagia. Intravenous drug users usually develop a right-sided tricuspid valve endocarditis(Vandenesch *et al.,* 2003).

e. Meningitis, Cerebritis and brain abscess

Patient with these disease show symptoms like high fever, stiff neck, headache, coma and focal neurological signs.

f. Septicemia

It can be originate from any localized lesion, especially wound infection or as a result of intravenous drug abuse (Gonzalez *et al.,* 2005; Lowely, 1998).

3.4.3. Disease caused by exotoxins release

a. Gastroenteritis

Staphylococcal gastroenteritis is caused by ingestion of food contaminated with enterotoxin-producing *S. aureus*. Symptoms such as nausea, vomiting, and diarrheoa develop in short period around six hours (Madigan *et al.,* 2003).

b. Toxic shock syndrome

Toxic shock syndrome is characterized by fever, hypotension, and diffuse, macular, sunburn- like rash that goes on to desquamate, and involvement of three or more of the following organs: liver, kidney, gastrointestinal tract, central nervous system, muscle or brain. An outbreak of toxic shock syndrome occurred in the late 1970s among menstruating women (Strohl *et al.,* 2003). The use of hyper absorbent tampons was responsible for the disease causation. It stimulated toxin production by *S. aureus,* which were later absorbed to the vaginal mucosa. Tampons use not only cause of this syndrome, since men and non- menstruating women can also be infected (Gladwin, *et al,* 2003).

c. Scalded skin syndrome

It is characterized by fever, large bulled and an erythematous macular rash. Large areas of skin slough, serous fluid exudes, and electrolyte imbalance can occur. The pathogenic strain produces exfoliatin toxin, which establishes a localized infection and releases a diffusible toxin that exerts distant effects.

3.5 Methicillin Resistant *S. aureus* (MRSA)

Methicillin resistant *S. aureus* or MRSA are Staphylococcal bacteria that have become resistant to all β- lactam antibiotics including penicillin, ampicillin, amoxicillin, methicillin, oxacillin, dicloxacillin, cephalosporins, carbapenems (emipenem), and monobactams (e.g. aztreonam). MRSA is also known as oxacillin-resistant *Staphylococcus aureus* (ORSA) and multiple –resistant *Staphylococcus aureus* (Boyce, 2011).

Methicillin is a synthetic antibiotic related to penicillin with modified radicals designed to protect the penicillin ring against the bacterial enzyme penicillinase. MRSA strains were first identified in the early 1961s and they now have a worldwide distribution and have evolved resistant to multiple antibiotics (Lee *et al.*, 2005). MRSA causes the same variety of infections as staphylococcal strains that are sensitive to β–lactam antibiotics.

Originally, all *S. aureus* were sensitive to penicillin's but soon after penicillin was put in clinical use, *S. aureus* had developed the ability to inactivate the β– lactam ring of penicillin by producing plasmid encoded β-lactamase (de Lencastre *et al.*, 2007). Currently more than 95% of *S. aureus* are resistant to penicillin (Chambers, 1997). Many scientific literatures report the widespread of MRSA in hospitals and health care institutions. Because MRSA are often resistant to many antibiotics like Gentamicin, tetracycline, erythromycin etc, the infections caused by them are difficult to treat. In particular, the majority of MRSA strains are not susceptible to macrolides and aminoglycosides, because the genes ermA and aadD encoding resistance to these drugs are usually conserved within *mecDNA*, and located upstream and downstream, respectively of the *mecA* gene (Chambers, 1997).

MRSA related infections contribute substantial costs for antibiotic treatment, screening, disinfection procedures, isolation procedures and extended hospital stays (NNIS, 2000). MRSA strains colonize easily in the hospital particularly in immunodeficient patients, which can cause a variety of serious difficult to control infections including septicemia, pneumonia, endocarditis, meningitis, and postoperative intra-abdominal infection.

3.5.1 Mechanism of resistance

Unlike penicillin resistance that results from a plasmid encoded enzyme (β-lactamase), methicillin resistance is genetically and biochemically complex and mediated by staphylococcal cassette chromosome (*SCCmec*), a mobile genetic element encoding for an altered penicillin-binding protein (PBP2a, mecA) with decreased affinity to β-lactams (Gorden *et al.,* 2008). *SCC mec* probably evolved in penicillase-negative staphylococci which was acquired by *S. aureus* following inter – species horizontal gene transfer under the pressure of penicillin and now eight different *SCCmec* types are known on the basis of *mecA* class and new class continue to emerge (de Lencastre *et al.,* 2007; Gordon *et al.,* 2008).

β- lactam antibiotics act by inhibiting enzymes involved in assembling the bacterial cell wall. These enzymes are found in the membrane and catalyze the cross-linking reaction between the peptidoglycan polymers. This cross-linking gives the wall additional rigidity. Many of these enzymes co-valently bind β-lactam antibioticsat their active site and have been termed PBPS. Five PBPs have been described for susceptible strains of *S. aureus*. Methicillin resistance is associated with production of a unique PBP that is not present in susceptible staphylococci.

The *mecA* gene is a component of a large DNA fragment designated *mec DNA,* which is located at the specific site of the *S. aureus* chromosome and has been suggested to be transmitted from other bacterial species (Hiramastu *et a*l., 1996). The PBP 2a gene has been shown to be part of *mec* DNA. PBP 2a may have evolved from the fusion of the genes for β- lactamase and a PBP from a non staphylococcal source.

Expression of PBP 2a is controlled by two regulator genes on mec DNA, mecI and mecRI, located upstream of *mecA,* which encode *mecA* repressor protein and signal transducer protein, respectively. An MRSA carrying intact *mec I* and *mecRI* together with mecA has been called pre –MRSA, which is represented by protype *S. aureus* strain N315. Since intact *mecI* product strongly represses the expression of PBP2a, the pre –MRSA is apparently methicillin susceptible (Kuwahara *et a*l., 1996). Hence, it is hypothesized that removal of the repressor function for mecA is a pre-requisite for constitutive expression of methicillin resistance in *S. aureus* with *mec* DNA. Indeed, the deletion of mecI or point mutation in the *mecI* gene has been found in a number of methicillin resistant staphylococci isolates. In some strains, point mutations were

detected in *mecA* promoter region corresponding to a presumptive operator of *mecA*, i.e. the binding site of the repressor protein. Furthermore, genetic alteration on the chromosome which causes high Methicillin- resistance was presented as another mechanism of evolution of MRSA, although the details are not known.

Some strains of *S. aureus* over express β– lactamase and appear to be resistant to oxacillin and, rarely methicillin despite being *mecA* negative. They have slightly raised minimum inhibitory concentrations (MICs) and may thus be described as "minimally resistant". Other strains express modified PBPs (not PBP2a) and exhibit varying degrees of β– lactam antibiotic resistance (Chambers, 1997). *mecA* is the primary determinant of intrinsic methicillin resistance but additional genes are required for a high level resistance phenotypes, besides other environmental factors (Berger,1990). Genes *murF, fmtA-C, sigB, hrmA* and *hrmB, dlt, pbp2* and *cta2*, as well as the auxiliary genes alias factors essential for methicillin resistance (genes or mutants *femA-F, femR* and *femX*) have been described.

3.6 Hospital- associated MRSA (HA-MRSA)

HA-MRSA has been regarded as a nosocomial pathogen which characteristically colonizes or infects hospitalized individuals with predisposing risk factors (Deleo *et al.*, 2010). The strains usually harbour *SCCmec* type I, II and III and are multidrug resistant (Gorden and Lowly, 2008). In health facilities, there is selective advantage for MRSA survival as a result of antibiotic use (Lee *et al.*, 2011). In 2005, the proportion of MRSA among *S. aureus* hospital associated infections in the US was estimated at 53% with large local variations (Styres *et al.*, 2006) and now it is more. MRSA is highly transmissible among hospitalized patients and the infected or colonized patients as well as colonized employee are the main reservoir of the bacteria in the hospital worldwide (Tambic *et al*., 1997). A number of factors have been found to associated with a higher risk for nosocomial acquisition of MRSA: prolonged hospitalization, care in an intensive care unit, prolonged antimicrobial therapy using broad spectrum antibiotics, surgical procedures, having a surgical wound and intravenous (IV) line, severe underlying illness and close proximity to other ill patients who infected or colonized with MRSA (Boyce *et al.*, 2011). Certain patient population with complicating medical conditions such as diabetes mellitus, HIV infection, chronic dermatological diseases, indwelling catheters haemodialysis, intravenous drug use and post surgical wound infections are at increased risk of

serious MRSA infections (Lowly, 1998; Boyce *et al*., 2011).These patients also have increased rate of MRSA carriage.

Some strains associated with outbreaks are called epidemic MRSA (EMRSA) because once introduced they have a remarkable ability to spread within a hospital (Reboli *et al.*, 1989). Many studies have characterized EMRSA appear to be well adapted to the hospital environment, are established in several hospitals within a country or have spread internationally. Currently in the US, 50% of nosocomial *S. aureus* infections in ICU are due to MRSA (NNIS, 2002). ICU nursing staffs are found to harbor MRSA in the nose, nails and uniforms (Cookson *et al.*, 1989). On the other hand, some authors have suggested that contaminated environmental surfaces may serve as a reservoir for MRSA in hospitals.

3.6.1 Prevalence of MRSA in the hospitals

MRSA is a major cause of hospital acquired infections that are becoming increasingly difficult to combat because of emerging resistance to all current antibiotics classes (Enright *et al.*, 2002). In the following decades it quickly became an important cause of health care associated infections worldwide (Lee *et al*., 2011) and accounts for 40-70% of *S. aureus* infections in ICU (Olivera *et al*., 2002). However, infection rates varied from 1% to 80% and were dependent on location, emphasizing the need to be cognisant of the local microbial resistance patterns.

Not all countries and regions are equally affected by MRSA. MRSA has been found in hospitals of all sizes, but the highest rates are in hospitals with more than 500 beds. In American hospitals, MRSA ranged from 2.4% to 29% between 1975 and 1991 and reached up to 41% in 1998. According to some estimates as many as 80,000 patients worldwide a year acquires MRSA infections after they enter a hospital (CDC). A 2007 report in Emerging Infectious Diseases, a publication of the Centers for Disease Control and Prevention (CDC), estimated the number of MRSA infections in hospitals doubled nationwide, from approximately 127,000 in 1999 to 278,000 in 2005, while at the same time annual deaths increased from 11,000 to more than 17,000. Another study led by the CDC and published in 2007 estimated that MRSA would have been responsible for 94,360 serious infections and associated with 18,650 hospital stay-related deaths in the United States in 2005.These figures suggest that MRSA infections are responsible for more deaths in the U.S. each year than AIDS. In

England, the percentage increased from 1.5% to 13.2% between 1989 and 1995 (Write *et al.*, 1997) and now the mean figure is about 45 % (Boyce *et al.*, 2005). A study by Dickinson in England and Wales concluded an increase in trend of death due to MRSA infections (Dickinson *et al.*, 2002). UK levels of MRSA bloodstream infections are higher in Europe.

Denmark and Netherlands have maintained low infections rates about 1%, primarily due to an aggressive "search and destroy" policy to identify patients and health care workers colonized with MRSA whereas in southern Europe more than 25% of *S. aureus* isolate are MRSA (EARSS data, 2010). These differences are at least partly explained by varying antibiotic use and infection control practices (Mackenzie *et al.*, 2007).

Japan has one of the highest prevalence of MRSA in the world (Boyce *et al.*, 2005). Among *S. aureus* bloodstream isolates in 2001, nearly 70% were MRSA. In South Africa, the exact data on prevalence of MRSA is unavailable. A study done in KwaZulu-Natal province showed 26.9% of isolates were MRSA (Shittu *et al.*, 2006).

In India, the epidemiology of MRSA over different parts is not uniform. Some studies showed alarmingly high incidence of MRSA infections whereas some was reported low. The prevalence rate of 34.7%, 31.0%, 29.1% and 51.8% were reported from Assam, Tamil , Mangalore and Central India (Wagdha) respectively (Saikai *et al.*,2009, Rajadurapandi *et al.*,2006,Pai *et al.*, 2010,Sanjaya *et al.*, 2010) whereas , in some studies the rate is comparatively low. Another study in Nagpur the rate of MRSA (19.5%) was low compared to other studies (Tahankiwale *et al.*, 2002). However, in another study it was very high (80.8%) (Verma *et al.*, 2002). In India, the prevalence of MRSA was also variable within the same hospital at different time (Tiwari *et al.*, 2008).

Scanty data regarding MRSA was available from Nepal. In Nepal, the prevalence rate ranges from 11.7% to 54.9% (Lamichane *et al.*, 1999; Pokharel *et al.*, 1993; Rajbhandari *et al.*, 2002; Sapkota, 2005; Kumari *et al.*, 2008; Sajana *et al.*, 2010). Many studies done in Nepal about MRSA and their antibiotic susceptibility pattern suggest the gradual emergency of MRSA in the hospital.

3.7 Community-acquired MRSA (CA-MRSA)

Cases of MRSA documented in healthy community dwelling persons without established risk factors for MRSA acquisition is referred to as community acquired (CA-MRSA). In the 1990s, the first case of MRSA were seen in people who were not hospitalized in Australia, followed by USA and is now highly prevalent worldwide (Otter *et al.*, 2010). The CA-MRSA infects healthy individuals without any health care contact, harbor smaller and more mobile *SCCmec* types (IV and V), is usually Panton-Valentine leucocidin (PVL) positive, susceptible to non-β-lactam drugs and typically manifests as skin and soft tissue infections. Community acquired MRSA isolates have been associated with many of the same clinical presentations known to occur with traditional *S. aureus* infection. However, several outbreaks of epidemic furunculosis and severe invasive pediatric infections have been particularly noteworthy (Eguia *et al.*, 2003).

In past, acquisition of MRSA colonization or infection was generally considered to be restricted to the nosocomial setting. However, recent reports regarding CA-MRSA showed the increasing prevalence in multiple countries with substantial morbidity and mortality associated infections caused by them suggest that CA-MRSA will continue to develop into a challenging public health problem (Zetola *et al.*, 2005). MRSA is now found in up to 70 percent of people in the community who diagnosed with a Staph infection (Morgan *et al.*, 2006).

Although CA-MRSA has emerged as important cause of skin and soft tissues infections throughout the world in otherwise healthy adults without prior health care contact (Morgan *et al.*, 2006), they also cause life threatening conditions such as bacteremia, endocarditis and osteomyelitis, fatal sepsis and necrotizing pneumonia in less frequent(Miller *et al.*, 2005; Deleo *et al.*, 2010). Nearly CA-MRSA often carry virulence factors such as the exotoxin Panton –Valentine Leukocidin (PVL) that may play a role in fulminate infections such as severe necrotizing community onset pneumonia or necrotizing fasciitis (Van Belkum *et al.*, 2009, Hidron *et al*., 2009). CA-MRSA also cause toxic shock syndrome like illness in which PVL and other toxins like SEB and SEC have key role. The pathogenesis of these factor in CA-MRSA infections was first suggested in the report of serious CA-MRSA infections from the community that have been described in children in Minnesota and North

Dakota who have died from these infections in 1997, 1998 and 1999 (Herold, 1998; MWR, 1999). These serious invasive infections have characterized the course of most cases of necrotizing pneumonia and skin and soft tissue infections.

CA-MRSA has been arisen from diverse genetic backgrounds rather than the worldwide spread of single clone (Okuma *et al.*, 1994). But in United States, there are several outbreaks of CA-MRSA, most of these outbreaks have been associated with a single clone strain (MW2) (MMWR, 2005).

Following conditions have been contributed for acquisition of MRSA outside the hospital such as skin trauma (e.g..turf burns, cuts or sores), athletes, being overweight or obese, shaving body hair physical contact with a person who has a draining cut or sore or is a carrier of MRSA, sharing personal items or equipment that is not cleaned or laundered between users (towels or protective sports pads) and public bath use (Lee *et al.*, 2005; Boyce *et al.*, 2011). In addition, transmission via animals have been noted which may further complicate the epidemiology of these organisms (Manian, 2003; Strommenger, 2006; Juhas-Kaszanyitzsk *et al.*, 2007). Outbreaks of cellulites, abscesses and other skin and soft tissue infections (SSTs) caused by CA-MRSA have been occurred in a well defined population of young, healthy, children in day care centers, children and adult in reservations, athletes who played on a team, military personnel and men having sex with men (MSM) (Lakachaman *et al.*, 2010).

Recent investigations have revealed several characteristics that differentiate CA-MRSA from HA-MRSA strains. The antimicrobial patterns of community isolates are unique and differ from HA-MRSA. Community isolates tend to be susceptible to a variety of non-β lactam antibiotics, whereas HA-MRSA is typically resistant to multiple antibiotics (Dietrich *et al.*, 2004). Most of them are susceptible to trimethoprim-sulphamethoxazole, rifampicin, doxycycline, minocycline and fusidic acid. Reports based on genotype evidence have suggested that CA-MRSA is likely spreading within hospital as well as blurring the line between CA-MRSA and HA-MRSA infections (Papovinch *et al.*, 2008). It is hypothesized that the evolution of CA-MRSA is a recent event due to the acquisition of *mec* DNA by previously Methicillin susceptible strains that circulated in the community (Laskhamam *et al.*, 2010). Skin infections caused by *S. aureus* are generally believed to follow colonization of the skin and nares of the host.

3.7.1 Prevalence of MRSA in the community

Because of different definitions of community acquired infections used in the literature and limited number of population based studied that include molecular typing techniques, the reported prevalence of MRSA in the community varied widely (Zetola *et al.*, 2005). However, various studies had shown the increasing trend of CA-MRSA regardless of definitions.

In recent years, there are several reports of community associated MRSA (CA-MRSA) infections throughout the world including several outbreaks in United States. A study among inmates in California prisons from 1997 to 2000 found that 54% of all Staphylococcal isolates were community associated (Pan *et al.*, 2003). Several other groups have also reported outbreaks of MRSA infections occurring outside of health care facilities involving athletes, military personnel and inmates leading to the term community acquired MRSA (lindenmayer, 1998; MMWR, 2003; Zinderman, 2004; Kazakov, 2005; Nguyen, 2005; Aiello, 2006).

Many studies suggest the association between HIV and MRSA and majority of MRSA infections in HIV positive patients are being acquired in the community. An HIV cohort study at the Owen clinic, San Diego, from 2000 through 2003, 60% of MRSA isolates were determined to have been community associated by epidemiological characteristics (Mathews, 2005). In a recent retrospective study from 1993 to 2005 of CA-MRSA infections among 425 HIV patients, in San Diego, California, CA-MRSA infections increased with a 17 fold from 2003 through 2005 and 65% of all Staphylococcal SSTIs were due to CA-MRSA in this cohort (Crum-Cianflone, 2006). These studies and other suggest that poor HIV control, antibiotic usage and perhaps high sexual risk behaviors may place HIV patients at heightened risk for CA-MRSA infections. In addition, some studies suggest that use of public hot tubs or saunas increased the risk of CA-MRSA (Lee, 2005).

In Louisiana, it is estimated that about 5.0-20% of *S. aureus* in the community are MRSA (US Navy Guidelines, 2005). In United States, there is evidence that CA-MRSA strains might replace traditional hospital –associated strains, an assumption also supported by mathematical models (Seybold *et al.*, 2006, Patel *et al.*, 2008; Popovinch *et al.*, 2008; Agata *et al.*, 2009). A retrospective study from Chicago analyzed hospital onset MRSA bacteremias between 2000 and 2006 and found that CA-MRSA strains caused an increasing proportion of bacteremias (from 24-49%),

whereas total hospital onset MRSA bloodstream infection (BSI) rates were stable (Popovinch *et al.*, 2008). This phenomenon may not be limited to the US as demonstrated by a study from Greece that ated MRSA infections in 2 University hospitals in 2004 to 2005(Chinin *et al.*, 2008). In addition, livestock related MRSA, particularly transmission from pigs to humans, is a recently recognized problems and may increasingly be imported into the hospital (Vossa *et al.*, 2005; Koch *et al.*, 2009).

The past epidemic waves of *S. aureus* demonstrated that the epidemiology of this pathogen is complex and unpredictable (Schinefield *et al.*, 2009; Chambers *et al.*, 2009). Only time will replace the traditional hospital strains and what the clinical consequences of this replacement will be (Andie *et al.*, 2011). In Nepal, the epidemiological surveys of CA-MRSA as well as HA-MRSA were lacking, so there is no exact data on CA-MRSA.

3.8 Glycopeptide intermediate resistant *S. aureus* (GISA)

Vancomycin has been used to treat severe MRSA infections. In 1996, the first documented case of infection caused by a strain of *S. aureus* with intermediate levels of resistance to vancomycin (VISA; minimum inhibitory concentration MIC =8µg/ ml) was reported from Japan (CDC, 1996) and later from Europe and USA. Since these pathogens also been known to be resistant to teicoplanin, the term glycopeptides intermediate *S. aureus* (GISA) is more appropriate. The recent description of clinical strains highly resistant to glycopeptides following the acquisition of the *vanA* gene has increased fears that will soon be impossible to treat patients infected with these epidemic strains (CDC, 2002).

The source of the resistance seems to be a transfer of resistance genes, particularly Van A from VRE. Since 1989, a rapid increase in the incidence of infection and colonization with VRE has been reported by United States (US) hospitals. This increase poses important problems such as the possibility that the vancomycin-resistant genes present in VRE can be transferred to other gram positive bacteria (Nobel, 1992). It is of utmost importance to prevent the dissemination of VRSA.

The target site for vancomycin is the binding with cell wall precursors. The bacterial peptidoglycan is synthesized in series of steps that take place both inside and outside of the cell membrane. Just outside the membrane lie the transglycosylases, enzymes that put together GlcNAc subunits to form the glycan chains and the transpeptidases,

which perform the peptide cross- linking between these chains. While penicillin affects the active sites of the transpeptidase enzymes themselves, vancomycin binds to peptide substrate and prevents it from binding to the enzyme's active site. (Walsh *et al.*, 2001)

To accurately detect *Staphylococci* with reduced susceptibility to vancomycin, antimicrobial susceptibility should be determined with a quantitative method (broth dilution, agar dilution, or agar gradient diffusion) using a full 24 hours of incubation at 35˚C. Strains of *Staphylococci* with vancomycin MICs of 8µg/ml cannot be detected using disk diffusion procedures.

Various measures should be done to avoid the emergence of VRSA. The Hospital Infection Control Practices Advisory Committee (HICPAC) for preventing and controlling the spread of vancomycin resistance, with a special focus on VRE was issued in 1995 (HICPAC, 1995).

3.9 Nasal carriage of *S. aureus* and MRSA

S. aureus is an endogenous microorganism colonizing the nasal cavities, skin, gastrointestinal, anuses, finger tips and vaginal vaults of healthy women. The anterior nares are a common colonization site because its primary habitat is moist squamous epithelium of the anterior nares and can lead to dissemination of other body sites (Sanford *et al.,* 1994). Colonization may be either transient or persistent (Sanford *et al.,* 1994). *S. aureus* is the most virulent and is associated with a wide spectrum of diseases including skin and soft tissues, systemic infections and exotoxin related diseases (Shittu *et al.*, 2006). About 20% of the population is always colonized with *S. aureus*, 60% are intermittent carriers and 20% never carry the organism (Paecock *et al.*, 2001; Von Eiff *et al.*, 2001) and a prevalence of 25% was found among hospital personnel (John *et al.*, 1993). *S. aureus* nasal carrier rate of 20-50% can be found in normal adults (Hizeh *et al.*, 1997). Approximately 85% of carriers can be identified with a swab taken from the anterior nares, higher carrier rates are seen in injection drug users, persons with insulin dependent diabetes, patients with dermatological conditions and patients with long term indwelling intravascular catheters (Haddadin *et al.*, 2002).

Carriage of *S. aureus* in the nose appears to play a key role in the epidemiology and pathogenesis of infection (Verghese *et al.*, 1999). Healthy individuals have a slight risk of invasive infection caused by *S. aureus* during their carrier state but they can be carriers of the organism (Mainous *et al.*, 2006). Zimakoff, 1996 reported that staphylococcal infection occurred significantly more frequently among carriers and more than half of the patients were infected by the same or possibly the same strain as they carried in the nose or on the skin.

MRSA now represents global problem. Ever its isolation, MRSA has emerged as one of the commonest causes of hospital acquired infection and continues to remain as important factor contributing to failure of management. Transmission of isolates of epidemic MRSA has traditionally been associated with hospital facilities (Thompson *et al.*, 1982). In recent years, dissemination of MRSA has been increasingly recognized in other health care settings including primary health care (Kollef *et al.*, 2006). Nasal carriage rates of MRSA have been reported to be 0.8 to 3.0% among adults in the community elsewhere in the world (Abudu *et al.*, 2001; Grundman, 2002; Jernigan *et al.*, 2003). Hospital personnel tend to have higher colonization rates than general population (Frank *et al.*, 1990). Among health care workers in hospital setting, it ranges from 6.0 to 17.8% (Mulqueen, *et al.*, 2007; Eveillard *et al.*, 2004; Cesur *et al.*, 2004). Health care personnel may become transient or persistent MRSA carriers while working in the hospitals in which MRSA is endemic (Mitsuda *et al.*, 1999). Risks factors for nasal carriage of MRSA are poorly understood. The possibility that exposure to hospital environments may be one of these (EI-Jalil *et al.*, 2008). Similarly risks factors for CA-MRSA carriage are not understood and some studies have suggested that recent anti-microbial drug use plays a role in CA-MRSA colonization (Bagget *et al.*, 2004, Ellis *et al.*, 2004). Usually within the hospital, colonized and infected patients are common reservoirs of MRSA transmission but nasal carriage of MRSA among hospital personnel and visitors may become the important sources for MRSA infections to patients as well as their own families and may harbor organism for many months. This group of people may create a problem especially in ICU, post operative wards and surgical wards increasing the vulnerability of infections with MRSA further complicating the treatment and recovery. Persistent or transient carriers among hospital personnel and visitors/patient attendants may also disseminate organism into the hospital environment contributing

to disseminate organism. The increase emergence of community acquired MRSA further increases the chances of infection of the patients with community acquired MRSA, following transmission from visitors/patient attendants. Moreover, the patients are also susceptible to staphylococcal infection from endogenous source during hospital stay due to own nasal carriage. *S. aureus* is usually transmitted by direct skin to skin contact with a colonized or infected individual and occasionally via fomites and transmission may be endogenous or endogenous. Five factors or "Cs" have been implicated in MRSA outbreaks-contact; lack of cleanliness; compromised skin integrity; contaminated objects; and crowding living conditions (Deleo *et a*l., 2010; Otter *et al.,* 2010). Among patients, colonization rate is often higher in newborns, diabetic patients, those who are frequently hospitalized, patients with skin diseases, those on dialysis or on advancing age and AIDS patients.

In context of Nepal, there were not much more studies regarding the nasal carriage rate of MRSA among health personnel. Early, the MRSA among patients, staff and hospital environment of a tertiary medical care centre (teaching hospital) in Kathmandu, Nepal has reported to be 29.1% (Rai *et al.,* 1990). This study signifies the role of hospitals ambience in increasing dispersion of MRSA in the hospital. In another study, Rai and Pant from Nepal reported nasal carriage rate of 43.8% among health personnel of medical college teaching hospital, Kathmandu (Pant *et al.*, 2007). Sapkota had reported 39.51% nasal carriage rate on health personnel of Bir hospital but none of them were colonized with MRSA. Recently, a study done in National Medical College Teaching Hospital, Birgunj, Nepal found to be 8% MRSA carriage rate of *S. aureus* among patients, visitors/patient attendants and health personnel. This study also showed highest nasal colonization rate (25%) and high MRSA prevalent rate (10%) among health personnel followed by visitors/patient attendants (8.2%).

A study on nasal carriage rate of *S. aureus* for both doctors and nurses was 15.8% and combined throat carriage was 16.6% (Hollis *et al.,* 2003). But none of the strains were found to be MRSA. A study on staphylococcal strains between parents and the off springs show the same type of strains suggesting the familial cross contamination (Amir, 2002). MRSA nasal carriage rates of hospital staff and outpatient control group were found to be 6.0% and 2.6% respectively in a hospital in Turkey (Dimitrov *et al.,* 2003). Similarly in an Iranian hospital, 19.7% hospital personnel were nasal carriers of *S. aureus*, of whom 8% were MRSA colonized and 96.2% were persistent

carriers (Armin *et al*., 2007). Some studies also showed relatively higher nasal carriage rate of *S. aureus* and MRSA among health workers which indicates the dispersion of MRSA among healthcare personnel is global trend. The study carried out in children hospital, Pakistan reported the prevalence of *S. aureus* carriage was 48%, out of these 29% were MRSA (Farzana *et al.*, 2008). A case study done on mother and child with recurrent infection and breast abscess found to harbor MRSA. In addition, family members were also infected with MRSA. Some cases of recurrent infections may be related to nasal carriage in mother or infant (Akoua *et al.*, 2004).

3.9.1 Assessment of Nasal Colonization (US Navy guidelines, 2005)

If nasal screening is conducted, culture should be obtained using the following protocol:

1. Remove swab collection device from its packaging material.
2. Confirm that swab collection device has been pre- labeled with appropriate identifiers.
3. Moisten swab with sterile saline.
4. Insert moistened swab approximately 2cm into one nare.
5. Rotate the swab against the anterior nasal mucosa for 3 seconds.
6. Using the same swab, repeat for the other nares.
7. Return swab to transport sleeve

3.10 Sources and transmission of MRSA

MRSA is primarily transmitted from person to person by direct contact, usually from the hands of an infected or colonized individual. It can also be transmitted by sharing towels, personal hygiene items, athletic equipment, clothes, and public used bath and used equipments. Environmental surfaces are not thought to play major role in transmission except in special populations, such as patients in burn units or intensive care units. Droplet-borne transmission is less common but may be important in patients with tracheostomies who are unable to control their secretions. Person with pneumonia in close contact with others can transmit MRSA by coughing up large droplets of infectious particles. MRSA can be found on the skin, in the nose, and in blood and urine. Colonized domestic pets such as horses, pigs can transmit MRSA to people in the community (Voss *et al.*, 2005).

3.11 Infection Control for MRSA

Rapid assessment of clinical specimens for the presence of MRSA is an important part of the infection control measures to control the spread of MRSA and, thus to decrease hospitalization costs. The use of molecular techniques is a valuable resource for the understanding of the hospital epidemiology of these infections and is of help in the application of efficient control measures. In the hospital, MRSA transmission can be limited by decontamination of reservoirs including decontamination of hands, hospital environment, decolonization of hospitalized patients, hospital staffs and staff education regarding MRSA transmission (US Navy guidelines, 2005).

3.12 Resistance and treatment

The MRSA has markedly influenced the empirical therapy for suspected staphylococcal infections. Most β-lactam antibiotics are ineffective against both HA and CA-MRSA. The CA-MRSA strains are often susceptible to non-β-lactam drugs. Cutaneous abscesses need surgical incision and drainage irrespective of the antibiotic susceptibility pattern and in most cases antibiotics provide little or no benefit, and are not recommended except for patients of advanced age or those with severe disease symptoms of systemic illness, immunosupression or abscess in an area that is difficult to drain (Deleo *et al.,* 2010). The CA-MRSA associated skin and soft-tissue infections are often treated with using oral antibiotics including doxycycline, minocycline, clindamycin, trimethoprim-sulfamethoxazole (TMP-SMX), rifampicin and fusidic acid (Nathwani *et al.,* 2008). However resistance to these drugs seems to be increasing. Fluoroquinolones are usually not recommended for MRSA treatments because therapy with these agents frequently results in selection of resistant mutants, and consequent relapse and treatment failure (Gortwiz *et al.,* 2011). Vancomycin remains the first-line intravenous drug for severe CA-MRSA and HA-MRSA infections. However, high rates of microbiological and clinical failure, nephrotoxicity and emergence of non susceptible strains have limited effectiveness of this drug (Chambers and Deleo, 2009). Linezoid exhibits an excellent antistaphylococcal activity, comparable to that of Vancomycin and can be administered orally, and has been approved by Food and Drug Administrator (FDA) for the treatment of serious MRSA infections. In addition, daptomycin and tigecycline have been approved by FDA for MRSA management (Chamber and Deleo, 2009). Similarly other drugs such as glycopeptides (televancin, dalbavancin and oritavancin) and two cephalosporins

(ceftobiprole and ceftaroline) are also effective against MRSA. However, choice of antibiotics in the treatment of MRSA infection is determined by the susceptibility of the organism and the severity of the infection (Chambers and Deleo, 2009; Ray *et al*., 2011).

CHAPTER-IV

4. MATERIALS AND METHODS

4.1 Materials

A complete list of materials, equipments, chemicals, reagent, antibiotics and media used for this study are listed in Appendix I.

This cross-sectional study was carried out in Microbiology Laboratory of Pathology Department, Shree Birendra Hospital, a central army hospital providing service to Nepalese army personnel and their families, Chhauni, Kathmandu. The different clinical samples were analysed from Out-patients as well as In–patients of the hospital. Nasal swabs were taken from the staffs and patient visitors of different wards and laboratory of the hospital, which were then microbiologically analyzed in the microbiology department of the hospital. The study was carried out from October, 2010 to April, 2011.

4.2 Sample size and types

Altogether 142 *S. aureus* were isolated from various clinical samples requested by medical officer for laboratory investigation were analyzed for Methicillin resistance. During study a total of 2012 clinical samples were analyzed. The clinical samples for study were selected randomly from the clinical samples submitted at laboratory. About 14-15 samples were analyzed per day. Altogether 200 nasal swabs, 100 from staffs and 100 from visitors of different wards. The specimens were cultured for the isolation of *S. aureus* and Methicillin resistance was analyzed thereafter.

4.3 Questionnaire sheet and sample collection protocol

A standard questionnaire sheet was made to record age, sex, education, and occupation and service years for nasal screening of MRSA. A simple nasal swab taking protocol was also developed to guide staffs who want to collect nasal swab by them. The complete questions and protocol is given in appendix VIII.

4.4 Sample collection

a. Clinical samples

Pus and swab from wound, eye, ear, throat, and urethra was collected aseptically by experienced medical officer, nurse or laboratory technicians. Blood and body fluids like synovial, peritoneal fluid, CSF were collected aseptically by skilled medical personnel. Semen and urine were collected by the patients as per instruction form to avoid contamination.

b. Nasal swabs

For nasal swab, sterile cotton swab dipped in sterile physiological saline was used for the collection of samples from anterior nares. The same swab was used to sample both nostrils. The swab was introduced into first nostril 1-2 cm inside, which was rotated 2-3 times with gentle pressure for 3-5 seconds and the swab was transferred to the second nostril and the process was repeated. After collecting the sample, the swab was kept immediately in the sterile test tube or sterile swab coverings and was plugged with cotton.

The samples were labeled with patient's identification number and other required information. In case of delay processing, the samples were usually stored at 4℃ in the refrigerator.

4.4.1 Sample transportation

The collected nasal swabs were put into sterile cotton capped test tube or into sterile swab coverings, and transported to the laboratory immediately for inoculation into culture media.

4.4.2 Sample processing

All the samples selected for the study were processed using standard protocols. After receiving and labeling the samples, they were inoculated into MacConkey agar, CLED agar, Chocolate agar and Blood agar based on the nature of samples. In case of mixed colonies, pure culture was isolated and streaked into Mannitol salt agar. For nasal MRSA screening, the swab was inoculated into Mannitol salt agar directly.

4.5 Bacteriological identification of *S. aureus*

The inoculated culture plates were incubated at 37˚C for 24 hours. *S. aureus* colonies were identified using established microbiological methods, which include colony morphology, Gram's stain reaction and biochemical tests. Isolates that were Gram-positive cocci, catalase positive, yellow colonies on MSA, VP-positive and coagulase positive were considered as *S. aureus* in this study.

4.5.1 Sub- culture on Nutrient agar (NA)

Mannitol fermenting colonies from MSA were sub cultured on nutrient agar (NA) at 37˚C for 24 hours for further processing. Colony having round, convex, opaque, smooth-glistening surface with colony diameter 2-3 mm were indicative of Staphylococci. Most staphylococci produced soft butyrous colony with golden yellow pigment. For further confirmation of *S. aureus*, various tests like Gram staining, Catalase test, Slide and Tube Coagulase test, VP test and O/F test were performed from isolated colonies. Standard protocol provided by Chessbrough, 2002; Collee *et al.,* 1996 and Forbes *et al.*, 1998 was followed for confirmatory identification of *S. aureus*.

The procedures for Gram's staining, Catalase test, Coagulase test, VP test and O/F test for the confirmatory identification of *S. aureus* are mentioned in the Appendix IV and V.

4.6 Antibiotic susceptibility testing

All *S. aureus* isolated from clinical samples and nasal screening process were subjected to in-vitro antimicrobial susceptibility test by Kirby-Bauer disc diffusion method as recommended by CLSI (formerly NCCLS). In this study the antibiotics used were Amoxycillin, Ciprofloxacin, Cloxacillin, Erythromycin, Gentamicin, Methicillin, Oxacillin, Penicillin and Co-trimoxazole. The MRSA strains were identified by testing with Oxacillin and resistant strains were also screened against Methicillin disc, those strains resistant to both discs were taken as MRSA strains in this study. Only MRSA strains were further tested with Vancomycin. Standardized overnight culture of each isolate was inoculated in nutrient broth at 37˚C for 4 hours to obtain turbidity equivalent to the density of Mac-Farlands Nephelometer standard number 0.5. Sterile cotton swab dipped into the culture tube of the organism; excess

inoculums removed by pressing and rotating the swab firmly against the tube wall. Swabbing was performed uniformly all over the surface of the sterile MHA plate and allowed to dry while the lid was in plate. Standard antibiotic discs were aseptically placed at reasonable equidistance on the inoculated MHA plate with the help of forcep and allowed to stand 30 minutes. The plates were then incubated at 35˚C for 24 hours. After incubation, the diameter of the zone of inhibition of each disc was measured, recorded and the isolates were classified as "resistant", "intermediate" and "sensitive" based on the standard interpretative chart updated according to the current NCCLS standard (Hi Media Company, 2010). The procedure of Kirby-Bauer disc diffusion method and zone interpretative chart are mentioned in the appendices VI and VII.

Multi-drug resistant (MDR) strains are those which are resistance to two or more than two of the following antistaphylococcal drug classes; β-lactams, aminoglycoside, glycopeptides, fluoroquinolones and synthetic antimicrobial agent (Cotrimoxazole).

4.7 Quality control for tests

In this study, quality and accuracy of all test was maintained by following standard procedures of collection, isolation and identification. For identification and standardization of the Kirby-Bauer test, standard culture of *S. aureus* ATCC 25923 was used as a reference strain.

For quality control, media, antibiotics and reagents were prepared, stored and utilized as recommended by the manufacturing company. Antibiotics discs were stored at refrigerator temperature. For each batch of test, a positive and negative known culture was used for colour reaction, biochemical test and antibiotic sensitivity test.

4.8 Data analysis

Most of the patient's data received from laboratory investigation slip documented by medical officer. A questionnaire was made for collecting epidemiological information for nasal screening.

All raw data obtained from laboratory investigation were tabulated and presented in defined tables to explore the major findings. The data were statistically analyzed by Chi-square (χ^2) test at 5 % level of significance by entering the data in the computer based PASW (Predictive Analytical Soft Ware), version 18.0, the premier vendor for

(Statistical Package for the Social Sciences) program. A P-value of less than or equal to 0.05 is considered to be statistically significant ($P \leq 0.05$).

CHAPTER-V

5. RESULTS

5.1 Clinical samples

5.1.1 Distribution of *S. aureus* in clinical samples

In this study all the clinical samples taken into consideration where the growth of *S. aureus* was possible. Various clinical samples from outpatients as well as inpatients were taken into daily record for analysis.

The higher *S. aureus* was isolated from pus and wound samples, 35.1% (n=113) including 64.6% (n=73) from outpatient and 35.4% (n=40) from inpatients. The pus sample in this study included was pus culture, wound swab, ear swab, throat swabs, high vaginal swab (HVS) and fine needle culture (FNC) material. Similarly, the percent isolation of *S. aureus* was 6.1%, 5.8% and 5.4% in sputum, invasive devices and genital specimens respectively. In case of invasive devices, all isolates were from inpatients. In case of body fluids, urine and blood, the *S. aureus* isolation was less than 2%. Body fluids included in this study were pleural fluid, ascetic fluid, synovial fluid, CSF and pericardial fluid.

Table1: Distribution of *S. aureus* in clinical samples

Clinical samples	Total samples	Total *S. aureus* isolates	Percent
Pus and wound	322	113	35.1
Urine	1025	10	0.9
Blood	424	3	0.7
Sputum	114	7	6.1
Body fluids	55	1	1.9
Genital specimen	37	2	5.4
Invasive devices	34	2	5.9

5.1.2 Distribution of *S. aureus* among outpatients and inpatients

In outpatients the prevalence of *S. aureus* was found to be higher in the age group >50 yrs, 6.9% (n=28). In the same age group the prevalence of *S. aureus* was higher, 10.3% (n=24) in case of inpatients. The prevalence of *S. aureus* was higher in inpatients (8.5%) as compared to that of outpatients (6.1%). In both types of patients, the isolation was lower in the age group <15 yrs.

Table 2: Distribution of *S. aureus* among outpatients and inpatients

Age Groups (Yrs)	Outpatients		Inpatients	
	Total Sample	Positive Isolates (%)	Total Sample	Positive Isolates (%)
<15	101	4 (4.0)	89	2 (2.2)
16-49	881	53 (6.0)	301	27 (9.0)
>50	407	28 (6.9)	233	24 (10.3)
Total	1389	85 (6.1)	623	53 (8.5)

5.1.3 Antibiotic susceptibility pattern of *S. aureus* isolates

All the isolated strains of *S. aureus* from different clinical samples were tested with specific antibiotics by using Kirby Bauer disc diffusion method. The following table depicts sensitivity and resistance pattern shown by *S. aureus* isolates towards different antibiotics.

A total of 138 *S. aureus* were isolated from clinical samples. It was found that 18.1% (25/138*) S. aureus* were Methicillin-resistant (MRSA). Additional antibiotics were also used to find out the susceptibility pattern of the positive isolates.

From the data observed, it was observed that the most sensitive drug for *S. aureus* strains were Gentamicin (85.5%) followed by Erythromycin (73.2%), Ciprofloxacin (68.8%) and Amoxycillin (67.4%). The isolated strains showed highest resistant to Penicillin (92.8%) followed by Cloxacillin (63.8%), Cotrimoxazole (38.4%), Amoxycillin (32.6%) and Ciprofloxacin (31.2%).

Table 3: Antibiotic susceptibility pattern of *S. aureus* isolates

Antibiotics	Total *S. aureus* tested	Sensitive		Resistant	
		Number	Percent	Number	Percent
Oxacillin(1mcg)	138	113	81.9	25	18.1
Methicillin(5mcg)	138	113	81.9	25	18.1
Amoxycillin(30mcg)	138	93	67.4	45	32.6
Cloxacillin(10mcg)	138	50	36.2	88	63.8
Erythromycin(15mcg)	138	101	73.2	37	26.8
Gentamicin(10mcg)	138	118	85.5	20	14.5
Cotrimoxazole(25mcg)	138	85	61.6	53	38.4
Ciprofloxacin(30mcg)	138	95	68.8	43	31.2
Penicillin(10 units)	138	10	7.2	128	92.8
Vancomycin(30mcg)	25	25	100.0	0	0.0

5.1.4 Comparison of *S. aureus* isolates and MRSA strains isolated from different clinical samples

In this study, all the clinical samples requested for culture and sensitivity were analyzed for the growth of *S. aureus*. Higher number of *S. aureus* was isolated from Pus and Wound samples (113) followed by Urine (10), Sputum (7), Blood (3), Genital specimen (2), Invasive devices (2) and body fluids (1). Among a total of pus and wound samples processed, only 113 *S. aureus* was isolated. Similarly among 1690 other samples, only 25 *S. aureus* was isolated. The chances of finding of *S. aureus* from pus and wound sample was statistically significant (P<0.05).

The highest prevalence of MRSA was isolated from urine 30.0% (3/10) followed by sputum 28.6 % (2/7) and pus samples 17.7% (20/113). The association of MRSA in pus and wound with reference to MRSA in other sample was statistically insignificant (P=0.77).

Table 4: Distribution of *S. aureus* and MRSA in different clinical samples

Clinical samples	Total samples	No. and % of *S. aureus*	p-value	No. and % of MRSA	p-value
Pus and wound	322	113 (35.1)	P <0.05	20(17.7)	P>0.05
Urine	1025	10 (0.96)		3(30.0)	
Blood	430	3 (0.69)		0(0.0)	
Sputum	103	7 (6.8)		2(28.6)	
Body fluids	55	1 (1.8)		0(0.0)	
Genital specimen	40	2 (5.0)		0(0.0)	
Invasive devices	34	2 (4.3)		0(0.0)	

5.1.5 Distribution of MRSA in outpatients and inpatients

In this study total MRSA isolated was 25 out of 138 *S. aureus* isolates. This accounted 18.1% MRSA of the total isolates. Among 85 *S. aureus* isolates from outpatients, 11.9% (n=11) were MRSA whereas among 53 *S. aureus* isolated from inpatients, only 26.4 % (n=14) were MRSA. Among total MRSA isolates, 56% (n =14) were from inpatients whereas 44% (n =11) were from outpatients. The association between the MRSA occurrence in inpatients patient was statistically

significant (P =0.046). This data clearly showed that the chances of finding MRSA in admitted patient is higher as compared to outpatients.

Table 5: MRSA in outpatient and inpatients

MRSA	Type of patients			p-value
	Outpatients (%)	Inpatients (%)	Total	
Positive	11 (12.9)	14 (26.4)	25	p<0.05
Negative	74 (87.1)	39 (73.6)	113	
Total	85	53	138	

The isolated *S. aureus* were categorized into different age groups on the basis of outpatient and Inpatients. The highest prevalence of *S. aureus* (n=53) was isolated from outpatient of age group 16-49 yrs. This age group accounted 13.2 % (7/53) MRSA isolates while age group >50 yrs accounted 14.3 % (4/28). Similarly among inpatients, age group 16-49 yrs accounted 27 isolates among which 33.3% (9/27) were MRSA while age group >60 yrs accounted 20.8% (5/24). However, the distribution of *S. aureus* was not homogenous among the different age groups. In both groups of patient, no MRSA was found in the age group <15 yrs and there was also low number of *S. aureus* presence in this age group.

Table 6: MRSA in outpatient and inpatients of different age groups

Age group (yrs)	Outpatients		Inpatients	
	No. of *S. aureus*	No. and % of MRSA	No. of *S. aureus*	No. and % of MRSA
<15	4	0(0.0%)	2	0(0.0%)
16-49	53	7(13.2%)	27	9(33.3%)
>50	28	4(14.3%)	24	5(20.8%)
Total	85	11(12.9%)	53	14(26.4%)

5.1.6 Antibiotic susceptibility pattern shown by MSSA and MRSA

The isolated strains of *S. aureus* were categorized into two groups: Methicillin Sensitive *S. aureus* (MSSA) and Methicillin Resistant *S. aureus* (MRSA). Both the group of *S. aureus* showed marked variation in sensitivity pattern to common antibiotics.

With regard to MSSA, the most effective antibiotic was Gentamicin 93.8% (106/113) followed by Amoxycillin 82.3% (93/113), Erythromycin 81.4% (92/113),

Ciprofloxacin 73.5% (83/113), Cotrimoxazole 68.1% (77/113), Cloxacillin 42.5% (48/113) and the least effective drug was penicillin 8.8% (10/113).

With regard to MRSA, the most effective antibiotic was Vancomycin 100.0% (25/25) followed by Gentamicin 48.0 % (12/25) and Ciprofloxacin 48.0% (12/25). The drug resistance of MRSA was highest with Penicillin, Amoxycillin (100.0%) and Cloxacillin 100.0%, Cotrimoxazole 68.0%, Erythromycin 64.0%, Gentamicin and Ciprofloxacin 52.0%.

Table 7: Antibiotic susceptibility pattern of MSSA and MRSA

Antibiotics	MSSA (n=113)		MRSA (n=25)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Amoxicillin	93(82.3)	20(17.7)	0(0.0)	25(100.0)
Cloxacillin	48(42.5)	65(57.5)	0(0.0)	25(100.0)
Erythromycin	92(81.4)	21(18.6)	9(36.0)	16(64.0)
Gentamicin	106(93.8)	7(6.2)	12(48.0)	13(52.0)
Cotrimoxazole	77(68.1)	36(31.9)	8(32.0)	17(68.0)
Ciprofloxacin	83(73.5)	30(26.5)	12(48.0)	13(52.0)
Penicillin	10(8.8)	103(91.2)	0(0.0)	25(100.0)
Vancomycin	Not tested	Not tested	25(100.0)	0(0.0)

5.1.7 Multi- drug resistant (MDR) *S. aureus* isolates

Multi- drug resistant (MDR) *S. aureus* were identified by their antibiotic sensitivity pattern. The *S. aureus* resistant to two or more than two classes of commonly prescribed antibiotics were considered as MDR. Among 138 *S. aureus* isolates, only 91(65.9%) were MDR. Out of the 113 Methicillin sensitive *S. aureus (*MSSA), only 68 (60.2%) were MDR. But in case of total MRSA isolates (n=25), 92.0% (n=23) were MDR. The remaining two isolates of MRSA which isolated from out patient's pus sample were only resistant towards beta- lactam antibiotics used during study.

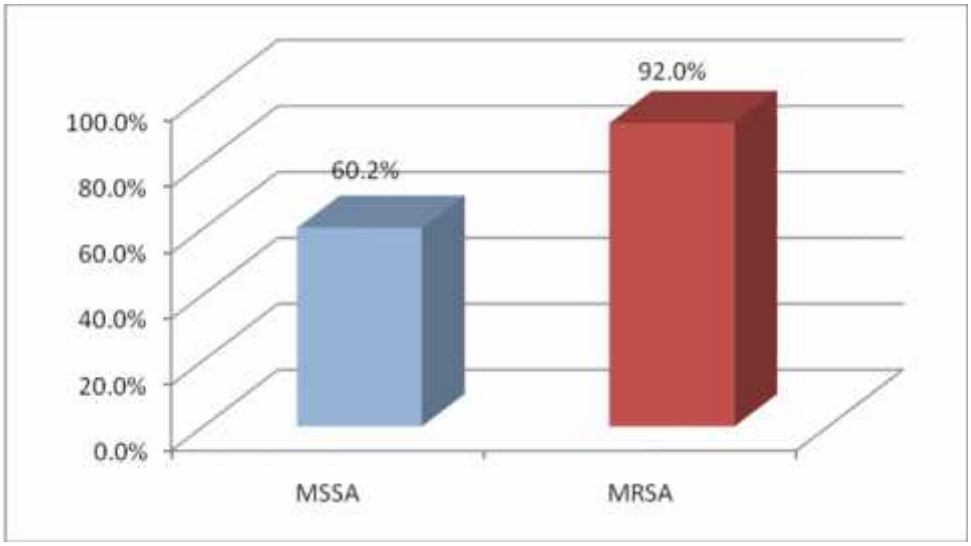

Figure 2: MDR strains among MSSA and MRSA strains

5.2 Nasal Swabs

Nasal swabs from health personnel

A total of 100 nasal swabs were taken from hospital personnel that included male health staffs, female health staffs, resident doctors, intern doctors, volunteer nurses, laboratory personnel and sweepers of different wards where admitted patients were presented. In this study nasal carrier rate of *S. aureus* was found to be 25.0% (n =25).

5.2.1 Sex wise nasal carriage of *S. aureus* among health personnel

Out of 57 nasal swab samples from male health personnel, 28.1% (n=16) *S. aureus* were isolated. Similarly, a total of 43 nasal samples from female health personnel, 20.9% (n=9), *S. aureus* was isolated. Statistically, there is no relationship of *S. aureus* nasal carrier rate between male and female (P=0.414).

Table 8: Sex- wise nasal carrier rate of *S. aureus* among health personnel

Sex	No. of samples taken	Carrier of *S. aureus* (%)	p- value
Male	57	16 (28.1)	P >0.05
Female	43	9 (20.9)	
Total	100	25 (25.0)	

5.2.2 Age wise nasal carrier of *S. aureus*

In male, the prevalence of nasal carrier of *S. aureus* was found to be highest in the age group of 21-39 yrs (32.6 %) followed by >40 yrs (9.1 %). No *S. aureus* was isolated in the age group >20 yrs.

In female, the higher prevalence of *S. aureus* nasal carrier was found to be in the age group >40 yrs. This percent was followed by the age group >20 yrs (25.0%). In female, the age group 21-40 yrs shows the low prevalence as compared to that of male.

Table 9: Age group wise nasal carrier rate of *S. aureus* among health personnel

Age group (yrs)	Male		Female	
	Total sample	No. and % of positive isolates	Total sample	No. and % of positive isolates
< 20	0	0(0.0)	12	3(25.0)
21-39	46	15(32.6)	25	3(12.0)
> 40	11	1(9.1)	6	3(50.0)
Total	57	16(28.1)	43	9 (20.9)

5.2.3 Occupation –wise nasal carrier of *S. aureus*

The occupation was recorded in the questionnaire set during a short interview while collecting nasal swabs. The involved hospital personnel related to health care facility were further classified in to female health workers (nursing staffs), resident doctors, intern doctors, volunteer nurses, laboratory personnel and sweepers. The maximum samples were collected from male health workers (n=40) followed by female health workers (n=22) and volunteers nurses (n=12).

Among health personnel, highest percent nasal carrier rate of *S. aureus* was found in male health workers 30.0% (n=12) followed by laboratory personnel 25.0% (n=3), sweepers 25.0% (n=1), female health workers 22.7% (n=5), intern doctors 20.0% (n=1) and resident doctors 20.0 % (n=1). The association of nasal carrier of *S. aureus* among different health occupational group was not statistically significant (P = 0.978).

Table 10: Occupation- wise nasal carrier of *S. aureus* among health personnel

Occupational groups	No. of sample taken	No. and % of *S. aureus*	p-value
Female health staffs	22	5 (22.7)	P >0.05
Male health staffs	40	12(30.0)	
Resident doctors	5	1(20.0)	
Intern doctors	5	1(20.0)	
Volunteer nurses	12	2(16.7)	
Laboratory personnel	12	3(25.0)	
Sweepers	4	1(25.0)	
Total	100	25(25.0)	

5.2.4 Ward wise distribution of *S. aureus*

The nasal swab samples were collected from different wards where admitted patients were presents. The wards included in this study were surgical ward (S ward), Intensive Care Unit (ICU), Intensive Trauma Care Unit (ITCU), Medical ward (M ward), New Family Ward (NFW), Gyno ward, Surgical Officer Cabinet (SOC) and Post Operative ward (POP). The highest nasal carrier rate of *S. aureus* among health personnel was from Gyno ward (66.7%) followed by ITCU (33.3%), Medical ward (28.0%), Surgical ward (26.3%), SOC (25.0%), Pediatric ward (25.0%), Pathology (23.1%), POP (20.0%) and ICU (18.2%). No *S. aureus* was isolated from NFW.

Table 11: Ward wise distribution of *S. aureus* among health personnel

Wards of hospital	Total swabs taken	Total *S. aureus* isolates (%)
S ward	19	5 (26.3)
ICU	11	2(18.2)
ITCU	3	1 (33.3)
M ward	25	7 (28.0)
NFW	4	0 (0.0)
Gyno ward	3	2 (66.7)
SOC	4	1(25.0)
POP	8	2 (20.0)
Pathology	13	3 (23.1)
Pediatric	8	2 (25.0)
Total	100	25 (25.0)

5.2.5 Rank wise distribution of *S. aureus* among health personnel

The rank was recorded in the questionnaire set during a short interview while collecting nasal swabs. The involved hospital personnel related to health care facility were further classified into doctors, Intern doctors, Technical Major, Captain, Subedar, Haldhar, Nayak, Seepy, Volunteer nurses and Sweepers based on ranking system made in the army hospital.

Among health personnel, highest percent nasal carriage rate of S. aureus was found in Technical Major 50.0% (1/2) followed by Technical Seepy 33.3% (6/18), Sweepers 25.0% (1/4), Technical Captain 25.0% (2/8), Technical Nayak 23.5% (4/17), Technical Subedhar 22.2% (4/18), Technical Haldhar 22.2% (9/2), Volunteer nurses 21.4% (3/14), Intern doctors 20.0% (1/5) and Doctors 20.0% (1/5). The association of nasal carrier of S. aureus among different rank of health personnel was not statistically significant (P >0.05)

Table12: Rank wise distribution of *S. aureus* among health personnel

Ranks	Total sample taken	No. and % of *S. aureus*	p-value
T/Maj.	2	1 (50.0)	P>0.05
Intern doctors	5	1(20.0)	
Doctors	5	1 (20.0)	
T/Cap.	8	2 (25.0)	
T/ Sub.	18	4 (22.2)	
T/Hav.	9	2 (22.2)	
T/ NK	17	4(23.5)	
T/Sep.	18	6 (33.3)	
Volunteer nurses	14	3 (21.4)	
Sweepers	4	1 (25.0)	

5.2.6 Antibiotic susceptibility pattern of *S. aureus* isolated from Nasal swab

Among 100 nasal samples *S. aureus* was isolated from 25.0% (n=25) samples. All the isolates were sensitive towards Methicillin and Oxacillin i.e. all isolates were MSSA. The isolates showed highest resistance towards Penicillin (84.0%) followed by Cloxacillin (36.0%), Amoxycillin (24.0%), Erythromycin (12.0%), and Cotrimoxazole (12.0%) and least towards Gentamicin (8.0%) and Ciprofloxacin (8.0%).

Table 13: Antibiotic susceptibility pattern of *S. aureus* isolated from health personnel

Antibiotics (n=25)	Sensitive (%)	Resistance (%)
Penicillin (10 units)	4 (16.0)	21 (84.0)
Cloxacillin (10 mcg)	16 (64.0)	9 (36.0)
Amoxycillin (30 mcg)	19 (76.0)	6 (24.0)
Cotrimoxazole (25 mcg)	22 (88.0)	3 (12.0)
Erythromycin (15 mcg)	22(88.0)	3 (12.0)
Ciprofloxacin (30 mcg)	23 (92.0)	2 (8.0)
Gentamicin (10 mcg)	22 (92.0)	2 (8.0)
Oxacillin (1mcg)	25 (100.0)	0 (0.0)
Methicillin (5 mcg)	25 (100.0)	0 (0.0)

5.2.7 MDR *S. aureus* isolated from hospital personnel

The *S. aureus* isolates resistant to two or more than two classes of commonly prescribed antibiotics were considered as MDR. Out of 25 *S. aureus* isolates, only 32.0% (n =8) were MDR.

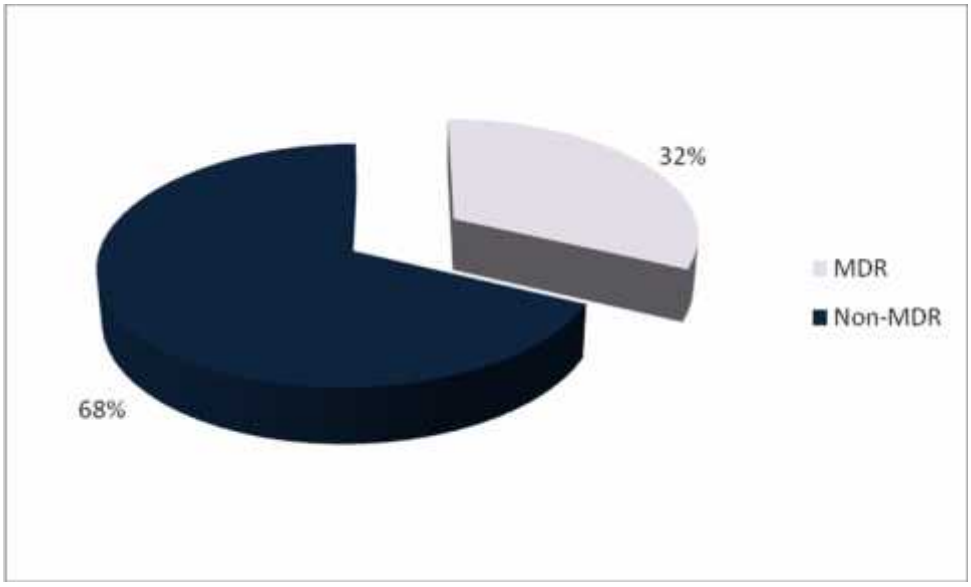

Figure 3: MDR strains among nasal isolates

5.3 Nasal swabs from visitors

A total of 100 nasal swabs were taken from patients visitors from different wards of hospital where admitted patients were present.

5.3.1 Sex wise nasal carriage of *S. aureus* among patient visitors

Out of 58 nasal swab samples from male, 22.4% (n=13) *S. aureus* were isolated. Similarly, a total of 42 nasal samples from female, 11.9% (n=5), *S. aureus* was isolated. Statistically, there is no relationship of *S. aureus* nasal carrier rate between male and female (P=0.177).

Table 14: Sex- wise nasal carrier rate of *S. aureus* among patient visitors

Sex	No. of samples taken	Carrier of *S. aureus* (%)	p-value
Female	42	5 (11.9)	P >0.05
Male	58	13 (22.4)	
Total	100	18 (18.0)	

5.3.2 Age wise nasal carrier of *S. aureus*

A total of 13 (22.4%) *S. aureus* isolates from male and 5 (11.9%) from female was isolated. In male, the prevalence of nasal carrier of *S. aureus* was found to be highest in the age group of < 20 yrs (50.0%) followed by 21-39 yrs (21.4%) and >40 yrs (16.7 %).

In female, the higher prevalence of *S. aureus* nasal carrier was found to be in the age group >40 yrs (18.1%). This percent was followed by the age group 21-39 yrs. No *S. aureus* was found in the age group < 20 yrs. But in male the higher percent was isolated from this <20 yrs age group.

Table 15: Age group wise nasal carrier rate of *S. aureus* among visitors

Age group (yrs)	Male		Female	
	Total sample	No. and % of positive isolates	Total sample	No. and % of positive isolates
< 20	4	2(50.0)	4	0 (0.0)
21-39	42	9(21.4)	27	3 (11.1)
>40	12	2(16.7)	11	2 (18.1)
Total	58	13 (22.4)	42	5 (11.9)

5.3.3 Ward wise distribution of *S. aureus*

The nasal swab samples were collected from different wards where admitted patients were presents. The wards included in this study were surgical ward (S-I, S-II ward and S-III (HCU), Intensive Care Unit (ICU), Intensive Trauma Care Unit (ITCU), Medical ward (M ward), New Family Ward (NFW), Gyno ward, Pediatric ward and Post Operative ward (POP). The highest nasal carrier rate of *S. aureus* among patient visitors was from M ward (50.0%) followed by S-II (31.3%), ITCU (20.0%), ICU (20.0%) and Gyno ward (18.2%). The least isolation was from S-I (5.9%) and NFW (6.3%). No *S. aureus* was isolated from POP and Pediatric ward.

Table 16: Ward wise distribution of *S. aureus* among patient visitors

Wards of hospital	Total swabs taken	Total *S. aureus* isolates (%)
S –I ward	17	1 (5.9)
ICU	5	1(20.0)
ITCU	5	1 (20.0)
M ward	12	6 (50.0)
NFW	16	1 (6.3)
Gyno ward	11	2 (18.2)
POP	7	0 (0.0)
S-II ward	16	5 (31.3)
Pediatric	5	0 (0.0)
HCU	6	1 (16.7%)
Total	100	18 (18.0%)

5.3.4 Antibiotic susceptibility pattern of *S. aureus* isolated from Nasal swab

Among 100 nasal samples, *S. aureus* was isolated from 18.0% (n=18) samples. All the isolates were sensitive towards Oxacillin and Methicillin i.e. all isolates were MSSA. The isolates showed highest resistance towards Penicillin (77.8%) followed by Cloxacillin (27.8%), Cotrimoxazole (27.8%), Amoxycillin (22.2%), Erythromycin (16.7%), and Ciprofloxacin (16.7%) and least towards Gentamicin (11.1%).

Table 17: Antibiotic susceptibility pattern of *S. aureus* isolated from visitors

Antibiotics (n=25)	Sensitive (%)	Resistance (%)
Penicillin (10 units)	4 (22.2)	14 (77.8)
Amoxycillin (30 mcg)	14 (77.8)	4 (22.2)
Cloxacillin (10 mcg)	13 (72.2)	5 (27.8)
Cotrimoxazole (25mcg)	13 (72.2)	5 (27.8)
Erythromycin(15mcg)	15 (83.3)	3 (16.7)
Ciprofloxacin (30mcg)	15 (83.3)	3 (16.7)
Gentamicin (10 mcg)	16 (88.9)	2 (11.1)
Oxacillin (1mcg)	18 (100.0)	0 (0.0)
Methicillin (5 mcg)	18 (100.0)	0 (0.0)

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 Discussion

This study was carried out at central army hospital to determine the prevalence of MRSA in clinically suspected samples and to assess the nasal carriage of MRSA among healthy carriers. A total of 2012 different clinical samples from admitted and OPD patients submitted to microbiological laboratory were analyzed using microbiological procedures. The isolates of *S. aureus* from clinical samples were categorized as MRSA and MSSA by testing their susceptibility towards Oxacillin and then with Methicillin.

Among 322 pus samples, the prevalence of *S. aureus* was 35.1%. This finding shows that *S. aureus* is a major cause of pyogenic infections. Kumari *et al*., 2008; Sanjana *et al*., 2010 reported the higher isolation of *S. aureus* from pus and wound samples. *S. aureus* frequently colonize skin areas as normal flora and under immunocompromised condition it can penetrate skin barrier resulting the skin and soft tissue infections. Similarly during skin trauma (e.g. Turf burns, cuts or sores) *S. aureus* directly enter the body.

A total of 1025 urine samples, *S. aureus* were isolated in less than1% urine samples. This shows that *S. aureus* is less frequently involved in cases of urinary tract infection. Therefore, significant growth of *S. aureus* from patient's urine cannot be ignored clinically but it is not considered as frequent pathogen of UTIs.

Among 430 blood samples, *S. aureus* was isolated in less than 1% samples. Blood is generally sterile tissue, but its infection results when organism gets access to bloodstream and usually results in bacteremia, infective endocarditis. In addition, dissemination to other tissues and organs also occurs. Similarly among 114 sputum samples, *S. aureus* was isolated from 6.1% samples. Among isolates from sputum, 85.7% was from inpatients. In the hospital, nosocomial respiratory infections such as pneumonia are common and mostly caused by *S. aureus* in critically ill and mechanically ventilated patients. Among ICU patients with hospital acquired pneumonia, *S. aureus* was identified as the frequent pathogen (Haddadin *et al.*, 2002). Similarly respiratory tract colonization with *S. aureus* plays an important role in the

development of nosocomial pneumonia in ICU patients. A study by Sirvent *et al.*, 2000 examined the role of tracheal colonization on ICU admission for head trauma in the production of early onset ventilator associated pneumonia, they found that more than half of patients with pneumonia were colonized with *S. aureus*. In this study, *S. aureus* was mostly isolated from sputum obtained from critically ill patients of ICU and ITCU. Therefore, *S. aureus* is the major problem for patients which are on intensive care units due to their decrease resistance.

Out of 34 invasive devices, *S. aureus* were isolated in 5.8% samples. Since invasive devices are artificial medical materials used in the treatment process, they pose a great risk of colonization by *S. aureus* leading to subsequent infections. This also indicates the possibility of higher incidence of nosocomial infection. Of 40 genital specimens, only 5.4% samples from outpatient were positive for *S. aureus* growth. From a total of 55 body fluid sample, only 1.9% sample showed positive growth for *S. aureus* which was isolated from pleural fluid of outpatient. *S. aureus* is an opportunistic pathogen and can colonize, and establish infection in a wide range of body sites if the bacteria enter the bloodstream through an opening in the skin including blood, indwelling biomaterials, mucosal surfaces, bone and other tissues (Boyce, 2011).The life threatening conditions and others infections usually follow the course of skin and soft tissue infections (Zetola *et al.*, 2005).

The prevalence of *S. aureus* was higher in inpatients (8.5%) as compared to that of outpatients (6.1%). This finding indicates the higher prevalence of *S. aureus* infections in the hospital as nosocomial pathogen. Sapkota, 2005 found that the higher prevalence of *S. aureus* from inpatient setting accounting 7.7% as compared to outpatients (5.1%). *S. aureus* is a leading cause of nosocomial infections and over the past 50 years it has acquired resistance to previsouly effective antimicrobials including the penicillinase resistant ones like Methicillin (Duckworth, 1993). In the hospital, infected patients and healthy carriers are important source of nosocomial *S. aureus*. Hospital acquired infection gives an enormous burden to the health care system significantly affecting the patient's mortality and morbidity.

In this study, higher number of *S. aureus* was isolated from pus and wound samples followed by urine, sputum, blood, genital specimen, invasive devices and body fluids. Among a total of 322 pus and wound samples processed, only 113 *S. aureus* was

isolated. Similarly, among 1690 other samples, only 25 *S. aureus* was isolated. The chances of finding of *S. aureus* from pus and wound sample was statistically significant (P<0.001) indicating that *S. aureus* is mainly involved in pyogenic infections.

The present study revealed that the total identified MRSA was 18.1%. The result of the present study correlates with the findings of study conducted by Tahnkiwale *et al*. (2002) where a prevalence of Methicillin-resistant *S. aureus* was 19.5% and with findings from Fraise *et al*. (1997) in which the researchers found 17.0% MRSA in nursing homes in major UK city but the finding of this study does not correlate with the finding from CMS-teaching hospital, Nepal (Sanjana *et al.*, 2010) where 39.9% MRSA was isolated from different clinical samples. Although it's extremely difficult to explain these conflicting data with regards to both time and place of study, the variation is probably due to differential clonal expansion and drug pressure in community.

The highest prevalence of MRSA was isolated from urine 30.0% (3/10) followed by sputum 28.6 % (2/7) and pus samples 17.7% (20/113). Pai *et al.*, 2010; Kumari *et al.*, 2008 reported 48.8% and 33.3% MRSA were in urine samples respectively. In addition, Onanuga *et al.*, 2005 reported 69.0% MRSA carrier rate in urine samples collected from healthy women. Though the maximum isolation of MRSA was from pus and wound sample, the association of MRSA in pus and wound with reference to MRSA in other sample was not statistically significant (P =0.77) indicating that MRSA can establish infections in other body sites.

Among 85 *S. aureus* isolates from outpatients, 11.9% were MRSA whereas among 53 *S. aureus* isolates from inpatients, 26.4 % were MRSA. Among MRSA isolates, 56.0% was from inpatients and 44.0% was from outpatients. The association between the MRSA occurrence in inpatient was statistically significant (P = 0.046). This data clearly showed that the chance of finding MRSA in inpatient is higher as compared to outpatients. This difference could be due to various hospital associated risk factors such as prolonged hospital stay, antibiotic treatment, underlying immunocompromised condition, instrumentation and other invasive devices which predispose patients to MRSA acquisition. Sanjana *et al*, 2010; Sapkota, 2005 reported that 41.14% and 46.5% MRSA respectively were present among inpatients. MRSA

infections would be higher in the hospitalized patients due to various hospital associated risk factors which facilities MRSA acquisition in hospitalized patients.

With regard to MSSA, the most effective antibiotic was Gentamicin 93.8% (106/113) followed by Amoxycillin 82.3% (93/113) and Erythromycin 81.4% (92/113) and the least effective drug was penicillin, with which MSSA showed 91.2% resistivity. Likewise, Shittu *et al*., 2006 reported 88.6% MSSA resistance towards the same antibiotic. The least effectiveness of penicillin and Cloxacillin is probably due to indiscriminate and empirical use of these drugs leading to emergence of resistant strains. Furthermore, these drugs are relatively cheaper and easily available as over-the-counter in Nepal (Kumari *et al.,* 2008). Therefore, now Penicillin is not appropriate drugs to treat staphylococcal infections due to increased resistance. Among tested antibiotics, Gentamicin found to be more effective which correlates finding of Parsa *et al*., 2010 and Shittu *et al.,* 2006. These studies showed that 100.0% and 96.4% sensitive pattern towards Gentamicin by clinical isolates of MSSA respectively. Therefore, these agents can be prescribed for MSSA treatment by testing their susceptibility pattern towards these agents.

With regard to MRSA, the most effective antibiotic was Vancomycin 100.0% (25/25) followed by Gentamicin 48.0 % (12/25) and Ciprofloxacin 48.0% (12/25). The drug resistance of MRSA was highest with Penicillin, Amoxycillin and Cloxacillin (100.0%). This finding corroborates with the finding of Sapkota, 2005. It is due to fact that MRSA strains are often resistant to all beta-lactams. This study showed that all MRSA isolates were significantly less sensitive to antibiotics as compared with MSSA isolates.

In this study all MRSA strains from clinical samples were sensitive (100.0%) towards Vancomycin. Many studies done in hospitals of Nepal reported similar results (Sanjana *et al.,* 2010; Kumari *et al.,* 2008; Thapa, 2004). Hence Vancomycin is the drug of choice for MRSA infection. Resistance to aminoglycosides (Gentamicin) was also more (48.0%). This result is less comparable to the result reported by Sanjana *et al*., 2010 in which 38.0% of MRSA was resistant to Gentamicin. Similarly, resistance to quinolones like Ciprofloxacin was also high (48.0%) in this study. In the study reported by Kumari *et al*., 2010, the resistance rate was also higher (67.75%). But in the study done by Sanjana *et al*., the resistance rate was higher (71.0%) than this

study. This variation may be due to the indiscriminate and empirical use of these drugs. In this study the most effective drug was Vancomycin followed by Gentamicin and Ciprofloxacin, however, Gentamicin and Ciprofloxacin cannot be recommended for empirical treatment of MRSA associated infections. Fluoroquinolones are usually not recommended for MRSA treatment and therapy with these agents frequently results in selection of resistant mutants, and consequent relapse and treatment failure (Gorwitz *et al.*, 2011).

Multi- drug resistant (MDR) *S. aureus* were identified by their antibiotic sensitivity pattern. The *S. aureus* resistant to two or more than two classes of commonly prescribed antibiotics were considered as MDR. Out of total 138 *S. aureus*, 91(65.9%) were MDR. Out of the 113 Methicillin sensitive *S. aureus* (MSSA), only 68 (60.2%) were MDR. But in case of total MRSA isolates, more than 90.0% were MDR. The remaining two isolates of MRSA which were isolated from out patient's pus sample were only resistant towards beta- lactams used in this study. The lack of legislation for prudent use of antibiotics which leads to indiscriminate and irrational use of antibiotics without prescription and termination of antibiotics before full course is one of the major cause of emergence of MDR organisms in our country. A study in South Africa reported that about 87.0% of MRSA were resistant to at least four classes of antibiotics and more than 40.0% were resistant to six classes of antibiotics (Shittu and Lin, 2006). This finding is comparable to the present study. A study from CMS-teaching hospital, Chitwan, Nepal reported many MDR strains of MRSA (Sanjana *et al.*, 2010). Multi-drug resistance pattern of MRSA is contributed by presence of *SCCmec* genes in these strains .It also indicates that the treatment of infections caused by MRSA strains may be difficult in this hospital, as there are reduced antimicrobial options, which could lead to substantial rates of in morbidity and mortality in hospital patients and increased health cost. Similarly, irrational use of antibiotics without surveillance and antimicrobial profile of MRSA may contribute to emergence of MDR strains of MRSA. A national surveillance study from Belgium demonstrated a direct association between MDR and use of fluoroquinoles (Crowcroft, *et al*, 1997).

Lack of regular surveillance within hospital premises, ineffective monitoring of antimicrobial susceptibility pattern of MRSA, lack of formulation of definite antimicrobial policy, ineffective hospital decontamination procedures and unhygienic practices have leaded to spread of antibiotic resistant *S. aureus* strains within wards

and to the community. MRSA related infections contribute to substantial costs for antibiotic treatment, screening, disinfection procedures, isolation procedures and extended hospital stays (NNIS, 2000). The availability of microbiological and epidemiological information would help clinicians in selecting the most appropriate antimicrobial agent for the treatment of infection. MRSA is not more virulent than Methicillin-sensitive *S. aureus* (MSSA), but it shows greater risk to the infection control because of its resistance to most antibiotics (Fluit *et al*, 2001).

Nasal carrier rate was found to be 25.0% and 18.0% among health personnel and visitors respectively. This indicates that one quarter of staffs of the hospital were colonized with *S. aureus*. This finding of nasal carrier rate among health care personnel was in the range of 10-40% as mentioned by Easmon, 1999. And this is comparable to research carried at National Medical College Teaching Hospital, Birgunj, Nepal (Shakaya *et al.,* 2010). However, some studies revealed higher (39.5%- 48.06%) nasal colonization among hospital staffs (Sapkota, 2005; Panta and Rai, 2007; Farazana *et al*., 2008). Staffs in the hospital tend to be colonized while working in the hospital depending upon on the length of their stay and carrier rate may increase during their prolonged stay and can act as source of infection (Armin *et al.*, 2007). The endemicity of *S. aureus* in the hospital also influences the rate of nasal colonization among healthy hospital staffs (Mitsuda *et al.,* 1999). Cruickshank *et al.,* 1975 showed the chances of nasal harboring *S. aureus* by hospital personnel and ward attendants was usually higher (50-60%) and could be easily transmitted to the patients due to frequent contact with them. Patient visitors may colonize with *S. aureus* from hospital environment and patients during care. In addition, they may colonize with community strains.

Among nasal swab samples from male health personnel, 28.1% *S. aureus* were isolated whereas 22.4% male visitors were carrier. Similarly, nasal samples taken from female health personnel and female visitors, 20.9% and 11.9% samples were positive for *S. aureus* respectively. In this hospital, nasal carrier rate was less or more equal in both sexes among health personnel. Statistically, there is no relationship of *S. aureus* nasal carrier rate between male and female of both groups (P=0.414; 0.177) indicating that gender is not a notable factor in *S. aureus* carriage. This finding correlates with the result reported by Armin *et al*., 2007. A little higher isolation of *S.*

aureus from male may be due to higher number of males who were included in the study and their long stay in the hospital.

Among health personnel, highest percent nasal carrier rate of *S. aureus* was found in male health workers 30.0% followed by sweepers 25.0%, laboratory personnel 25.0%, female health workers 22.7% intern doctors 20.0% and resident doctors 20.0 %. The association of nasal carrier of *S. aureus* among different health occupational group was not significant (P= 0.978). The higher carriage pattern of *S. aureus* in male health workers indicates that they were highly exposed to colonized or infected patients or may be their higher stay in the hospital. Similarly, the higher carrier pattern in sweepers may be due to highly expose to hospital wastes and dust in the hospital. Since all types of health staffs shows carrier pattern of *S. aureus*, decolonization is suggested as a precautionary action to reduce nosocomial infection because nasal carrier among health staffs has indicated the chances of transmission of the organism to patients during patient –care (Sapkota, 2005).

Regarding the ward wise distribution of nasal carrier, the highest nasal carrier rate of *S. aureus* among health personnel was from Gyno ward (66.7%) followed by ITCU (33.3%), Medical ward (28.0%) and Surgical ward (26.3%). As nasal carriage of *S. aureus* was found to be health staffs from each ward, ward personnel should be aware of contamination of patient's open surfaces such as wounds during care. As maximum isolates of *S. aureus* in this study belonged to the subjects at Gyno, ITCU, Medical and Surgical ward, transmission from staffs to patients of these wards cannot be ignored. It also indicates the need of the control in the frequency of their exposure with the vulnerable patients. Regarding the carrier rate of *S. aureus* among patient visitors, the higher carrier rate was found in M ward (50.0%) followed by S-II (31.3%), ITCU (20.0%), ICU (20.0%) and Gyno ward (18.2%). The least isolation was from S-I (5.9%) and NFW (6.3%). Visitors may carry community strains before entry into hospital or may colonize from infected patient at ward during contacts with patients.

Carriage of *S. aureus* in the nose appears to play a key role in the epidemiology and pathogenesis of infection (Verghese *et al,* 1999). Asymptomatic nasal carriers are at a high risk of subsequent *S. aureus* infection under immunocompromised condition and presumed to an important source of strains that spread and cause infection in contacts

(Deleo *et al*, 2010; Kennedy *et al.*, 2009; Chambers *et al.*, 2009). The nares are the body's primary MSSA and MRSA reservoir, and nares colonization is largely responsible for colonization at other body sites. Healthcare workers, who have direct contact with persistently colonized patients, or contaminated objects in the immediate environment of such patients, can colonize or contaminate their hands and subsequently transmit the organism to other patients complicating the treatment and recovery. Some may harbor the organism for prolonged periods (persistent carriers) and spread the organism to patients by direct contact constantly (Boyce, 1996). They may also disseminate the organism to hospital environment and to their own families (Armin *et al.*, 2007). Occasionally, health care workers, who carry *S. aureus* in their nares can cause outbreaks of surgical-site infections (Luzar *et al*, 1990; Cespedes, 2002). Some studies showed the significant association between the working place (health care), age and years of employment in the hospital with *S. aureus* and MRSA nasal colonization rate (Armin *et al.*, 2007). There is also existence of an association between MRSA carriage and antimicrobial utilization (Monnet, 1998).

As all groups of staffs and patient visitors were carrier of *S. aureus*, a universal precaution like hand washing before and after patient contact is essential and routine screening of MSSA and MRSA among health staffs and patients should be performed to reduce transmission and decrease the number of patients infected with this organism. Gupta *et al*., 2005 showed the reduction in MRSA infection was observed with the implementation of the barrier nursing protocols (wearing of protective caps, masks, changing into clean linen instead of the normal uniform, and frequent hand washing before and after attending each patient). Similarly, the healthcare personnel require awareness regarding nosocomial infection and should know their status of nasal carriage of MSAA and MRSA and accordingly take necessary universal control measures to prevent possible transmission (Shakaya *et al.*, 2010). Patients, their take cares and household members should take appropriate precautions such as good hygiene practices, proper cleaning, coverage and management of draining wounds for limiting the spread of infection in their households and close contacts (Gorwitz *et al*., 2011). Also clinicians and hospital control personnel should remain vigilant in using appropriate protocols for minimizing microbial transmission (Arch *et al.*, 2006).

All the isolates from nasal swabs were sensitive towards Methicillin i.e. all isolates were MSSA. But many studies showed nasal carriage of MRSA among health

personnel and visitors in different hospital (Shakya *et al.,* 2010). The isolates of both groups showed highest resistance towards Penicillin followed by Cloxacillin and least towards Gentamicin and Ciprofloxacin. Thus Gentamicin, Ciprofloxacin are found to be effective against *S. aureus* isolates from nasal swabs. There is a high percentage of resistance towards beta lactams including Penicillin, Cloxacillin and Amoxycillin. This indicates the higher expression of beta-lactamase enzyme among the isolates. Sapkota, 2005 found 86.6% and 71.6% resistant strains against Penicillin and Amoxycillin while Dimitrov *et al*., 2003 reported 90.0% resistant strains against Penicillin. The finding of this study about susceptibility pattern against Gentamicin and Ciprofloxacin correlates with Sapkota, 2005, but some study showed higher resistance pattern towards quinolones. This may due to empirical use of drugs to treat infections which induces drug resistance in organism. Antibiotic use provides selective pressure favoring resistant bacterial strains. And the drugs, which are commonly used, are generally inexpensive, and popular agents lead to development of bacterial resistance in developing countries (Fazarana *et al.*, 2008).

Among total isolates from health personnel, 32.0% were MDR. Some of the strains showed resistance to Ciprofloxacin, Gentamicin, Erythromycin and Cotrimoxazole. Sapkota, 2005 reported no MRSA carriage among hospital staffs and 23.5% MDR strains. Ahmed *et al.,* 1998 reported that medical persons were colonized with more antibiotic resistant isolates than non-medical persons. A study done on MRSA nasal carriage rates of hospital staffs and outpatient control group were found to be 6.0% and 2.6% respectively. This is probably due to continuous exposure to infected patients and contaminated hospital environment (Dimitrov *et al.*, 2003).

Early detection of MRSA colonization is very important to control nosocomial infection in the hospitals. The presence of higher percent of MDR strains of *S. aureus* in nasal cavity of health personnel may signifies the possibility of transmission of these strains to patients, further complicating treatment and recovery. Because nasal carriage in hospital nurses and other staffs is an important risk factor for infection spread to patients, routine screening and eradicating nasal carriage of *S. aureus* is a one of the useful control measures. Sampling of hospital personnel is most necessary during hospital outbreaks of MDR *S. aureus* infections. MDR strains are the biggest problem for hospitals because these are usually resistant to most of the common antibiotics. Similarly health staffs and patient education regarding nosocomial

infection, environment cleaning, applying useful protocols play an important role in preventing transmission in hospital settings.

6.2 Conclusion

MRSA infection is still one of the most threatening infections in the hospitals of Nepal as such infections are difficult to treat. Vancomycin remains to be the drug of choice. In absence of healthy carriers of MRSA also, there is still higher chances of acquiring MRSA infections. Regular surveillance of MRSA related infections including monitoring of antimicrobial susceptibility pattern of MRSA and formulation of a definite antimicrobial policy may be helpful for reducing MRSA prevalence in hospital setting. In addition, improvement of hygiene standards in hospitals among personnel and visitors will help to prevent *S. aureus* and MRSA transmission in the hospitals.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 Summary

1. A total of 138 *S. aureus* were isolated from 2012 clinical samples collected from outpatients and inpatients attending Shree Birendra Hospital, Chhauni, Kathmandu.

2. The higher *S. aureus* isolation was found in pus and wound samples (35.1%) followed by sputum (6.1%), invasive devices (5.9%), and genital specimens (5.4%) whereas in case of blood and urine, *S. aureus* was isolated in less than 1% samples.

3. The prevalence of S. aureus was higher in inpatients (8.5%) as compared to outpatients (6.1%). In both types of patients, the higher prevalence was found in age group of >50 years.

4. In this study, the prevalence of MRSA was found to be 18.1%.

5. The highest prevalence of MRSA was isolated from urine 30.0% (3/10) followed by sputum 28.6 %(2/7) and pus samples 17.7% (20/113).

6. Among total isolates (85) from outpatients, 11.9% were MRSA whereas among 53 S. *aureus* isolated from admitted group, only 26.4 % were MRSA. Among total MRSA isolates, 56.0% were from inpatients whereas 44.0% were from outpatients.

7. With regard to MSSA, the most sensitive antibiotic was Gentamicin 93.8% (106/113) followed by Amoxycillin 82.3% (93/113), Erythromycin 81.4% (92/113), Ciprofloxacin 73.5% (83/113), Cotrimoxazole 68.1% (77/113), Cloxacillin 42.5% (48/113) and the least effective drug was penicillin 8.8% (10/113).

8. With regard to MRSA, the most sensitive antibiotic was Vancomycin 100.0% (25/25) followed by Gentamicin 48.0 % (12/25) and Ciprofloxacin 48.0% (12/25). The drug resistance of MRSA was highest with Penicillin and Amoxycillin (100.0%) followed by Cloxacillin 92.0%, Cotrimoxazole 68.0%,

Erythromycin 64.0%, Gentamicin and Ciprofloxacin 52.0%. The more effective drug was Vancomycin.

9. Out of a total 138 *S. aureus*, 91(65.9%) were MDR. Among MSSA, only 68 (60.2%) were MDR. But in case of total MRSA isolates, 23 (92.0%) were MDR.

10. In this study nasal carrier rate of *S. aureus* was found to be 25.0% among health personnel.

11. Among positive isolates from nasal swabs, 64.0% were from males and 36.0% were from females.

12. Among total nasal swab samples from male health personnel, 28.1% *S. aureus* were isolated. Similarly, a total of 43 nasal samples from female health personnel, 20.9% *S. aureus* were isolated.

13. Among health personnel, highest percent nasal carrier rate of *S. aureus* was found in male health workers 30.0% followed by laboratory personnel 25.0% , sweepers 25.0%, female health workers 22.7%, intern doctors 20.0% and resident doctors 20.0 %.

14. Regarding the ward wise distribution of nasal carrier, the highest nasal carrier rate of *S. aureus* among health personnel was from Gyno ward (66.7%) followed by ITCU (33.3%), Medical ward (28.0%), Surgical ward (26.3%), SOC (25.0%), Pediatric ward (25.0%), Pathology (23.1%), POP (20.0%) and ICU (18.2%). No *S. aureus* was isolated from NFW.

15. All the isolates from nasal swabs were sensitive towards Methicillin i.e. all isolates were MSSA. The isolates showed highest resistance towards Penicillin (84.0%) followed by Cloxacillin (36.0%), Amoxycillin (24.0%), Erythromycin (12.0%), and Cotrimoxazole (12.0%) and least towards Gentamicin (8.0%) and Ciprofloxacin (8.0%).

16. Among total of 25 *S. aureus* isolates from health personnel, only 32.0% were MDR.

17. Among visitors nasal carrier rate was found to be 18.0%.

18. Among 18 isolates, female accounted 27.8% whereas male accounted 72.3%.

19. The highest nasal carrier rate of *S. aureus* among patient visitors was from M ward (50.0%) followed by S-II (31.3%), ITCU (20.0%), ICU (20.0%) and Gyno ward (18.2%). The least isolation was from S-I (5.9%) and NFW (6.3%). No *S. aureus* was isolated from POP and Pediatric ward.

20. All the isolates were sensitive towards Methicillin and Oxacillin i.e. all isolates were MSSA. The isolates showed highest resistance towards penicillin (77.8%) followed by Cloxacillin (27.8%), Cotrimoxazole (27.8%), Amoxycillin (22.2%), Erythromycin (16.7%), Ciprofloxacin (16.7%) and least towards Gentamicin (11.1%).

7.2 Recommendations

i. Since colonized hospital staffs would be an important source of transmission, they should follow universal barrier precautions.

ii. Patients and their caretakers should take appropriate precautions such as good hygiene practices, and proper cleaning, coverage and management of draining wounds for limiting the spread of infection in their household and close contacts.

iii. More than thirty percent hospital personnel are colonized with MDR strains of *S. aureus*. Therefore their expose to patients' particularly vulnerable patients should be limited during patient care and their nasal decolonization should be done.

iv. MRSA strains showed variable susceptibility pattern to various drugs, so their testing with newer drugs would help to make local effective antimicrobial profile and to reduce reliance on Vancomycin alone.

CHAPTER- VIII

8. REFERENCES

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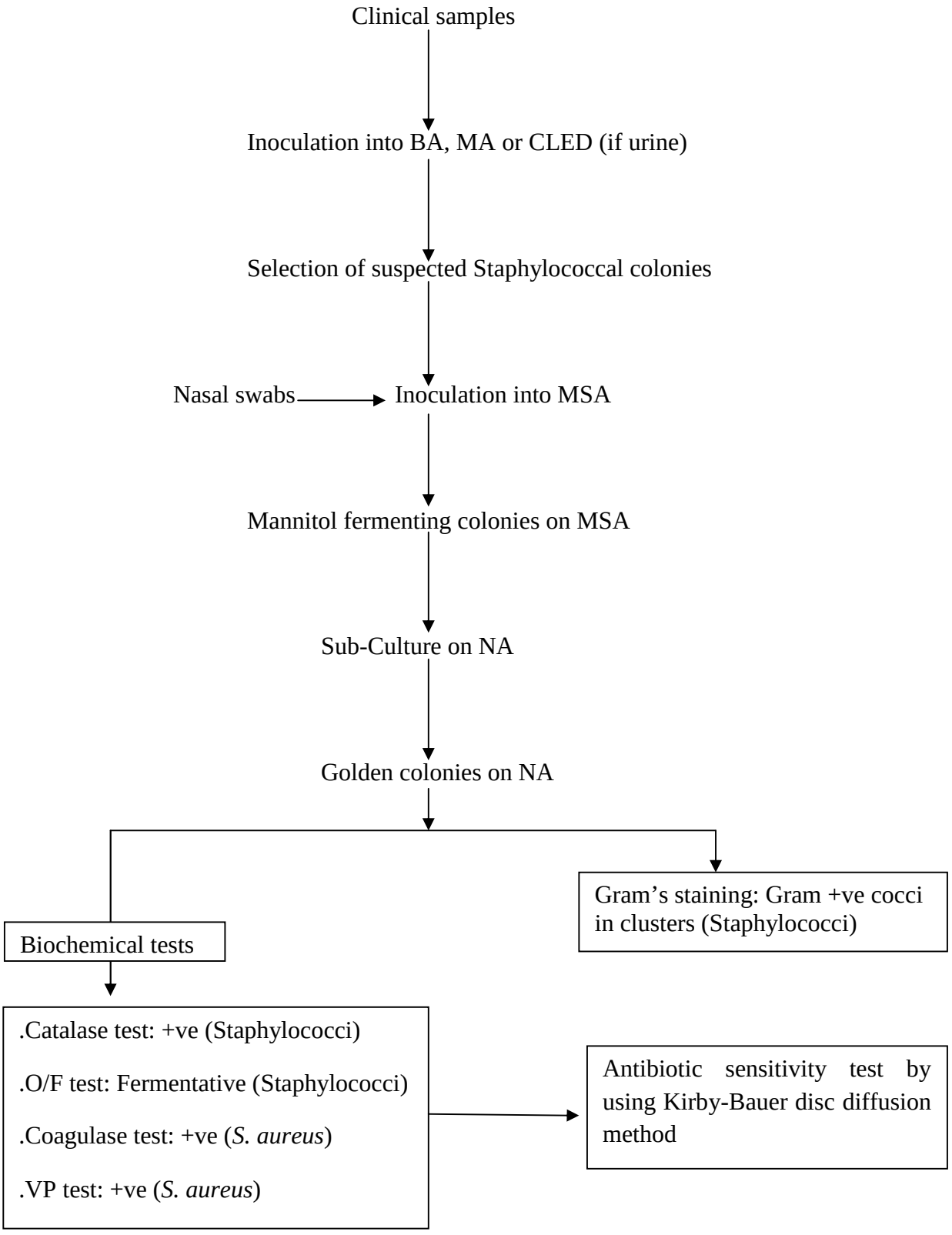

Figure 1: Flow chart for processing of clinical samples and nasal swabs

APPENDIX-I

Materials and Equipments

List of Materials

Glass wares

Beaker	Conical flask
Culture tubes	Glass rod
Slides	Pipettes
Test tubes	Measuring cylinder
Micropipette	Micropipette tips

Miscellaneous

Bacteriological loop	Labeling stickers
Bunsen burner	Staining rack
Sterile cotton swabs	Spirit lamp
Forceps	Gloves
Marker	Soaps
Tissue paper	Tube holder

Equipments

Autoclave	Incubator
Water bath	Refrigerator
Hot air oven	Compound Microscope
Water distillation plant	

Chemical and Reagents

Crystal violet	α- Napthol Solution
Gram's iodine	40% Potassium Hydroxide
Ethanol	1N Hydrochloride acid
Safranin	Distilled water
3% Hydrogen peroxide	MacFarland's Nephelometer Standard No.0.5

Physiological saline	Microscope oil
Paraffin oil	Lysol
Blood plasma	

Antibiotics (HiMedia Company)	**Media (Hi Media Company)**
Penicillin (10 units)	Nutrient Agar
Methicillin (5mcg)	Nutrient Broth
Amoxycillin (30mcg)	MacConkey Agar
Cloxacillin (10mcg)	Mannitol Salt Agar
Cotrimoxazole (25mcg)	Muller Hinton Agar
Ciprofloxacillin (30mcg)	Hugh-Leifson's medium
Gentamicin (10mcg)	MR-VP Broth
Erythromycin (15mcg)	Peptone
Vancomycin (30mcg)	
Oxacillin (1mcg)	

APPENDIX-II

Bacteriological media

Composition and preparation of different types of media

1. **Nutrient Agar (NA**)

Ingredients	**Gram/litre**
Peptone	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	15.0
Final pH (at 25℃)	7.4 ±0.2

28 grams of media was dissolved in 1000 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15lbs at 121℃ for 15 minutes.

2. **Nutrient Broth (NB)**

Ingredients	**Gram/litre**
Peptone	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final pH (at 25℃)	7.4 ±0.2

13 grams of media was dissolved in 1000 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15lbs at 121° C for 15 minutes.

3. **MacConkey Agar (MA)**

Ingredients	**Gram/litre**
Pancreatic digest of gelatin	17.0
Peptone	3.0
Lactose	10.0

Sodium chloride	5.0
Bile Salt	1.5
Agar	13.5
Neutral red	0.03
Crystal violet	0.001
Final pH	6.9-7.3

51.3 grams of the medium was suspended in 1000ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121℃ for 15 minutes.

4. Mannitol Salt Agar (MSA)

Ingredients	**Gram/litre**
Protease peptone	10.0
Sodium Chloride	75.0
D-Mannitol	10.0
Phenol red	0.025
Agar	15.0
Final pH	7.4±0.2

111 grams of the medium was suspended in 1000ml distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs at 121℃ for 15 minutes.

5. Huge-Leifson Medium

Ingredients	**Gram/litre**
Peptone	2.0
Sodium Chloride	5.0
Dipotassium Phosphate	0.3
Agar	2.0
Bromothymol blue	0.05

Glucose	10.0
Final pH	7.4±0.2

19.3 grams of the medium was suspended in 1000ml distilled water and boiled to dissolve completely. The medium was dispended in test tubes in duplicates for aerobic and anaerobic fermentations. The media was autoclaved at 15 lbs at 121℃ for 15 minutes.

6. Methyl Red Voges-Proskauer (MRVP) Broth

Ingredients	**Gram/litre**
Buffered peptone	7.0
Dextrose	5.0
Dipotassium Phosphate	5.0
Final pH (at 25℃)	6.9±0.2

1.7 grams of the medium was suspended in 1000ml distilled water and distributed in 10ml in test tubes and sterilized by autoclaving at 15 lbs at 121℃ for 15 minutes.

7. Muller –Hinton Agar (MHA)

Ingredients	**Gram/litre**
Beef extract	300.0
Casein acid hydrolysate	17.5
Starch	1.5
Agar	17.0
Final pH (at 25℃)	7.4±0.2

38 grams of the medium was suspended in 1000ml and boiled to dissolve completely. The medium was then autoclaved at 15 lbs at 121℃ for 15 minutes.

8. Peptone water

Ingredients	**Gram/litre**
Peptone	10.0
Sodium Chloride	5.0
Final pH (at 25℃)	7.2±0.2

1.5 grams of the medium was suspended in 100ml distilled water and dissolved completely. It was then sterilized by autoclaving at 15 lbs at 121℃ for 15 minutes.

APPENDIX-III

Reagents/Stains

i) Crystal violet stain

Solution A

Crystal violet	2.0 gm
95% ethyl alcohol	20.0 ml

Solution B

Ammonium oxalate	0.8 gm

Distilled water	30.0 ml

Crystal violet was dissolved in ethyl alcohol, and ammonium oxalate in distilled water. Then solution A and B were mixed properly.

ii) Gram's iodine solution

Iodine	1.0 gm
Potassium Iodide	2.0 gm
Distilled water	30.0 ml

iii) Ethyl alcohol (95%)

Absolute alcohol	95.0 ml
Distilled water	5.0 ml

iv) Safranin

Safranin (99% dye content	10.0 gm
Distiled water	1000.0 ml

2. Catalase reagent:

3% Hydrogen peroxide solution (100 ml)

Hydrogen peroxide	3 ml
Distilled water	97 ml

3. V. P reagent:

i) V.P reagent A (100 ml)

α- Napthol	5.0 gm
Absolute ethyl alcohol	100.0 ml

ii) V.P reagent B

Potassium hydroxide	40.0 gm
Distilled water	100.0 ml

3 volumes of VP reagent A and 1 volume of VP reagent B are added to prepare fresh Barrit's reagent.

4. 1 N hydrochloric Acid (1 mol/litre)

Concentrated hydrochloric acid	8.6 ml
Distilled water	100.0 ml

5. Physiological saline

Sodium Chloride	0.85 gm
Distilled water	100.0 ml

6. MacFarland Nephelometer Standards

1% V/V solution of Sulphuric acid was prepared by adding 1ml of concentrated Sulphuric acid to 99 ml of distilled water. 1% W/V solution of barium chloride was prepared by dissolving 0.5 gram of dehydrate barium chloride in 50 ml of distilled water. Then to the 99.5ml of 1% Sulphuric acid solution, 0.5 ml of barium chloride solution was mixed and stirred continuously. Then the solution was transferred in to the clean screw capped tube and stored at dark place until use. The test tube for the broth preparation should be of same size as of McFarland tube. The tubes can be stored and used for six months.

APPENDIX-IV

Gram's staining procedure

Procedure (Chessbrough, 2002; Collee *et al*., 1996)

i. With the help of inoculating loop, isolated pure colonies from NA was touched at the tip and transferred to a clear and grease free slide containing a drop of distilled water.
ii. A uniform smear was made on the slide, which was first air dried and then heat fixed gently.
iii. After cooling, the smear was flooded with crystal violet solution for 1 minute and then rinsed with distilled water.

iv. The slide was flooded with Gram's iodine solution for 1 minute, after which it was rinsed off with water.
v. The slide was then decolorized with acetone alcohol for 10-15 seconds and rinsed off with water.
vi. The decolorized smear was again flooded with safranin for 1 minute and then washed with water.
vii. Finally, the slide was blot dried with absorbent paper and examined under oil immersion.

Observation of Gram positive cocci arranged in grape like clusters is indicative of Staphylococci.

APPENDIX-V

Further identification tests

S. aureus ATCC25923 was included as the standard organism in all tests.

Catalase test

Procedure (Chessbrough, 2002)

a. A drop of 3% hydrogen peroxide was put in a clean slide.
b. The pure colony from NA plate was taken with the help of glass rod or plastic stick
c. The test organism was placed on the hydrogen peroxide drop.

The rapid evolution of gas bubbles is indicative of a positive test. The catalase positive organisms were tested for coagulase production for further confirmation of *S. aureus*.

Coagulase test

Positive coagulase test is the best single test to identify *S. aureus*. The following two tests were performed.

Slide coagulase test (Clumping factor test, Chessbrough, 2002)

i. A homogenous suspension of an isolated colony was made with a drop of physiological saline on a clean slide.
ii. A drop of plasma was added to the homogenous suspension.
iii. The suspension was mixed with plastic stick or glass rod.
iv. Clumping observed within 5- 10 seconds was indicative of positive test.
v. For positive as well negative test, further confirmation was done by tube coagulase test.

Tube coagulase test (Free coagulase test, Collee *et al*., 1996)

i. 1ml of 1:6 diluted plasma in saline (0.85% saline) was placed in small tube.
ii. A colony of the organism was emulsified in the plasma containing test tube.
iii. Control organism was inoculated in the same way as above and a tube containing only plasma was also kept.
iv. All the tubes were incubated at 37˚C and clot formation was examined at intervals of one hour for up to 4 hours, by tilting the tube. The negative tubes may keep for 24 hours
v. The negative tube was examined for auto -agglutition of plasma.
vi. Stiff gel formation or large clots floating in the tube was the indication of positive test.

Voges- Proskaur (VP) test

This test detects the production of acetyl methyl Carbinol or Acetoin from carbrohydrate fermentation

Procedure (Forbes *et al.*, 1998)

i. 2 ml of sterile MRVP broth was taken in a test tube.

ii. A loopful of test organism was inoculated and incubated at 37˚C for 48 hours.
iii. Barrit's reagent was added to the incubated tubes.
iv. The tubes were shaken vigorously to provide maximum aeration.
v. Appearance of pink- red color within 30 minutes was indicative of positive test.

Oxidation-fermentation test (O/F test)

Procedure (Collee *et al*., 1996)

i. Two tubes each containing O/F medium were taken and the test organism was inoculation into both tubes by stabbing with a straight wire up to the bottom of the tube
ii. One tube of the pair was covered with sterile liquid paraffin oil to form a layer up to 1 cm in depth.
iii. Both tubes were incubated at 37˚C for 48- 72 hours.
iv. Two un- inoculated tubes of which one covered with paraffin as above was also used as control.
v. Observation was made for the appearance of the color in the test tube.

APPENDIX-VI

Antibiotic susceptibility test

All *S. aureus* isolated from clinical samples and nasal screening process were subjected to in-vitro antimicrobial susceptibility test by Kirby-Bauer modified disc diffusion method as recommended by CLSI (formerly NCCLS). In this study the antibiotics used were Amoxycillin, Ciprofloxacillin, Cloxacillin, Erythromycin, Gentamicin, Methicillin, Oxacillin, Penicillin and Co-trimoxazole. Only MRSA strains were further tested with Vancomycin.

Procedure

i. Muller Hinton agar (MHA) was prepared, sterilized and poured into sterile petriplate to reach 4mm in depth.
ii. Pure culture of the test organism was transferred into a sterile nutrient broth tube.

iii. The inoculated culture was incubated at 37˚ C for up to 4 hours to obtain turbidity equivalent to the density of MacFarlands Nephelometer standard number 0.5.

iv. Sterile cotton swab was dipped into the culture tube of the organism and excess inoculum was removed by pressing and rotating the swab firmly against the tube wall.

v. Swabbing was performed uniformly all over the surface of the MHA plate by rotating the plate.

vi. With closed petriplate lid, it was kept at room temperature for 3-5 minutes for the surface of the agar to dry.

vii. Antibiotic discs were taken out from their respective vials with the help of sterile forcep and placed carefully on the surface of the swabbed medium, at least 15mm away from the edge of one disc to another.

viii. The discs were pressed lightly with the forceps to make complete contact with the surface of the medium.

ix. The plates left for 30 minutes at room temperature.

x. The plates were then incubated at 35˚C for 24 hours.

xi. After incubation, the diameter of the zone of inhibition of each disc was measured.

The organism was considered as resistant, intermediate or sensitive based on the standard interpretative chart updated according to the current CLSI standard (HiMedia Company, 2010).

APPENDIX-VII

Zone Size Interpretative Chart (CLSI, 2010)

Antibiotics	Symbol	Disc content	Diameter of zone of inhibition (mm)			*S. aureus* ATCC 25923
			Resistant	intermediate	sensitive	
Penicillin G	P	10 units	≤28	-	≥29	26-37
Cloxacillin	COX	10mcg				
Amoxycillin	AMX	30mcg	≤19	-	≥20	28-36
Oxacillin	OX	1mcg	≤10	11-12	≥13	18-24
Methicillin	MET	5mcg	≤9	10-13	≥14	17-22
Gentamicin	GEN	10mcg	≤12	13-14	≥15	19-27
Erythromycin	E	15mcg	≤13	14-22	≥23	22-30
Ciprofloxacillin	CIP	30mcg	≤15	16-20	≥21	27-35
Cot-moxazole	COT	25mcg	≤10	11-15	≥16	24-32
Vancomycin	VA	30mcg	-	-	≥15	17-21

Note: CLSI =Clinical Laboratory Standard Institute

Source: Hi Media Company, 2010

APPENDIX-VIII

Questionnaire sheet for nasal sample collection

This is the questionnaire sheet for data collection. The name is optional. Other fields should be filled correctly.

For laboratory identification purposes:
S. No.
Laboratory code for sampling:
Date of sample collection

Name (Optional):
Occupation:
Age:
Sex:
Education level:
Department/ward:
Years of service:
History of any known infection of MRSA:
If yes, When:
Which site:

Instructions for taking nasal swab
a. Take the moistened sterile swab from the test tube
b. Gently insert the swab in the first nostril