SCREENING OF *Staphylococcus aureus* AS NASAL CARRIER FROM HOSPITAL PERSONNEL OF SHREE BIRENDRA HOSPITAL, CHHAUNI

A

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By

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RECOMMENDATION

This is to certify that **Ms. Juni Thulunga** has completed this dissertation work entitled "**Screening of *Staphylococcus aureus* as nasal carrier from hospital personnel of Shree Birendra Hospital, Chhauni**" as a partial fulfillment of M.Sc. degree in Microbiology under our supervision. To the best of our knowledge this work has not been submitted for any other degree.

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CERTIFICATE OF APPROVAL

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ABSTRACT

Staphylococcus aureus is one of the most common human pathogen and is capable of causing wide range of infections in human. *S. aureus* is the normal flora of the nasal cavity which is carried in the nose of about 40% of healthy people. *S. aureus* as a nasal carrier has been identified as a risk factor for community acquired and nosocomial infection. The present study was conducted from June 2007 to December 2007 to screen out *S. aureus* as nasal carrier from hospital personnel of Shree Birendra Hospital Chhauni. All together 264 nasal swab samples were collected from hospital personnel of different wards. All nasal swab samples were cultured on Mannitol Salt Agar and suspected isolates were identified as *S. aureus.* Antibiotic susceptibility test of the isolated *S. aureus* was done by Kirby Bauer disc diffusion method.

Among 65 isolates of *S. aureus* from nasal swabs; 27.02% (47/174) isolates were from males and 20% (18/90) were from females. The distribution of *S. aureus* as a nasal carrier between male and female was not statistically significant (P = 0.21). In both male and female, the highest prevalence (31.66%) of nasal carrier of *S. aureus* was found in the age group of 26-30 years. Among hospital personnel, maximum nasal carrier rate was found in nursing assistant 33.87% (21/62) followed by intern doctors 31.82% (7/22) and medical trainee 27.59% (16/58). Regarding the department wise distribution of nasal carrier, highest nasal carrier rate of *S. aureus* was from Medical III 53.84% (7/13) followed by orthopedic 50% (2/4) and surgical officer cabin 42.85% (6/14). The isolates *S. aureus* showed highest resistant to amoxicillin (76.9%) followed by penicillin (38.5%), cotrimoxazole (21.5%), gentamycin (13.8%), erythromycin (9.2%), ciprofloxacin (9.2%), chloramphenicol (4.6%) and least towards tetracycline (3.1%). Out of 65, only 20% (n=13) *S. aureus* isolates were multi-drug resistant (MDR) and highest number of MDR *S. aureus* was isolated from Intensive Care Unit 75% (n=3). No methicillin resistant *S. aureus* (MRSA) was found among the isolates of *S. aureus*.

Multidrug Resistant (MDR) *S. aureus* was found but methicillin resistant *S. aureus* (MRSA) was not found among the isolates. However, regular monitoring of methicillin sensitivity should be carried out.

Keywords: *S. aureus*, MDR, MRSA, Nasal carrier, Shree Birendra Hospital

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LIST OF ABBREVIATIONS

ATCC	:	American Type Culture Collection
CDC	:	Centre for Disease Control and Prevention
CONS	:	Coagulase Negative *Saphylococci*
DNA	:	Deoxyribonucleic Acid
ENT	:	Ear, Nose Throat Ward
GOPD	:	General Out Patient Department
HAI	:	Hospital Acquired Infection
ICU	:	Intensive Care Unit
ITCU	:	Intensive Trauma Care Unit
MI	:	Medical I
MDR	:	Multi drug resistance
MIC	:	Minimum Inhibitory Concentration
MHA	:	Mueller Hinton Agar
MRSA	:	Methicillin Resistant *Staphylococcus aureus*
MSA	:	Mannitol Salt Agar
NCCLS	:	National Committee for Clinical Laboratory Standard
NFW	:	New Family Ward
NHANES	:	National Health and Nutrition Examination Survey
OPD	:	Out Patient Department
ORSA	:	Oxacillin Resistant *Staphylococcus aureus*
PBPs	:	Penicillin Binding Proteins
PCR	:	Polymerase Chain Reaction
RFLP	:	Restriction Fragment Length Polymorphism
SI	:	Surgical I
SOC	:	Surgical Officer Cabin
TSST	:	Toxic Shock Syndrome Toxin
VISA	:	Vancomycin Intermediate *Staphylococcus aureus*
VRSA	:	Vancomycin Resistant *Staphylococcus aureus*
WHO	:	World Health Organization

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CHAPTER-I

1. INTRODUCTION

Carrier harbors a specific infectious agent in the absence of discernible clinical disease and serves as a potential source of infection for others. Carriers are significantly dangerous to community and in hospital. Among different carrier's categories, nasal carriers are those who harbour infectious agents in their nasal cavity. Important pathogens are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Niesseria meningitidis*, *Haemophilus influenzae* (mostly non-capsulate) (Cheesbrough, 2000).

S. aureus is one of the most common human pathogens and is capable of causing a wide range of infections in human. Although primary staphylococcal infections are not common, a great deal of the virulence from this organism occurs through cross-infection by spread from patient to patient in hospitals and other institutional settings. In contrast, healthy individuals have a small risk of contracting an invasive infection caused by *S. aureus*, but they can be carrier organism. Because its primary habitat is most squamous epithelium of the anterior nares, most invasive *S. aureus* infections are assumed to arise from nasal carrier (Von Eiff, 2001).

S. aureus is the normal flora of the nasal cavity, which is carried in the nose of about 40% of healthy people (Cheesbrough, 2000). *S. aureus* as a nasal carrier has been identified as a risk factor for community acquired and nosocomial infection. Healthy hospital personnel may carry pathogenic strains in their nose and skin, and may spread these pathogens to the community leading to more dreadful condition. The earlier research done in this case has reported that medical personnel were colonized with more antibiotic resistant isolates than non-medical personnel.

Multidrug-resistant (MDR) strains of *S. aureus* have been reported with increasing frequency worldwide, including isolates that are resistant to methicillin, lincosamides, macrolides, aminoglycosides, fluroquinolones, or combination of these antibiotics. In 1960s, *S. aureus* acquired methicillin resistance by changing the configuration of the penicillin binding protein. *S. aureus* resistant to oxacillin, methicillin and few others related antibiotics are all known under the generic term methicillin resistant *S. aureus* or MRSA.

Many studies done in Nepal about *S. aureus* and their antibiotic sensitivity pattern suggest the gradual emergence of MRSA in hospitals. The prevalence of MRSA in Nepal ranges from 11.7% to 54.9% (Lamichhane, 1999; Pokhrel *et. al.,* 1993; Rajbhandari, 2002; Thapa, 2004). Many studies have also shown the emergence of MDR *S. aureus* in hospitals (Anupurba *et. al.*, 2003; Sapkota, 2007; Shah, 2002). These studies clearly indicate about the appropriate steps to be taken to reduce MRSA and MDR strains in hospital settings to minimize nosocomial infections.

The fact that huge portions of healthy population carry *S. aureus* in their nose and body surfaces is responsible for fast spread of Staphylococcal infections and the situation seems worse in hospitals. So, study of *S. aureus* as nasal carrier is of importance, especially in people concerned with hospitals to explore the clear picture regarding its existence. It not only gives information about the nasal carrier rate but also gives idea about the preventive measures to be initiated against the *S. aureus* infections in hospitals.

This study focuses on *S. aureus* as nasal carrier in hospital personnel of Shree Birendra Hospital, Chhauni and it has been assumed that this study will help to bring health awareness among the staffs of the hospital especially in nasal health as well as personnel hygiene. Since this study has been performed as a part of surveillance of nosocomial infection, it will help to analyse the current microbial status of the hospital personnel and can aid in control of nosocomial infections.

CHAPTER-II

2. OBJECTIVES

2.1 General objective

Screening of *Staphylococcus aureus as* nasal carrier from hospital personnel of Shree Birendra Hospital, Chhauni.

2.2 Specific objectives

- To isolate and identify of *S. aureus* from nasal cavity of healthcare personnel of Shree Birendra Hospital, Chhauni.
- To describe distribution pattern of *S. aureus* isolates from nasal carriers.
- To screen out the multidrug-resistant (MDR) and methicillin resistant *Staphylococcus aureus* (MRSA) strain from nasal carriers.

CHAPTER III

3. LITERATURE REVIEW

3.1 Staphylococcus

Staphylococci were first seen in pus by Robert Koch in 1878. In 1880, Sir Alexander Ogston showed that many pyogenic diseases in humans were caused by cluster forming bacteria. The name staphylococcus was given by Ogston, which has the origin from Greek (Staphyle = bunch of grapes; kokkos = grain or berry). Ogston noticed that non-individual staphylococci were also present on skin surfaces (Ananthanarayan and Panikar, 2000).

Staphylococci are gram-positive cocci that occur singly, in pairs, tetrads, short chains, and irregular "grape like" clusters. Clusters formation results from sequential division of bacteria in irregular fashion, in more than one plane, at right angles to each other. Staphylococci are non-motile, non-spore forming and spherical or somewhat ovoid ranging from 0.5-1.5 μm in diameter (Holt *et al*., 1994).

The staphylococcal cell wall contains a thick peptidoglycan layer with distinctive pentaglycine bridges linking the amino acid side chains. 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 human skin and mucosa. Some of the species are normal flora of humans. Species of Staphylococci found on human skin include *S. epidermidis, S. haemolyticus, S. hominis, S. warnari, S. captits, S. lugdunesis, S. cohnii, S. simulans and S. xyulosus* (Collee *et al.,* 1996). The organisms can also be isolated from a variety of environmental sources e.g. fomites, sewage, soil, water, air and animal products such as cheese, egg, meat and milk (Easmon and Goodfellow, 1990).

Staphylococci 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. On nutrient agar; they form white, yellow or golden yellow colonies with or without pigment production. They are facultative anaerobic and catalase positive organisms. The metabolism is both respiratory and fermentative type, and most strains grow in the presence of 10% sodium chloride and between 18-40°C temperature (Easmon and Goodfellow, 1990).

3.2 Classification of *Staphylococcus*

Staphylococci can be classified on the basis of pigment production, cultural characteristics, coagulase production, biochemical characteristics, pathogenicity and chemical composition of their cell wall components.

3.2.1 Classification on the basis of pigment production

Three major types of staphylococci are identified, however, the pigment production variable (Gupta, 1999).

i. *S. aureus* producing golden yellow colonies and are pathogenic.

ii. *S. albus* producing white colonies and are non-pathogenic.

iii. *S. citreus* producing yellow colonies and are non-pathogenic.

3.2.2 Classification on the basis of pathogenicity

They are classified as follows (Gupta, 1999)

i. Pathogenic species: *S. aureus,* the common cause of suppuration

ii. Non-pathogenic species*: S. epidermidis, S. citreus, S. saprophyticus*

Table 3.1: Distinguishing characteristics of three species of *Staphylococci*

Characters	*S. aureus*	Albus Staphylococci	
		S. epidermidis	***S. saprophyticus***
Colony Colour	Yellow to orange	Usually white	Usually white
Coagulase	+	-	-
DNase	+	(+) weak	-
Mannitol fermentation (anaerobically)	+	-	-
Novobiocin susceptibility	S	S	R

S= sensitive, R= resistant , + = positive, - = negative

(Source: Collee *et al,* 1996*)*

3.2.3 Classification on the basis of coagulase production

Staphylococci are classified into two groups according to their ability to produce coagulase in plasma (Chakraborty, 1995)

i. Coagulase positive: *S.aureus*

ii Coagulase negative: *S.epidermidis, S.citreus* etc.

3.2.4 Baired-Parker classification

Baired-Parker classified staphylococci into six sub-groups(I-VI).The tests used for classification include coagulase and phosphatases tests, acid production from glucose, arabinose, lactose, maltose ,mannitol and pigment production(Gupta,1999).

Table 3.2: Baired-Parker's classification of Staphylococci

Tests	Subgroups of Staphylococcus					
	I	**II**	**III**	**IV**	**V**	**VI**
Hugh and Leifson	F	F	F	F	F	F
Coagulase	+	-	-	-	-	-
Phosphates	+	+	+	-	-	-
VP	+	+	-	+	+	+
Arabinose	-	-	-	-	-	-
Lactose	**A**	**A**	**V**	-	**A**	**V**
Maltose	**A**	**A**	-	**V**	**A**	**V**
Mannitol	**A**	-	-	-	-	**A**
Pink pigment	-	-	-	-	-	-

Note: A=acid production, F= fermentative, V=variable (Source: Gupta, 1999)

3.3 Cultural characteristics

S. aureus can grow easily on basic media like nutrient agar within the temperature range of 12-44ºC.The optimum temperature and pH for growth is 37ºC and 7.5 respectively. It can be cultured readily on mannitol salt agar, blood agar and most other media (Collins and Lyne, 1983).Common media used for *S. aureus* are nutrient agar, nutrient broth, blood agar, Mac Conkey agar, Mannitol salt agar, phenolphthalein phosphate agar.

3.4 Biochemical characteristics

S.aureus ferments a number of sugars namely glucose, lactose, sucrose, maltose and mannitol with the production of acid but no gas. Sugar fermentation is of no diagnostic value except for mannitol fermentation by *S. aureus*. They are catalase and coagulase positive, oxidase negative. The organism hydrolyses urea, reduces nitrate to nitrite,

liquefy gelatin and MRVP positive but indole negative. Urease and esterase production and lactose fermentation are variable characters useful in the type 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 coagulase test positive.

Table 3.3: Some virulence factors of *S. aureus*

Virulence factor	Mode of action
I.Cellwall polymers	
· Peptidoglycan	-Inhibit inflammatory response, endotoxin-like activity
·Techoic acid	-Phage adsorption, reservoir of bound divalent cations
II.Cell surface proteins	
· Protein A	-Reacts with Fc region of IgM
· Clumping factor	-Binds to fibrinogen
· Fibronectin-binding protein	-Binds to fibronectin
III. Exoproteins	
· α lysine, β lysine, γ lysine,δ lysine, pantonvalentine leucocidin	-Impairment of membrane permeability; cytotoxic effects on phagocytic and tissue cells
·Epidermolytic toxin	-Causes blistering of skin
·TSST	-Induces multi system effects; super antigen effects
·Enterotoxins	-Induce vomiting and diarrhea, super antigen effects
·Coagulase	-Converts fibrinogen to fibrin in plasma
·Staphylokinase	-Degrades fibrin
·Lipase	-Degrades lipid
·Deoxyribonuclease	-Degrades DNA

(Source: Greenwood *et al.,* 2006)

3.5 Virulence factors and pathogenesis

S. aureus causes abscess, various pyogenic infections, food poisoning, and toxic shock syndrome. Varieties of disease establishing factors are responsible for causing infection and diseases. *S. aureus* possess a large number of cells associated and extra cellular factors, which overcome the body's defense and invade, survive and colonize the tissue. The organism possess such virulence factors which oppose destruction by the component of innate immunity i.e. complement and phagocytosis (Greenwood *et al.*, 2006).

S.aureus produces disease in humans by two types of mechanism: infection and intoxication. In infection, the cocci gain access to damaged skin, mucosal or tissue sites; colonize by adhering the cells or extra-cellular matrix, evade host defense mechanism, multiply and cause tissue damage. In intoxication, the disease is caused by the bacterial toxins produced either in the infected host or performed in vitro (Ananthanarayan and Panikar, 2000).

1. Cell wall associated factors

Peptidoglycan

It gives rigidity to the cell. 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.

Teichoic acid

It confers antigenicity and behaves as surface receptors for staphylococcal bacteriophage. It mediates adherence of the staphylococci to mucosal cells.

2. Cell surface proteins

Protein A

This cell wall protein is an important virulence factor because it binds to the Fc portion of IgG at the complement-binding site, thereby preventing the activation of complement. As a result, C3b production is hampered and the opsonization and phagocytosis of the organism is greatly reduced. Coagulase negative staphylococci do not produce protein A (Greenwood *et al.*, 2006).

Clumping factor

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

Fibronectin-binding protein (FBP)

It promotes binding to mucosal cells and tissue matrices.

3. Microcapsule

Most strains of *S. aureus* are coated 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).

4. Exoproteins

Cytolytic exoproteins

α, β, γ and δ toxins attack mammalian members (including red blood cells) and are often called hemolysins. α –toxin is chromosomally encoded with polymerizes into

tubes and forms holes in the membrane causing loss of important molecules and eventually osmotic lysis of the cell.

Superantigen exotoxins

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

Enterotoxins

Enterotoxin types A-E, G, H, I and J are commonly produced by up to 65% of strains of *S. aureus* (Greenwood *et al.*, 2003). When ingested as performed toxins in contaminated food, they stimulate the vomiting center in the brain by binding the neural receptors in the upper GI tract. These toxic proteins are heat resistant and withstand up to 100ºC for several minutes.

Toxic shock syndrome toxin (TSST)

TSST is a superantigen 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. Toxic shock occurs especially in tampon using women or in individuals with wound infections who do not have antibody against TSST.

Exfoliatin (Exfoliative toxin)

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

Hyaluronidase

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

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.

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.

Deoxyribonuclease

This enzyme helps in the degradation of host cell DNA.

Coagulase

It is an enzyme, which causes plasma to clot by activating prothrombin, which in turn converts fibrinogen to fibrin

Phosphatase

This enzyme breaks down phospholipids of the host cell.

3.6 Human Diseases due to *Staphylococcus aureus*

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, soft tissue, bone, joint, lung, heart and kidney infections.

Table 3.4: Diseases due to *S. aureus*

Pyogenic infections	**Toxic mediated infections**
Boils, Carbuncles, Wound infections Abscesses, Impetigo, Mastitis, Bacteraemia/sepsis, Meningitis, Osteomyelitis/septic arthritis, Pneumonia Endocarditic and Urinary tract infection	Scaled skin syndrome Pemphighus neonatorum Toxic shock syndrome Gastroenteritis

(Greenwood *et al.*, 2006)

1. Localized skin infections

The most common *S. aureus* infections are small, superficial abscesses involving hair follicles (folliculitis) or sweat or sebaceous glands. The infection to 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 carbuncles. Carbuncles are larger, deeper, multilocated skin infections that can lead to bacteremia and require antibiotic therapy and debridement.

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.

Cellulitis

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

Wound infection

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

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.

2. Disease due to organ invasion

Pneumonia

S. aureus is a rare but severe cause of community acquired bacterial pneumonia. Pneumonia is a 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 empyema.

Oesteomyelitis

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.

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.

Acute endocarditis

It is generally associated with intravenous drug abuse and is caused by injection of contaminated preparations or by needles that are contaminated with *S. aureus.* It causes destructive infection of the heart valves with the sudden onset of high fever, chills and myalgias. Intravenous drug users usually develop a right-sided tricuspid valve endocarditis.

Meningitis, cerebritis and brain abscess

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

Septicemia

It can originate from any localized lesion, especially wound infection or as a result of intravenous drug abuse.

3.7 Epidemiology of *S. aureus*

Epidemiological study of *S.aureus* is necessary to explore the sources of contamination and modes of dissemination of the bacteria and hence helps to reduce the infections.

3.7.1 Infection

Infection entails the entry and development or multiplication of an infectious organism in the body tissues of a host. There are several levels of infections. If this results in overt clinical manifestations, the state is known as disease (clinical infection). However, if the infection only provokes an immune response without overt clinical disease, it is referred to as an in apparent or sub-clinical infection. Colonization implies the presence of a microorganism in or on a host with growth and multiplication of the organism, but without any overt clinical expression of detected immune reaction at the time it is isolated e.g. *S. aureus* in anterior nares (Bennett and Brachman, 1989).

3.7.2 Carrier

A carrier is an infected individual colonized with a specific microorganism and from whom the organism can be recovered (i.e. cultural) but who shows no overt expression (clinical disease) of the presence of the microorganism at the time of isolation. Carriers serve as potential source of infection for others. Although carriers are less infectious than infected cases; epidemiologically, they are more dangerous than cases because they escape recognition, and continuing as they do to live a normal life among the population or community. They readily infect the susceptible individuals over a wider area and longer period of time, under favourable conditions.

Healthy carriers develop their carrier state without suffering from overt disease, but are nevertheless shedding the disease agent. *S. aureus* is carried in the nose of about 40% or more of people as healthy carriers (Cheesbrough, 2000). About 8 to 22% of the subjects yielded staphylococci from other skin areas (Williams, 1963). It has usually been found that the numbers of staphylococcal colonies on cultures from the nose are far greater than the numbers from skin swabs, and it is generally accepted that the nose is, in most carriers, the principle site of multiplication of the cocci. Chances of harboring of *S. aureus* by hospital personnel and transmitting them to the patients may be high due to

frequent contact with the patients. This carrier state can serve as a reservoir for infection of hospitalized patients, but most carriers do not disseminate the organism and are not a risk to others. They can also contaminate food, which may result in food poisoning (Weems and Beck, 2002).

Three human nasal *S.aureus* carriage patterns can be distinguished: persistent carriage, intermittent carriage, and non-carriage (Riewarts *et al.,* 1995). In culture studies of hospital staff for nasal carriage of *S. aureus*, approximately 15-20% are non-carriers, 60-70% are intermittent carriers and 10-15% are persistent carriers (Bennett and Brachman, 1989). The relatively small proportions of carriers who are intermittent or persistent with heavy shedders of high numbers of organisms, especially in conjunction with any overt staphylococcal disease, appear to pose the greatest threat to susceptible patients (Easmon & Goodfellow, 1990). Nasal carriage of *S. aureus* is a major risk factor for the development of *S. aureus* infection, including skin and soft tissue infections and foreign body infections, such as catheter-associated bacteraemia and peritonitis (Weems and Beck, 2002; Von Eiff, 2001).

3.7.3 Dissemination

Dissemination or shedding of microorganisms refers to the movement of organisms from an individual carrying the organisms into the immediate environment. The demonstration by culturing techniques that an individual is carrying a certain organisms defines a potential problem, whereas epidemiologic demonstration by surveillance and investigative techniques defines the real problem. In some hospitals, routine culture survey of all or selected asymptomatic staff may be conducted in an attempt to identify carriers of certain organisms, but such surveys lack practical relevance unless the results are related to specific cases or an outbreak of disease.

Large numbers of organisms are disseminated in pus and dried exudates discharged from large infected wounds, burns, skin lesions, and in sputum coughed from the lung

of a patient with bronchopneumonia. Animals may disseminate *S. aureus* and so cause infections in humans, e.g. milk from a dairy cow with mastitis can cause staphylococcal food poisoning.

3.7.4 Mode of transmission

Direct contact is the most important mode of spread, but air-borne dissemination may also occur. Small discharging lesions on the hands of doctors and nurses are special danger to their patients. Cross-infection is commonly seen in crowded hospital bed settings. Food handlers may introduce enterotoxin-producing strains into food.

3.8 Hospital infections

Hospital infection, also known as hospital acquired infection (HAI) or nosocomial infection is defined as infection acquired in hospital by a patient who was admitted for a reason other than that infection. A generally accepted definition is that it is an infection acquired four days or more after admission to hospital (Glenister *et al.,* 1992).

Nosocomial infection is one of the most important public health problems in the world today. It is the single largest factor that adversely affects both the patient and hospital. Nosocomial infection involve not only the patients but also any one else who is in contact with the hospital including staff members, volunteers, visitors, workmen, salesman and delivery personnel. It has been reported that even in developed country like United States nosocomial infection appears in one in ten patients admitted and affects approximately 2 million people annually (CDC, 1992). It is said that at any time 9% of hospital in-patients have a hospital acquired infection (National Audit Office, England, 2000). Additionally, 2% of nosocomial infections probably result directly in the death of the patient (Bennett and Brachman, 1979). The highest frequency of nosocomial infection is reported in eastern Mediterranean regions and South East Asian Regions (11.8% and 10.0% respectively), with a prevalence of 7.7% and 9.0%

respectively in Europe and Western Pacific regions. In a study performed in a tertiary care hospital in Nepal, the overall point prevalence of nosocomial infection is reported to be 2.4% (Lamichhane and Shrestha, 2001). Tuladhar, 1990 has reported the prevalence of nosocomial infection to be 10.5%, majority of these being endemic infection.

The three principle factors determine the likelihood that a given patient will acquire a nosocomial infection: susceptibility of the patient to the infection, the inoculum and virulence of the infecting organism, and the nature of the patient's exposure to the infecting organism. The occurrence rate of nosocomial infection varies according to the type of hospital. Large, tertiary-care hospitals that treat the most seriously ill patients often have higher rates of nosocomial infection than do small, acute-care community hospitals. This difference in the risk of infection is related to several factors including the severity of illness, the frequency of invasive diagnostic and therapeutic procedures and variation in the effectiveness of infection control programs (Forbes *et al.,* 2000).

Many different pathogens may cause nosocomial infections. The infecting organisms vary among different patient populations, different health care settings, different facilities, and different countries. In Semmelwei's era, Group A streptococci created most nosocomial problems. For the next 50 to 60 years, gram positive cocci, particularly Streptococci and *S. aureus,* were the hospital pathogen of major concern. These problems culminated in the pandemic of 1940 to 1950, when *S. aureus* phage type 94/96 caused major nosocomial problems. In the 1970s, gram negative bacilli, particularly *P. aeruginosa* and E*nterobacteriaceae*, become synonymous with nosocomial infection. By the late 1980s and early 1990s, several different classes of antimicrobial drugs effective against gram negative bacilli provided a brief respite. During this time, methicillin resistant *S. aureus* (MRSA) and Vancomycin resistant enterococci (VRE) emerged. In 1990 to 1996, the three most common gram positive pathogens, *S. aureus*, coagulase-negative staphylococci and enterococci accounted for 34% of nosocomial infections, and the four most common gram negative pathogens - *E.*

coli, *P. aeruginosa*, *Enterobacter* spp and *K. pneumoniae* accounted for 32% (Weinestein, 1998).

3.8.1 *Staphylococcus aureus* and Hospital infection

S. aureus is another very important pathogen associated with nosocomial infection. It is one of the most resistant non-sporing bacteria and survives well in the environment under both moist and dry condition (Forbes *et al.*, 2002). Especially multi-drug resistant strains are an important cause of hospital acquired infection. The emergence of *S. aureus* strains resistant to methicillin and other antibiotics has become a major concern, especially in the hospital environment, because of higher mortality due to hospital acquired systematic methicillin resistant *S. aureus* (MRSA) infection. Infection associated with such organism is particularly a threat to vulnerable patients such as neonates, cancer patients, elderly or patients from ICU, burn units, high dependency units and infectious disease care centres. Methicillin resistance *S. aureus* (MRSA) has become an important hospital pathogen, MRSA are endemic in healthcare settings in the United States and many other countries of the world. Nosocomial transmission of MRSA serves as a source of hospital outbreaks and recent reports of Vancomycin resistant *S. aureus* (VRSA) in the United States emphasizes the need for the better control for the MRSA and other resistant bacteria within healthcare settings. In a study done at St. Thomas hospital, London, UK 267 cases of MRSA bacteraemia were detected during two year period (2001-2003) giving a rate of 0.37 per 1000 occupied bed per day (Jevaratnam *et al.*, 2006). *S. aureus* bloodstream infections are common and serious causes of morbidity and mortality that incur considerable healthcare costs and are potentially preventable. Patient to patient transmission of MRSA within the healthcare settings primarily occurs via carriage on the hands of healthcare workers. It has been suggested that the best approach to control the transmission of MRSA in a hospital or a healthcare settings is hand hygiene plus a careful assessment of an institution's particular circumstances, applying more aggressive procedures such as patient isolation, staff cohorting, and active surveillance culture, as indicated. Zhang *et*

al., has reported that there could be cross infection of *S. aureus* between the medical staff, inpatients and the infected patients.

It has been found that *S. aureus* was isolated in 19.8% of patients with ICU-acquired infection, particularly in relation to pneumonia in mechanically ventilated patients. Mortality in patients with *S. aureus* infection was higher than that in patients with infections due to other microorganisms and patients without infection. In contrast there was no significant difference in the outcome in the infections caused by methicillin sensitive and methicillin resistant *S. aureus* (Alvarez *et al.,* 2006).

High level vancomycin-resistance has now been reported in a single clinical isolate of *S. aureus* emphasizing the need to increase efforts to control nosocomial spread. Effective control of *S. aureus* within the hospital and community will require more aggressive measures that include earlier diagnosis of colonized patients, better hand washing and barrier precaution measures and renewed efforts to eradicate the carriage state (Chiang *et al.,* 2002).

3.8.2 Nasal carriage and nosocomial infection

Nasal carriage of *S. aureus* has been identified as a risk factor for community-acquired and nosocomial infections (Collee *et al.,* 2001). Healthy hospital personnel may carry pathogenic hospital strains in their nose and skin and may spread these pathogens to the community leading to more dreadful condition (Nakanishi *et al.,* 1996). In some other conditions these carriers may disseminate the pathogens to the hospitalized patients and increase the duration of hospitalization for those due to hospital acquired infections. Patients who develop persistent nasal carriage may be colonized on their hands or other areas of intact skin and can disperse the organism into the environment around them. Healthcare workers who have direct contact with persistently colonized patient or contaminated objects in the immediate environment around them can contaminate their hands and subsequently transmit the organism to other patients. A subset of these will

remain as nasal carrier for a prolonged period of time and may spread the organism to patients by direct contact transmission (Boyce, 1996). Hospital acquired infections are transmitted to patients by hospital personnel and other patients, or they may arise from patient's own endogenous flora. The fact that huge portions of healthy population carry *S. aureus* in their nose and body surfaces is responsible for the fast spread of the staphylococcal infections and the situation seems worse in hospitals. So, study of *S. aureus* as nasal carrier is of importance especially in people concerned with hospitals to explore the clear picture regarding its existence. A substantial proportion of cases of *S. aureus* bacteraemia appear to be of endogenous origin since they originate from colonies in the nasal mucosa. These results provide support for strategies to prevent systemic *S. aureus* infections by eliminating nasal carriage of *S. aureus* (Von Eiff, 2001).

It has been reported that medical personnel were colonized with more antibiotic-resistant isolates than non-medical personnel, and the strain profiles indicated that they tended to be more clonal in origin, suggesting that exposure to hospital isolates alters the colonization profile. *S. aureus* carriage among patients and staffs has been found to be directly correlated with the occurrence of nosocomial infection due to the organism (Chiang *et al.,* 2002; Nouwen *et al.,* 2005). It has been found that nasal carriage of *S. aureus* is a well-defined risk factor for subsequent infection in nearly all categories of hospitalized patients that have been studied. Studies have found that screening and eradication of nasal carrier for *S. aureus* decrease the incidence of nosocomial infections (Kluytmans and Wertheine, 2005).

S. aureus is one of the most common causes of hospital-acquired infections. At the same time, 25% of healthy persons are symptom-free *S. aureus* carriers, and they have an increased risk of developing nosocomial *S. aureus* septicaemia (Holtfreter *et al.,* 2006). 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). However, none of the strains were found to be MRSA. A study on staphylococcal strains between parents and the

offspring 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 2.0% respectively in a hospital in Turkey (Dimitrov *et al*., 2003). A case study done on mother and child with recurrent infection and breast abscess found to harbor MRSA. Some cases of recurrent infections may be related to nasal carriage in mother or infant (Akoua *et al.,* 2004).

Anwar *et al*., 2004 studied the nasal swabs of 1660 community subjects and 14.8% (n=246) of them were found positive for *S. aureus*. Among the total isolates, 19.5% (n=48) were MRSA. Nasal carriage was higher in males (15.4%) in comparison to females (13.2%), in urban areas (16.9%) as compared to rural areas (19.3%). Similar study done in 2003 by Saxena *et al*., found a nasal carriage rate of 29.4% with a prevalence of 18.1% MRSA among positive isolates.

National Health and Nutrition Examination Survey (NHANES) 2001-2002 to estimate carriage of *S. aureus* and MRSA for the non-institutionalized US population including children and adults showed that nearly one third of the population is currently colonized by this organism. Although, the prevalence of MRSA remains low, more than 2.2 million people carry this resistant organism (Mainous, 2006). A total of 1,838 subjects from the community and 393 subjects from health care related facilities in Taiwan were evaluated for the prevalence of nasal *S. aureus* colonization and to identify risk factors associated with *S. aureus* and methicillin resistant *S. aureus* (MRSA) colonization. Subjects from health care-related facilities had a lower *S. aureus* colonization rate (19.1%) than community subjects (25.2%) but had a significantly higher rate of colonization with MRSA (7.63%) (Lu *et al.,* 2005). In context of Nepal there are few studies regarding the prevalence of nasal carrier of MRSA among hospital personnel. Shah 2002 studied 250 nasal swab samples taken from patients, visitors and staffs of TU teaching hospital. *S. aureus* were isolated from the nose of 31.4% (33/105), 35.2% (37/105) and 47.5% (19/40) of patients, visitors and staffs respectively. Sapkota 2006 studied 205 nasal swabs from hospital personnel of Bir Hospital. Among 123 positive

isolates from nasal swabs, 39% (n=48) were from males and 40.2% (n=33) were from females. Pant 2007 studied 48 nasal swabs from staffs of different wards in Nepal Medical College Teaching Hospital; *Staphylococcus aureus* was isolated from 21 samples that are 44% of the total samples.

3.9 Methicillin resistant *S. aureus*

Methicillin resistant *Staphylococcus aureus* or MRSA are staphylococcal bacteria that have become resistant to β-lactam antibiotics, including penicillin, ampicillin, amoxicillin, methicillin, oxacillin, dictoxacillin, cephalosporin, carbapenems (e.g. imipenem) and the monobactams (e.g. aztreonam). MRSA is also known as oxacillin-resistant *S. aureus* (ORSA) and multiple-resistant *S. aureus.*

Methicillin is a synthetic antibiotic related to penicillin with modified radicals designed to protect the penicillin ring against the bacterial enzyme penicillinase. In the 1960s, *S aureus* acquired methicillin resistance by changing the configuration of the penicillin binding protein (PBP). 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 but soon after penicillin was put in clinical use penicillin resistance developed. *S. aureus* had acquired the ability to inactivate the ℘lactam ring of penicillin. Currently more than 95% of *S. aureus* are resistant to penicillin (Chamber, 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. MRSA related infections contribute to substantial costs for antibiotic treatment, screening, disinfection procedures, isolation procedures and extended hospital stays (NNIS, 2002). MRSA strains colonize easily in the host particularly in immunodeficient patients, which can cause a variety of serious difficult-

to-control infections including septicemia, pneumonia, endocarditis, meningitis, and post operative intra-abdominal infection. MRSA are predominantly hospital pathogens in severely weakened patients, such as those in intensive care units, where the combination of multiple courses of antibiotics and the use of invasive devices contribute greatly to the risk of acquisition. Vancomycin is the only drug of choice for MRSA. Nowadays, vancomycin resistant *Staphylococcus aureus* (VRSA) has also been reported.

3.10 Mechanism of antibiotic resistance

The genetic determinant which confers methicillin resistance is termed mec and it is chromosomal derived. Methicillin resistance rises by acquisition of a staphylococcal cassette chromosome SCCmec, which has the *mecA* gene. Expression of this gene yields PBP2a, a penicillin binding protein with reduced affinity for ℘lactam rings. Methicillin resistance is genetically and biochemically complex. The PBP2a is encoded by 2.1 kilobase of DNA mec region (Chambers, 1997).

Beta 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 covalently bind ℘lactam antibiotics at 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. Unlike the other PBPs, this additional PBP (PBP2a) has a low binding affinity for beta-lactam antibiotic (Brown and Reynolds, 1980).

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 (Hiramatsu *et al.*, 1996). The acquisition of mec DNA is considered to be the first genetic requisite for methicillin resistance of staphylococci. The PBB2a gene has been shown to be part of mec DNA. PBP2a may have evolved from the fusion of the genes for ℘lactamase and a PBP from a non staphylococcal source, PBP2a is an inducible by beta lactam antibiotics, although the protein can be produced constitutively. Although all cells within a heterogenous strain produce PBP2a, only rare cells, perhaps as few as 1 in 10^6, express methicillin resistance.

Expression of PBP2a is controlled by two regulator genes on mec DNA, mecI and mecR1, located upstream of mecA, which encode mecA repressor protein and signal transducer protein, respectively. An MRSA carrying intact mecI and mecR1 together with mecA has been called pre-MRSA, which is represented by prototype *S. aureus* strain N315. Since intact mecI product strongly represses the expression of PBP2a, the pre-MRSA is apparently methicillin susceptible (Kuwahara *et al.,* 1996). Hence, it is hypothesized that removal of the repressor function for mecA is a prerequisite for constitutive expression of methicillin resistance in *S. aureus* with mec DNA. Indeed, the deletion of mecI or point mutations in the mecI gene has been found in a number of methicillin-resistant staphylococcal isolates. In some strains, point mutations were detected in the 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.

CHAPTER- IV

4. MATERIALS AND METHODS

4.1 Materials

A list of materials used for this study is given in the Appendix I.

4.2 Study period

This study was carried out from June 2007 to December 2007.

4.2.1 Laboratory Setting

Microbiology section of Pathology Department of Shree Birendra Hospital, Chhauni.

4.2.2 Study Objects

Nasal swab samples were taken from doctors, paramedical officers, nurses, nursing assistants, nursing volunteers, laboratory technicians, medical trainees and sweepers.

4.2.3 Sample size

Altogether 264 nasal swab samples were taken.

4.2.4 Data collection

A structured questionnaire form (Appendix VI) was used to record age, sex, occupation and service years etc. for screening of *Staphylococcus aureus* as nasal carrier from hospital personals.

4.2.5 Sample collection

Sterile cotton swab dipped in physiological saline was used for the collection of sample from anterior nares. The cotton swabs were used to sample both the nostrils. The swab was introduced into first nostril 1-2cm inside, which was rotated 2-3 times with gentle pressure for 3-5 seconds in same manner; nasal swab from the second nostril was collected. Then, the swabs were kept immediately in the sterile test tube and plugged with cotton. All the tubes were labeled with personnel's identification number and other required information. In case of delay, samples were usually stored at 4°C in the refrigerator.

A nasal swab samples were collected as described by standard protocols (Cheesbrough, 2000).

4.2.6 Sample processing

The collected swab samples were put into the sterile cotton capped test tubes and transported to the laboratory immediately for inoculation into Mannitol Salt Agar (MSA) for the isolation of *Staphylococcus aureus.*

4.3 Isolation of organism

The inoculated MSA plates were incubated at 37°C for 24 hours, *S. aureus* colonies were identified on the basis of colony characteristics, Gram staining and biochemical tests. Mannitol fermenting colonies of *S. aureus* surrounded by yellow zones due to acid production were selected for further processing.

4.3.1 Subculture on Nutrient Agar (NA)

Mannitol fermenting typical yellow colonies from MSA were subcultured on nutrient agar (NA) at 37°C for 24 hours for further processing. Colony having round, convex, opaque, smooth glistening surface with colony diameter 2-3mm were indicative of Staphylococci. Most staphylococci produce 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, Voges-Proskauer (VP) test, deoxyribonuclease (DNase) test were performed from isolated colonies.

Following biochemical tests were performed for the confirmatory identification of *S. aureus.*

A. Gram's staining

Procedure (Cheesbrough, 2000; 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 clean 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.
iii. After cooling, the smear was flooded with crystal violet solution for 1 minute and then rinsed with distilled water.
iv. The smear was flooded with Gram's Iodine solution for 1 minute, after which it was rinsed off with water.
v. The smear was then decolorized with acetone alcohol for 10-15 seconds and rinsed off with water.
vi. The decolorized smear was again flooded with safranine for 1 minute and then washed with water.
vii. Finally, the slide was blot dried with absorbent paper and then examined under oil immersion lens at 100X.

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

B. Catalase test

Procedure (Cheesbrough, 2000)

i. A drop of 3% hydrogen peroxide was put in a clean slide.
ii. The pure colony from nutrient agar was taken with the help of glass rod or plastic stick.
iii. The test organism was put 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*.

C. Coagulase test

Coagulase test is the best single test to confirm *S. aureus*. It can be performed by slide or tube method.

I. Slide coagulase test (Clumping factor test)

Procedure (Cheesbrough, 2000)

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 coagulase positive test.
v. For positive as well as negative coagulase tests, further confirmation was done by tube coagulase test.

II. Tube coagulase test (Free coagulase test)

Procedure (Collee *et. al.*, 1996)

i. One ml of 1:6 diluted plasma in saline (0.85% NaCl) was placed in small test tube.
ii. The test organism was mixed in the plasma containing test tube.
iii. Control organism was inoculated in the same way as above and a tube containing plasma only was also kept.
iv. All the tubes were inoculated at 37°C and clot formation was examined at intervals of one hour for upto 4 hours by tilting the tubes.
v. The negative tubes i.e. no clot formation were kept at room temperature overnight and were re-examined.
vi. Stiff gel formation or large clots floating in the tube was indicative of positive test.

D. Voges-Proskauer (VP) test

This test detects the production of acetyl methyl carbinol or acetoin from carbohydrate fermentation.

Procedure (Forbes *et. al.*, 1998)

i. Two ml of sterile MRVP broth was taken in a test tube.
ii. A loopful of test organism was inoculated at 37°C for 48 hours.
iii. Baritt's reagent (consisting 3 volumes of 5% alpha-napthol and 1 volume of 40% KOH solution) 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.

E.Oxidation-Fermentation (O/F) test

Procedure (Collee *et. al,* 1996; Forbes *et. al,* 1998)

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

F. Deoxyribonuclease (DNase) production test

Procedure (Collee *et. al.,* 1996)

i. Sterile DNase agar plate was taken and 8 equal compartments were made my marker.
ii. The organisms to be tested were spot inoculated and incubated for 24 hours at 37°C.
iii. After incubation, the plate was flooded with 1N hydrochloric acid to precipitate unhydrolysed DNA which produces white cloudiness in the medium.

DNase positive cultures were surrounded by clear areas where the DNA had been hydrolysed.

4.4 Antibiotic susceptibility test

All *S. aureus* isolated from nasal screening process were subjected to in vitro antibiotic susceptibility test by using modified Kirby-Bauer disc-diffusion method as recommended by NCCLS. The antibiotics used in this study were amoxicillin (10mcg),

chloramphenicol (30mcg), ciprofloxacin (5mcg), cotrimoxazole (25mcg), erythromycin (15mcg), gentamicin (10mcg), methicillin (5mcg), penicillin (10 units) and tetracycline (30mcg). Methicillin resistant isolates were further tested with Vancomycin (30mcg).

Procedure (Forbes *et. al.,* 1998; WHO, 1996)

i. Mueller Hinton Agar (MHA) was prepared, sterilized and poured into sterile plates.
ii. Pure culture of the test organism was inoculated into a sterile nutrient broth tube.
iii. The culture tube was incubated at 37°C for upto 4 hours to obtain turbidity equivalent to the density of Mac Farland's Nephalometer standard no. 0.5 ($1.5x10^8$ CFU/ml of cell density).
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. MHA plate was swabbed uniformly by rotating the plate.
vi. With the closed petridish 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 forceps and placed carefully on the surface of swabbed medium, at least 15 mm apart from 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 were then incubated at 37°C for 24 hours.
x. After incubation, the diameter of the zone of inhibition shown by each antibiotic disc was measured.

The organism was considered as resistant, intermediate or sensitive by comparing the zone sizes given in the chart Appendix IV.

4.5 Quality control for the tests

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* ATCC25923 was used as a reference strain. For quality control, media, antibiotics and reagents were prepared, stored and utilized as recommended by the manufacturing company. Antibiotic discs were stored at refrigerator temperature. For each batch of test, a positive and negative known culture was used for color reaction, biochemical test and antibiotic sensitivity test.

4.6 Data analysis

All data entries, association between dependent variables (*S. aureus* isolation) and independent variables (age, sex, ward) were analysed by descriptive analysis, cross tabulation and statistical analyses using SPSS version 11.5. Presumptive associations between or among attributes were examined by Chi square ($\chi 2$) test.

CHAPTER V

5. RESULTS

5.1 Distribution of Study Objects

Table 5.1: Gender wise Nasal carrier rate of S. *aureus*

Gender	No. of Samples Taken	Carrier *S. aureus*	Significance (P-value)*
Male	174	47 (27.02%)	0.21
Female	90	18 (20%)	
Total	264	65	

*Calculated by χ^2 test.

Out of the 264 nasal swabs, 174 nasal swab samples from male, 27.02% (n=48) *S. aureus* were isolated. Similarly, 90 nasal swab samples from female 20% (n=18) *S. aureus* were isolated. Statistically, there is no relationship of nasal *S. aureus* carrier rate between male and female carriers.

Table 5.2: Age wise distribution of nasal carrier rate of *S. aureus*

Age Group (year)	Male		Female	
	Total Samples	**No. and % isolates.**	**Total Samples**	**No. and % isolates.**
20-25	56	14 (25)	48	9(18.75)
26-30	60	19(31.66)	19	6(31.57)
31-35	30	8(26.66)	9	2(22.22)
36-40	10	3(30)	9	1(11.11)
41-45	14	2(14.28)	2	0
> 45	4	1(25)	3	0
Total	174	47	90	18

A total of 65 *S. aureus* was isolated from 264 samples. From male, 47 *S.aureus* and from female, 18 *S. aureus* were isolated. In male the prevalence of nasal carrier of *S.aureus* was found to be highest in the age group of 26-30 yrs. (31.66%) followed by 36-40 (30%), 31-35 (26.66%), 20-25 (25%), >45 (25%) and 41-45 (14.28%). In female, the highest prevalence of nasal carrier of *S.aureus* was found in the age group of 26-30 (31.57%) followed by 31-35 (22.22%), 20-25 (18.75%) and 36-40 (11.11%). No *S.aureus* was found in the age group of 41-45 years and > 45 years in female.

Table 5.3: Occupation-wise distribution of *S.aureus*

S.N.	Occupational group	No. of samples	Carrier of *S.aureus*	Significance (p-value)*
1	Doctors	19	1(5.27%)	
2	Intern doctors	22	7(31.82%)	
3	Paramedical officers	4	0(0%)	
4	Nurses	43	9(20.93%)	0.334
5	Nursing Assistants	62	21(33.87%)	
6	Nursing Volunteers	8	2(25%)	
7	Lab. technicians	30	6(20%)	
8	Medical trainees	58	16(27.59%)	
9	Sweepers	18	3(16.67%)	
	Total	264	65(24.62%)	

* Calculated by Fisher's exact test.

Hospital personnel related to health care was further classified into doctor, paramedical officer, nurses, nursing assistant, nursing volunteer, laboratory technician, medical trainee and sweeper. The maximum number of samples were collected from nursing assistant (n=62) followed by medical trainee (58) and nurses (n=43). Among the hospital personnel, highest percentage nasal carrier rate was found in nursing assistant 33.87% (n=21) followed by intern doctors 31.82% (n=7), medical trainee 27.59% (n=16), nursing volunteer 25% (n=2), nurses 20.93% (n=9), Laboratory technician 20% (n=6), sweeper 16.67 (n=3), doctors 5.27% (n=1) and paramedical officers 0%.But statistically, there is no significant relationship of nasal *S.aureus* carrier rate among different occupational group.

Table 5.4: Ward and department wise distribution of *S.aureus*

S.N.	Wards and Departments	Total no. of samples collected	Total *S.aureus* isolated
1	Pathology	42	10(23.80%)
2	Medical I	13	3(23.07%)
3	Medical II	15	2(13.33%)
4	Medical III	13	7(53.84%)
5	Surgical Officer Cabin	14	6(42.85%)
6	Surgical I	12	3(25%)
7	Surgical II	12	4(33.33%)
8	Surgical III	15	4(26.66%)
9	Post Operative Ward	11	3(27.27%)
10	Intensive Care Unit	18	4(22.22%)
11	New Family Ward	12	2(16.66%)
12	Pediatric	8	0
13	Haemodialysis	7	0
14	Gynecology	9	1(11.11%)
15	Very important person ward	7	2(28.57%)
16	Intensive Trauma Care	10	2(20%)
17	Ear Nose and Throat	8	3(37.5%)
18	Orthopedic	4	2(50%)
19	Physiotherapy	13	3(23.07%)
20	Trauma Hall	7	1(14.28%)
21	General Out Patient Department	14	3(21.42%)
	Total	264	65

The nasal samples were collected from different wards. The wards included in the study was pathology, medical I (MI), medical II (MII), medical III(MIII), surgical officer

cabin (SOC),surgical I(SI), surgical II(SII), surgical III (SIII) , post operative Ward (POP), intensive care unit (ICU), new family ward (NFW), pediatric ward, haemodialysis unit, gynecology Ward, Very Important Person ward (VIP), intensive trauma care unit (ITCU), ear, nose and throat ward(ENT), orthopedic ward, physiotherapy ward, trauma hall and general out patient department (GOPD).

Highest nasal carriage rate of *S.aureus* was from medical III 53.84% followed by orthopedic 50%, SOC 42.85%, ENT 37.5% and SII 33.33%.

5.2 Antibiotic Susceptibility Pattern of Isolated *S. aureus*

Table 5.5: Antibiotic Susceptibility pattern of *S.aureus* isolated from the nasal swab.

Antibiotic Tested	Total number of *S.aureus* isolates	Number and Percentage of *S.aureus*	
		Susceptible	Resistant
Amoxicillin	65	15(23.1%)	50(76.9%)
Penicillin	65	40(61.5%)	25(38.5%)
Ciprofloxacin	65	59(90.8%)	6(9.2%)
Cotrimoxazole	65	51(78.8%)	14(21.5%)
Erythromycin	65	59(90.8%)	6(9.2%)
Tetracycline	65	63(96.9%)	2(3.1%)
Chloramphenicol	65	62(95.4%)	3(4.6%)
Gentamycin	65	56(86.2%)	9(13.8%)
Methicillin	65	65(100%)	0(0%)

Among 264 nasal samples, *S.aureus* was isolated from 24.62% (n=65) samples. All the samples were cent percent sensitive towards Methicillin and Vancomycin.The bacterial

isolates showed highest resistant towards Amoxicillin (76.9%), followed by Penicillin (38.5%), Cotrimoxazole (21.5%), Gentamycin (13.8%), Erythromycin (9.2%), Ciprofloxacin (9.2%), Chloramphenicol (4.6%) and Tetracycline (3.1%).

Table 5.6: Gender wise distribution of MDR *S.aureus*

Gender	Total *S.aureus* isolates	No. and percentage of MDR *S.aureus*	Significance (P value)*
Male	47	10(21.27%)	0.721
Female	18	3(16.66%)	
Total	65	13(20%)	

* Calculated by Fisher's exact test

The *S.aureus* resistant to three or more than three commonly prescribed antibiotics were considered as MDR. Out of 65 *S.aureus* isolates only 20% (n=13) were MDR. Among 65 isolates, male constituted 21.27% (n=10) whereas female constituted 16.66% (n=3) MDR strains of *S.aureus*. The distribution of MDR strains of *S.aureus* among male and female was not found to be statistically significant.

Table 5.7: Ward wise distribution of MDR *S.aureus*

Department	antibiotic resistance pattern		
	Resistance towards 1 antibiotic	Resistance towards 2 antibiotics	Resistance towards 3 or more than 3 antibiotics
Pathology	4	5	1
Medical I	1	0	1
Medical II	2	0	0
Medical III	3	1	1
SOC	1	1	2
Surgical I	0	1	1
Surgical II	1	0	1
Surgical III	2	1	1
POP	1	0	0
ICU	1	0	3
NFW	1	0	0
Gynecology	1	0	0
VIP	2	0	0
ITCU	0	1	0
ENT	1	1	0
Orthopedic	2	0	0
Physiotherapy	1	0	1
GOPD	1	0	1
Total	**25**	**11**	**13**

The isolated MDR strains of *S.aureus* were further arranged according to respective wards from where samples were collected. The highest number of MDR strains of *S.aureus* was isolated from intensive care unit (ICU) 75% (n=3) followed by SOC 50%

(n=2), M I 50% (n=1), S I 50% (n=1), S II 50% (n=2), Physiotherapy 50% (n=1), GOPD 50 % (n=1), S III 25% (n=1), M III 20% (n=1) and Pathology 10% (n=1).

No MRSA (Methicillin Resistant *S.aureus*) was found in this study.

CHAPTER VI

6. DISCUSSION AND CONCLUSION

6.1 Discussion

The anterior vestibule of the nose is an important reservoir of *S. aureus* and dissemination of this organism by carriers is important in the perpetuation and spread of Staphylococcal disease. Staphylococci are responsible for more than 80% of the suppurative diseases found in medical practice and are a major problem in newborn nurseries and neonatal intensive care units. Additionally, the emergence of methicillin-resistant patterns highlights the importance of finding methods to treat staphylococcal disease, the staphylococcal carrier state and associated nososcomial outbreaks. Bryan *et al.,* 1980 has reported that 70% of hospitals continue to obtain cultures from person during staphylococcal disease outbreaks and about 40% prescribe tropical antibiotic ointment for person with staphylococcal positive culture.

Approximately 20% of individuals almost always carry one type of strain of *S. aureus* and are called persistent carriers. A large proportion of population (~60%) harbors *S. aureus* intermittently, and the strains change with varying frequency. Such persons are called intermittent carriers. Finally, minorities of people (~20%) almost never carries *S. aureus* and are called non-carriers (Williams 1963). Persistent carrier is more common in children than in adults and many people change their pattern of carriage between the ages of 10 to 20 years. The reasons for these differences in colonization patterns are unknown. The ecological niches of *S. aureus* strains are the anterior nares. When the nares are treated topically to eliminate nasal carriers, in most cases the organism also disappears from other areas of the body. (Petrez-Fontan *et al.,* 1993)

Methicillin-resistant *S. aureus* (MRSA) was first detected in 1960s, and since that time it has spread rapidly worldwide, becoming a leading cause of nosocomial infection. Due to increasing number of infections caused by MRSA strains, which are now most often multi-resistant, therapy has become problematic. Therefore, prevention of staphylococcal infection is now more important than ever. Carriage of *S. aureus* in the nose appears to play a key role in the epidemiology and pathogenesis of infection.

S. aureus is a well-recognized cause of serious community-acquired infections and is a leading cause of nosocomial infections. There is a substantial body of evidence that individuals who are asymptomatic nasal carriers of *S. aureus* are at increased risk of developing serious staphylococcal infections. Several studies conducted in the 1950s and 1960s demonstrated that the incidence of *S. aureus* surgical wound infections was higher among nasal carriers than among non-carriers. Similarly, a recent case-control study found that preoperative nasal carriage of *S. aureus* was significantly more common among cardiothoracic surgery patients who developed *S. aureus* wound infections then among controls. Recent reports from two surgical site infection surveillance systems indicate that the overall frequency of post-operative infections is approximately 3% to 4% but the incidence varies with the hospital and the type of surgery. Although this complication rate many seem low, surgical site infections are still very much dreaded, especially in cardiothoracic and orthopedic surgery, because of their very high morbidity and often devastating consequences (Boyce 1996).

In this study, a total of 264 nasal swabs were taken from doctors, intern doctors, paramedical officers, nurses, nursing assistants, nursing volunteers, laboratory technicians, medical trainees and sweepers of different wards in hospital.

Out of total 264 nasal samples, 24.62% (n=65) *S. aureus* was isolated. The finding of this nasal carrier rate was in the range of 10-40% as mentioned by Easmon (1990) but this rate was higher as reported by Baron (1998) 10-15% and lower as reported by Humpreys (1997) 30%, Cheesbrough (2000) 40% and Mirkin (2000) 44%.

Staffs in the hospitals tend to be colonized with *S. aureus* depending on the length of their stay in hospital and carrier rate of *S. aureus* may increase during their prolonged stay. Cruickshank *et al.*, (1975) showed 30-40% nasal carriage rate and 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. It has been previously established that *S. aureus* carriage among patients and staffs has been found to be directly correlated with the occurrence of nosocomial infection due to the organisms (Chiang and Climo, 2002).

In this study, among 65 positive isolates from nasal swab 27.02% (n=47) were from males and 20% (n=18) were from females. The distribution of nasal carriage of *S. aureus* between male and female was not found to be statistically significant.

Nasal carrier rate was also studied in different age groups starting from 20-25 years, 26-30 years, 31-35 years, 36-40 years, 41-45 years and above 45 years. In both male and female, highest nasal carrier rate of *S. aureus* was found in the age of 26-30 years. In male, it was 31.66% while in females 31.57%. This was followed by age group of 36-40 years (30%) and 31-35 (26.66%) years in male and age group of 31-35 (22.22%) and 20-25 (18.75%) in female.

Among hospital personnel, maximum nasal carrier rate was found in nursing assistant 33.87% (n=21) followed by intern doctors 31.82% (n=7) and medical trainee 27.59% (n=16).

The association of nasal carrier among different occupational group was not significant. Regarding the department or ward wise distribution of nasal carrier, highest nasal carrier rate of *S. aureus* was from Medical III 53.84% followed by Orthopedic 50%, SOC 42.85%, ENT 37.5%, VIP 28.57%, POP 27.27%, Surgical III 26.66% and Surgical I 25%. No *S. aureus* was isolated in pediatric ward and haemodialysis unit.

The carriage pattern of *S. aureus* in nursing assistant indicates that they are highly exposed to hospital environment, maximum contact with the patients in the hospitals. The highest carriage rate of *S. aureus* is seen in intern doctors and medical trainees. As *S. aureus* was isolated from maximum wards, ward personnel should be aware of contamination wounds of patient of their nasal flora. Because nasal carrier represents an important risk factor for infection in the affected individual, and serves as a source from which the organism can be spread to others, eradicating nasal carriage of *S. aureus* with mupirocin ointment is highly recommended. Many authorities currently consider it to be the agent of choice for eradicating *S. aureus* nasal carriage (Boyce, 1996). Rifampin may be an effective antistaphylococcal antibiotic and could be used to control the carrier state in high risk situation (Mcnally *et al.,* 1984). Lysostaphin appears to be slightly more effective than conventional topical antimicrobial therapy in reducing nasal carriage of staphylococci in this rigorously defined population of persistent carriers. In this study, we used nasal mupirocin in MDR hospital personnel 3 times a day in the anterior nares for 5 days and again nasal swabs were taken for isolation of MDR *S. aureus* but no *S. aureus* was isolated from the sample, from this we concluded that nasal mupirocin ointment eradicate the nasal *S. aureus* carrier.

S. aureus spreads mostly by direct contact. The transmission can be either exogenous or endogenous. Transmission by contact occurs when *S. aureus* or infectious material in transmitted to another patient through direct contact with an infected patient or with a healthy carrier carrying a virulent pathogen. *S. aureus* usually colonizes skin and mucous membranes.But more often common in newborns, diabetic, patients with skin diseases, and haemodialysis patients.

All the nasal samples were cent percent sensitive to Methicillin. The isolates showed highest resistant to amoxicillin (76.9%), followed by penicillin (38.5%), cotrimoxazole (21.5%), gentamycin (13.8%), erythromycin (9.2%), ciprofloxacin (9.2%), chloramphenicol (4.6%) and tetracycline (3.1%). Thus, tetracycline, chloramphenicol, ciprofloxacin and erythromycin are found to be most effective drugs of choice to treat

the *S. aureus*. But the isolates showed high percentage of resistance towards β-lactam agents which include Amoxicillin (76.9%) and penicillin (38.5%). This clearly indicates the higher prevalence of β-lactamase enzyme among the isolates. Uses of antimicrobial drugs in long-term care facilities have created a large reservoir of resistant strains in nursing homes (Weinstein, 1998).

In this study 20% (n=13) of the isolates were MDR *S. aureus*. In male hospital personnel 21.27% of *S. aureus* were found to be MDR; while in female hospital personnel, 16.66% of them were found to be MDR.The distribution of MDR *S. aureus* among male and female was not found to be statistically significant. Ahmed *et al.*, (1998) reported that medical personnel were colonized with more antibiotic resistant isolates than non-medial personnel.

The isolated MDR strains were arranged with respect to wards. All the ward personnel harbor *S. aureus* in their nasal cavity except haemodialysis and pediatric ward during the study period. The highest percentage of MDR *S. aureus* was isolated from Intensive Care Unit (ICU) 75% (n=3) followed by SOC 50% (N=2), MI 50% (n=1), SI 50% (n=1), SII 50% (n=2), MIII 20% (n=1) and pathology 10% (n=1).

In a study to detect healthy MRSA carrier in a hospital in Abidjan out of 269 *S. aureus* carriers 38.7% were MRSA carriers. A study done on MRSA nasal carriage rates of hospital staff and outpatient control group were found to be 6.0% and 2.6% (Dimitrov *et al.*, 2003). Yagci *et al.*, reported 17.3% *S. aureus* in Turkish children. Uemura *et al.*, found 36.0% *S. aureus* from nasal swabs of healthy volunteers. Another study canducted in Taiwan 25% children had *S. aureus* isolated from nasal swabs.

Study done on nasal carriage by Sapkota *et al.*, reported 23.5% MDR *S. aureus* in Bir Hospital, but no MRSA was isolated. However, Pant *et al.*, reported 9.5% MRSA from nasal carrier of hospital personnel in Nepal Medical College Teaching Hospital. Shah *et al.*, reported 30.30%, 10.5% and 2.7% MDR *S. aureus* as nasal carrier from patients,

staffs and visitors respectively in TU Teaching Hospital. But no MRSA was isolated in our study. No MRSA was found in Shree Birendra Hospital Chhauni, it may be due to absence of MRSA strains in this hospital. To be sure, further research and study should be done. However, the isolates were found to be MDR *S. aureus*.

Worldwide increase in MDR *S. aureus* is associated with the growing and frequent indiscriminate use of antibiotics in human. In recent years, drug resistant bacteria have given rise to several serious outbreaks of infection with many deaths. This has led to need for national and international surveillance programme to monitor antibiotic resistance in bacteria by susceptibility testing using reliable methods that generate comparable data. The availability of microbiological and epidemiological information would help clinicians in selecting the most appropriate antimicrobial drug for the treatment of the microbial infection.

MDR strains are the biggest problem for hospital personnels. The presence of high percentage of MDR in nasal cavity of hospital personnel may signify the possible danger of transmitting these strains to patients. Because nasal carriage in hospital nurses and doctors is an important risk factor for infection to patients, eradicating nasal carriage of *S. aureus* is a useful control measures. Screening of *S. aureus* as nasal carrier from hospital personnel is necessary in hospital outbreaks of MDR *S. aureus* infections.

6.2 Conclusion

The research study showed MDR *S. aureus* but methicillin resistant *S. aureus* was not found among the isolates. Methicillin showed 100% sensitivity. However, regular monitoring of methicillin sensitivity should be carried out. Similar type of research study should be conducted frequently,it will help to know the current microbial status of the hospital personnel and can aid in control of nosocomial infection.

CHAPTER VII

7. SUMMARY AND RECOMMENDATION

7.1 Summary

1. *S. aureus* as nasal carrier is the most common causative factor in the perpetuation and spread of staphylococcal disease and risk factor for community acquired and nosocomial infection.

2. In this study, altogether 264 nasal swabs were taken from hospital personnel of different wards. Among 65 positive isolates from nasal swab 27.02% (n=47) were from males and 20% (n=18) were from females.

3. In this study, the highest nasal carrier rate of *S. aureus* was found in the age group of 26-30 years of which 31.66% was male and 31.57% from female.

4. In this study, the male carrier rate of *S. aureus* was found to be highest in Nursing assistant group 33.87% (n=21) followed by intern doctors 31.82% (n=7) and medical trainee 27.59% (n=16).

5. In this study, the department wise distribution was found to be highest in Medical III 53.84% (n = 7) followed by orthopedic 50% (n = 2).

6. The *S. aureus* isolates showed highest resistant to amoxycillin (76.9%) followed by penicillin (38.5%), cotrimoxazole (21.5%), gentamycin (13.8%), erythromycin (9.2%), ciprofloxacin (9.2%), chloramphenicol (4.6%) and tetracycline (3.1%). All isolates were sensitive to methicillin.

7. Out of 65 *S. aureus* isolates isolated from nasal swab, only 20% (n=13) were MDR. No MRSA was found in this study.

7.2 RECOMMENDATIONS

1. Regular screening of hospital staffs and health care personnel for the carriage of *S. aureus* should be done to minimize the spread of the staphylococcal infection.

2. A number of procedures such as antibiogram, biotyping, phagetyping, serotyping and plasmid profiling of the isolates should be simultaneously carried out for complete characterization of the isolates to establish relationship with the epidemic strains in hospital.

3. Indiscriminate and overuse of antibiotics should be minimized to prevent the emergence of multi-drug resistant organism. For this, formulation and implementation of strict rules and regulations of antibiotic use seems to be necessary.

4. Surveillance should be done regularly from the hospital premises, operation theatre, air and dust of wards to stop the spread of multi-drug resistant staphylococcal infection.

References

Ahmed AA, Belkum A, Fahal AH, Abu Elnor AE, Abouigroun AM, Vandenbergh FQ and Verbrugh HA (1998) Nasal carriage of *S. aureus* and epidemiology of surgical-site infections in a Sudanese University Hospital. J. Clin. Microbiol. 36 (12): 3614-8

Akoua KC, Dje K, Toure R, Guessennd N, Acho B, Faye Kette H, LouKou YG and Dosso M (2004) Nasal carriage of methicillin-resistant Staphylococcus aureus among health care personnel in Abidjan, Dakar J. Med. Microbiol. 49 (1): 70-4

Alvarez FL, Palomar M, Insausti J, Olaechea P, Cerda E, Sanchez JG and De la Torre MV (2006) *Staphylococcus aureus* nosocomial infections in critically ill patients admitted in intensive care units. Med. Clin (Barc) 126: 641-6

Amir L (2002) Breastfeeding and *Staphylococcus aureus*: three case reports. Breastfeed Rev. 10(1): 15-8

Ananthanarayan R and Panikar CKJ (2002) Textbook of Microbiology. 6th edition. Orient Longman Ltd. Chennai, India. 210-18

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

Anwar MS, Jaffery G, Rehman KU, Tayyib M and Bokhari SR (2004) *Staphylococcus aureus* and MRSA nasal carriage in general population. J. Coll. Physicians Surg. Pak. 14 (11): 661-4

Baired Parker AC (1979) Methods for identifyiong Staphylococci and Micrococci. The society for Applied Microbiology Technical Series No. 14. Academic Press, London.

Baron EJ, Peterson LR and Finegold SM (1998) Bailey and Scott's Diagnostic Microbiology 10th edition. Mosby Publication, USA. 607-18

Bennett JV and Brachman PS (1989). Hospital infections. 1st edition. Little Brown and Company. Boston.

Boyce JM (1996) Preventing Staphylocococcal infections by eradicating nasal carriage of *Staphylococcus aureus*. Infection Control and Hospital Epidemiology. 17(12)

Brown DFJ and Reynolds PE (1980) Intrinsic resistance to beta lactam antibiotics in *Staphylococcus aureus*. FEBS Lett. 122: 275-8

Bryan CS, Wilson RS, Maede P and Sill LG (1980) Topical antibiotic ointment for staphylococcal nasal carriers survey of current practices and comparison of bacitracin and vancomycin ointments. Infect. Control (Thorofare) 1: 153-156

Centre for Disease Control (1992) Public health facts: Surveillance, prevention and control of nosocomial infections. Morbid. Mortal. Wkly. Rep. 41: 783-7

Center for Disease Control (2000) Monitoring Hospital-Acquired infections to promote patient safety United States, 1990-199. Morbid. Mortal. Wkly. Rep. 49: 149-53

Center for Disease Control and Prevention (2004) National nosocomial infections surveillance (NNIS) System report, data summary from January 1992 through June 2004, Issued October 2004, AJIC special article.

Chiang FY and Climo M (2002) *Staphylococcus aureus* carriage and health care-acquired infection, Curr. Infect. Disease Reports. 4: 498-504

Chakraborty M (2004) A textbook of Microbiology 2nd edition. New Central Book Agency Pvt. Ltd. Calcutta, India. 206-10

Chamber HF (1997) Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clinical Microbiology Reviews. 10: 781-91

Cheesbrough M (2000) District Laboratory practice in tropical countries Part II. Cambridge University Press, Low Price edition, India pp 196-274

Coia JE, Hussain NI and Platt DJ (1990) Plasmid profiles and sensitivity of Methicillin resistant isolates of *Staphylococcus aureus* from hospitals and the community. J. Med. Microbiol. 27: 2171-6

Collee JG, Fraser AG, Marmion BP and Simmon A (1996) Mackie and McCartney Practical Microbiology, 14th edition Churchill Livingstone, Longman Group, UK. pp 1210-15

Collins CH and Lyne PM (1983) Microbiological Methods. 6th edition. Butterworths and Company Publishers Ltd. London. pp 178-82

Cruickshank R (1975) Medical Microbiology. Vol II. The practice of Medical Microbiology, 12th edition. Churchill Livingstone, London, UK. pp 432-6

Dimitrov T, Udo EE and Frover S (2003) Point surveillance of *Staphylococcus aureus* carriage among medicine staff in infectious diseases hospitals. Medical Principles and Practice. 12: 139-44

Easmon CSF and Goodfellow M (1990) *Staphylococcus* and *Micrococcus.* In Topley and Wilson's Principles of Bacteriology, Virology and Immunity. 8th edition (Eds.) Parker MT and Duerdon BI. London. UK.2: 161-81

Forbes BA, Sahm DF, Weissfeld AS (2000) Bailey and Scott's Diagnostic Microbiology, 11th edition. Mosby Inc. pp 68-72

Glenister HM, Taylor LJ, Cooke FM and Bartlett CLR (1992) A study of surveillance methods for detecting hospital infection. Public Health Laboratory service. UK

Greenwood D, Slack RCB and Peutherer JF (2006) Medical Microbiology. 16th edition. Churchill Livingstone. UK. 168-73

Gupta S (1999) The short Textbook of Medical Microbiology. 7th edition. Jaypee Brothers Medical Publishers Pvt. Ltd. India.

Hiramatsu K, Kondo N and Ito T (1996) Genetic basis for molecular epidemiology of MRSA. J. Infect. Chemother. 2: 117-29

Hollis RJ, Barr JL, Deebbeling BN, Pfaller MA and Nenzel RP (2003) Familial carriage of methicillin-resistant *Staphylococcus aureus* and subsequent infection in a premature neonate. Clin Infect Dis. 21 (2): 328-32

Holt JG, Kreig NR, Sneath PHA, Stanley JT and Williams ST (1994) Bergey's Manual of Determinative Bacteriology. 9th edition. Williams and Wilkins. London.

Holtfreter S. Roschack K, Eichler P, Eske K, Holtfreter B, Kohler C, Engelmann S, Hecker M, Greinacher A and Broker BM (2006) *Staphylococcus aureus* carriers neutralize superantigens by antibodies specific for their colonizing strain: a potential explanation for their improved prognosis in severe sepsis. J. Infect. Dis. 193: 1275-8

Humpreys H (1997) Medical Microbiology. 15th edition. Churchill Livingstone. UK

Jevaratnam D, Edgeworth JD and French GL (2006) Enhances surveillance of Methicillin-resistant *Staphylococcus aureus* bacteraemia in a London teaching hospital. J Hosp Infect 7: Epub ahead of print.

Kaplan SL, Hulten KG and Gonzalez BE (2005) Three years surveillance of community-acquired *Staphylococcus aureus* infection in children. Clin. Infect Dis. 40: 1785-91

Kluytmans JA and Wertheim HF (2005) Nasal carriage of *Staphylococcus aureus* and prevention of nosocomial infections 33: 3-8

Kuwahara Arai K, Kondo N, Hori S, Tateda Suzuki E and Hiramatsu K (1996) Suppression of methicillin resistance in a mecA containing pre-methicillin resistant *Staphylococcus aureus* strain is caused by the mec-I mediated repression of PBP 2' production. Antimicrob Agents Chemother. 40: 2680-5

Lamichhane DR and Shrestha P (2001) Prevalence of Nosocomial Infection in TUTH. A report submitted to Nepal Health Research Council.

Lamichhane R (1999) Study of Methicillin resistance *Staphylococcus aureus* (MRSA) isolated from different clinical samples. A M.Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Lo WT, Lin WJ, Tseng MH (2007) Nasal Carriage of single clone of community acquired Methicillin-resistant *Staphylococcus aureus* among kindergarten attendees in northern Taiwan. Biomed Central Infect Dis. 7: 15-4.

Lowy FD (1998) *Staphylococcus aureus* infections. New Engl J Med. 339: 520-32

Lu P, Chn L, Peng C, Chiang Y, Chan T, Ma L and Siu LK (2005) Risk factors and molecular analysis of community methicillin-resistant *Staphylococcus aureus* carriage. J. of Clin. Microbiol. 43(1): 132- 39

M IN, Nagri BS, Hashmi K and Gauhar S (2006) Paediatric nosocomila infections: resistance pattern of clinical isolates. Pak J Pharm Sci. 19: 52-7

Mainous AG (2006) Nasal carriage of *Staphylococcus aureus* and methicillin-resistant *S. aureus* in the United States, 2001-2002. Ann Fam Med. 4: 132-37

Mayon-White RT (1991) An international survey of the prevalence of hospital-acquired infection. J Hosp. Infect. 18 (Supplement A): 43-8

McNally TP, Lewis RM and Brown RD (1984) Effect of rifampin and bacitracin on nasal carriers of *S. aureus* U.S. Air Force Medical Center Keesler, Mississippi, Antimicrobial Agents and Chemotherapy: 422-26

Mirkin G (2000) Health report. Available from www.drmirkin.com/morehealth/G167.htm

Nakahishi M, Shresta HG and Rai SK (1996) Textbook of Medical Laboratory Technology, 1st edition. Medical Education Project JICA, TUTH, Kathmandu Nepal pp 359

National Audit office (2000). The management and control of hospital acquired infection in acute NHS trusts in England. London: Stationary office.

National Nosocomial Infections Surveillance System (NNISS) (2002) NNIS System Report, data summary from January 1992 to June 2002. Am. J. Infect. Control 30: 458-75

Nouwen JL, Fieron MW, Snijders S, Verburgh HA, Belkum AV (2005) Persistent (not intermittent) nasal carriage of *S. aureus* is the determinant of CPD-related infections. Kidney Int. 67: 1084-92

Pant J (2006) Microbial study of hospital environment and carrier pattern study among staff in Nepal Medical College Teaching Hospital. A M.Sc. Dissertation submitted to Central Department of Microbiology. Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Petrez-Fontan M, Garcia-Falon T, Rasales M, Rodriguez-Caramona A, Adeva M, Rodgriguez Lozano I and Moncalian J (1993) Treat of *S. aureus* nasal carriers in continuous ambulatory peritoneal dialysis with mupirocin, long term results. Am. J. Kidney Dis. 22: 708-712

Pokhrel BM, Rawal S, Joshi HH and Kubo T (1993) Bacteriological study at Tribhuvan University Teaching Hospital, Kathmandu, Nepal. J. Inst. Med 15: 217-21

Rai SK, Pokharel BM, Tuladhar NR, Khadka JB and Upadhyay MP (1987) Methicillin resistant coagulase negative staphylococci. J. Inst. Med. 9: 23-8

Rajbhandari R (2002) Prevalence of antibiotic sensitivity pattern of methicillin resistance *Staphylococcus aureus* (MRSA) in Bir Hospital. A M.Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Riewerts Eriksen NH, Espersen F, Thamdrup R and Jensen K (1995). Carriage of *Staphylococcus aureus* among 104 healthy persons during a 19 month period. Epidemiol. Infect. 115: 51-60

Sapkota K (2007) Prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in clinical specimens from patients and screening of nasal carriage of MRSA from medical staffs of Bir Hospital. A M.Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Saxena S, Singh K and Talwar V (2003) Methicillin-resistant *Staphylococcus aureus* prevalence of community in the East Delhi area, Jpn. J. Infect. Dis 56: 54-6

Shah KB (2002) Study of nasal carriage of *Staphylococcus aureus* among the post-operative ward visitors, staff and patients of TU Teaching hospital with drug sensitivity pattern. A M.Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Shrestha I (1997) Study of β-lactamase activity by microbiological and biochemical methods in *S. aureus* isolated from healthy nasal carriers and hospital isolates. A M.Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Soysal A., Sahin H, Yogci A, Barlan I and Bakir M. (2006) The low rate of Methicillin-resistant *Staphylococcus aureus* in Turkish children. Japanese J. Infect. Dis. 59: 195-6.

Thapa S (2004) Prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in children visiting Kanti Children Hospital. A M.Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Thornsberry C (1998) The development of antimicrobial resistance in *Staphylococci.* J. Antimicrob. Chemotherap. 21 (Suppl. C) 9-16

Todar K (2002) *Staphylococcus aureus*. Research article. Deparment of Bacteriology. University of Wisconsin-Madison, USA.

Tuladhar NR (1990) Nosocomial infection in surgical patient at Tribhuvan University teaching hospital. J. Nep Med Ass. 28: 152-56|

Uemura E, Kakinohana S, Higa N, Toma C, Nakasone N. Comparative characterization of *Staphylococcus aureus* isolates from throats and nose of healthy volunteers. Japanese J Infect Dis 2004; 57: 21-24.

US Navy and Marine Crops guidelines (2005) Guidelines for the management of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections in the US Navy and Marins Crops. Navy Environmental Health Center 620 John Paul Jones Circle, Suite 1100 Portsmouth, VA 23708-2103

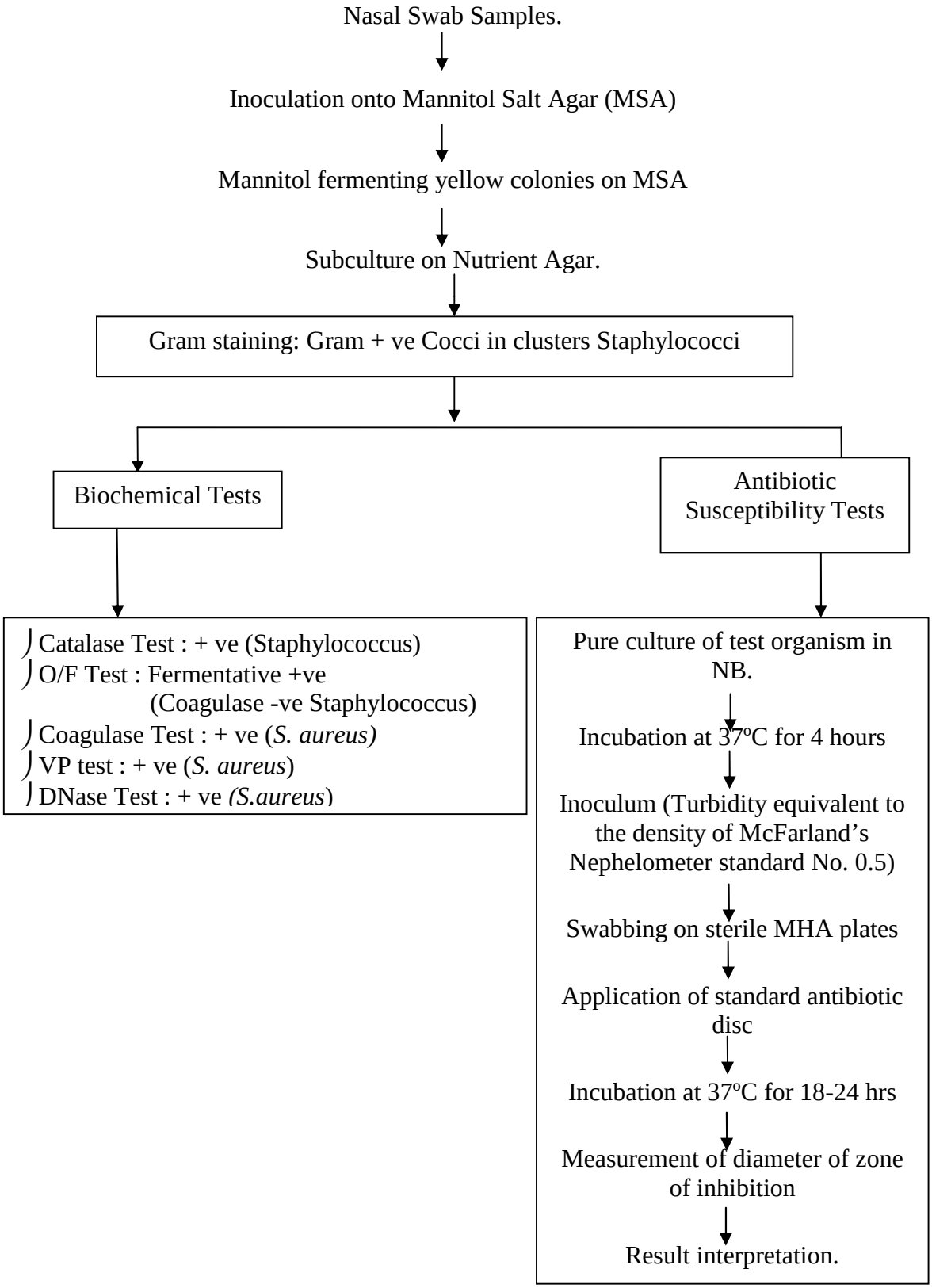
Von Eiff C, Becker K, Machka K, Stammer H and Peters G (2001) Nasal carriage as a source of *S. aureus* bacteremia. Study Group. N. Engl. J. Med. 344: 11-6

Weems JJ and Beck LB (2002) Nasal Carriage of *Staphylococcus aureus* as a risk factor for skin and soft tissue infections. Curr. Infect. Dis. Rep; 4(5): 420-425

Weinstein RA (1998) Emerging Infectious Diseases. Special Issue: Nosocomial Infection Update: 4(3) Cook County Hospital and Rush Medical College, Chicago. USA.

WHO (1996) Guidelines for antimicrobial susceptibility testing World Health Organization collaborating centre for surveillance of antimicrobial resistance. Egypt.

Williams REO (1963) Healthy carriage of *Staphylococcus aureus* : its prevalence and importance. Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, England. 27: 56-67



Flow Chart for screening of *S.aureus* from Nasal swab specimens.