CHAPTER –I

1. INTRODUCTION

A hospital is a residential establishment that provides short term medical care consisting of observational, diagnostic, therapeutic and rehabilitative services for persons suffering or suspected to be suffering from a disease or injury. However, at certain instances a hospital may not only be a place where a sick people get well but may make a sick person sicker.

Hospital acquired infection also called nosocomial infection is defined as infection acquired in hospital by a patient who was admitted for a reason other than that infection (Mayon-White *et al*, 1991). Or it can be defined as an infection occurring in a hospital or other healthcare facility in whom the infection was not present or incubating at the time of admission. This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility (WHO, 2002).

Infections spread easily and rapidly in hospital as many patients in the hospital have weakened resistance to infectious disease and many of them may be the reservoir of pathogens. Worst of all most of the common antibiotics are ineffective to control the infections due to hospital borne pathogens as many of these pathogens may be multidrug resistant strains. As such hospital infections pose a serious problem in an ICU setting.

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 infections involve not only the patients but also any one else who is in contact with the hospital including staff members, volunteers, visitors, workmen, salesmen 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). Despite rapid advances in therapeutics, diagnostics and a better understanding of the disease process, the problem of hospital acquired infections is constantly rising and its occurrence varies from hospital to hospital; it is estimated to be around 8.7% of

hospital admission. 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 (WHO, 2002). 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 *et al* (1990) has reported the prevalence of nosocomial infection to be 10.5%, majority of these being endemic infection. The occurrence rates of nosocomial infections vary 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; large medical school-teaching hospitals have higher infection rates than do small teaching 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).

1.1 Microorganisms causing hospital acquired infection

Urinary tract infections	*Escherichia coli* *Klebsiella, Serratia, Proteus* spp. *Pseudomonas aeruginosa* Fecal streptococci *C*and*ida albicans*
Respiratory infections	*Haemophilus inflenzae* *Streptococcus pneumoniae* *Staphylococcus aureus* *Enterobacteriacae* Respiratory viruses
Wounds and skin sepsis	*S. aureus* *E. coli* *Proteus* spp Anaerobes Fecal streptococci Coagulase negative staphylococci
Gastrointestinal infections	*Salmonella* spp. *Shigella sonnie* Viruses

1.2 Emergence of antibiotic-resistant microorganisms and hospital acquired infection

Antibiotic use and hospital infection control are closely associated with each other. At least 30% of hospital patients receive antibiotics and this exerts strong selective pressures on the microbial flora (Greenwood *et al,* 1997). Due to wide use of antibiotics, development of resistance is common among organisms in hospital environment. As an antimicrobial agent becomes widely used, bacteria resistant to this drug eventually emerge and may spread in the health care setting.

Bacterial resistance is clearly the major threat in hospital as a cause of nosocomial infection. Infections associated with such microorganisms can pose a serious threat to vulnerable patients such as neonates, immuno-debilitated patients, elderly patients etc. The increased occurrence of antimicrobial-resistant microorganisms (i.e. methicillin-resistant *S. aureus* (Ayliffe, 2000) or vancomycin-resistant enterococci (CDC, 1995) is a major medical concern. The spread of multiresistant strains of *S. aureus* and VRE is usually by transient carriage on the hands of health care workers Many strains of pneumococci, staphylococci, enterococci, and tuberculosis are currently resistant to most or all antimicrobials which were once effective. Multiresistant *Klebsiella* spp. and *P. aeruginosa* are prevalent in many hospitals. This problem is particularly critical in developing countries where more expensive second-line antibiotics may not be available or affordable (WHO, 2002). Thus multidrug resistant strains such as MRSA, Methicillin resistant coagulase negative staphylococci, enterococci, *E. coli*, *K. pneumoniae, P. aeruginosa, Enterobacter* spp., *Citrobacter* spp., and *Acinetobacter calcoaceticus* have become important hospital pathogens.

First emergence of methicillin resistant *S. aureus* has been reported in 1990 and that of Methicillin and multi drug resistant coagulase negative staphylococci in 1987 in Nepal (Rai *et al,* 1987; Rai *et al,* 1990).

1.3 Source of infection

Endogenous source (Self infection)

Exogenous source (Cross-infection / environmental infection)

In a health-care facility, the sources of infection, and of the preceding contamination, may be the personnel, the patients, or the inanimate environment. Human beings (patient, staff and visitors) represent the primary reservoir while all environments with appropriate condition for the growth of microbes such as air, dialysis equipment, and equipment fitted with air humidifiers, nebulizers of all types, food kept at inappropriate temperature etc. and others represent the secondary reservoir of microorganisms that may cause nosocomial infection. About 80-90% of the nosocomial infection originates from endogenous source while 10-20% is of exogenous origin (Greenwood *et al*, 1997).

1.4 Prevention

Two basic principles govern the main measures that should be taken in order to prevent the spread of nosocomial infections in health-care facilities:

- Separate the infection source from the rest of the hospital;
- Cut off any route of transmission.

The separation of the source has to be interpreted in a broad sense. It includes not only the isolation of infected patients but also all “aseptic techniques” - the measures that are intended to act as a barrier between infected or potentially contaminated tissue and the environment, including other patients and personnel. It has been reported that hospital infection control programs can prevent 33.0% of nosocomial infections especially those arising from

exogenous sources (Greenwood *et al*, 1997). Thus by monitoring for various factors and applying various precautions wherever needed, the incidence of nosocomial infection can be reduced to a great extent.

Though routine microbiological sampling of hospital air and environmental surfaces is not recommended by CDC, the study was performed since no such study have been performed previously in the hospital and it has been assumed that this study will definitely help to bring awareness about cleaning and disinfection of the environment as well as personnel hygiene to the staffs in the hospital.

So, this study was performed as a part of surveillance of hospital environment and carrier pattern of staffs in Nepal Medical College Teaching Hospital to know the current microbial status of the hospital environment and to aid in control of hospital acquired infections.

CHAPTER-II

2. OBJECTIVES

General objective

To study the microbiology of Hospital environment and carrier pattern of infectious agents among staffs in Nepal Medical College Teaching Hospital.

Specific objectives

i) To isolate and identify microorganisms from air of different wards in the hospital.

ii) To isolate and identify microorganisms from various surface samples from different wards

iii) To study the antimicrobial susceptibility pattern of the isolates isolated from the hospital environment.

iv) To isolate and identify microorganisms from hands of staffs working in the hospital.

v) To isolate and identify *S. aureus* from the anterior nares of staffs working in the hospital.

vi) To isolate and identify the *S. pyogenes* from the throat carriers of the above personnel.

vii) To study the antibiotic susceptibility pattern of the isolates.

CHAPTER-III

3. LITERATURE REVIEW

Since the very birth of the modern medicine nosocomial infection had established its identity as a dreaded event among physicians. The need to isolate patients with obvious infectious disease has been identified since ancient times. Nosocomial infection and the consequent mortality reached its peak in the 19th century (Smith and Easmon, 1990). With the growing scientific knowledge man began to conquer infection with the advent of isolation, antisepsis, asepsis and finally antibiotics. But still the efforts to lower infection risks has been continually challenged by the growing number of immunocompromised patients, antibiotic-resistant bacterial, fungal and viral superinfections, and invasive devices and procedures (Braunwald *et al*, 2001).

The segregation of fever in hospitals from general hospitals dates back to early 19th century, which was one of the early steps to control these infections. Semmelweiss (1861) on the basis of his work on puerperal sepsis then introduced hand washing with chlorinated lime which dramatically reduced infection rates. His work was largely disregarded at that time but it still holds its position in reducing maternal and child infection during delivery as one of the 5 C's (Clean surface, Clean hand, Clean cut, Clean tie, Clean stump). Florence Nightingale (1863) based on her experience stated in her book "Notes on hospitals" that mortality in hospitals especially in large crowded city was very much higher than of those treated elsewhere. She also established important principles of nursing and hospital design and hygiene. Simpson (1869) also established that sepsis, gangrene, and pyaemia were commoner in urban hospitals than in rural practice. The major change in the concept of control of nosocomial infection was brought about by "antiseptic surgery" introduced by Lister in 1867. However it has by far been replaced by aseptic surgery, but, still the step brought a major revolution in surgical practices and infection control. Aseptic surgery came into practice in the late 19th century with the introduction of surgical gloves in the USA (Smith and Easmon, 1990).

In the early 20th century cubicles and barrier nursing was introduced in the UK, which was shown to be effective in preventing spread of childhood infections. Also, Dukes (1929) recognized indwelling catheter as a means of introducing infection in the bladder. Aseptic surgery complacency was shattered by the first and second World Wars. Large open wounds and compound fracture became easily infected, even those mild antiseptics proved ineffective. The arrival of penicillin in the later years of World War 2 was a blessing for the practitioners at the time.

Penicillin with its arrival brought various miracles with it when practitioners began thinking that they conquered infections. But later with emergence of drug resistance, for the first time staphylococcal rather then streptococcal, infection dominated the scene. Penicillin resistant and later multi-drug resistant *S. aureus* strains with additional properties of transmissibility and virulence caused serious wound, burn and other sepsis. The importance of air borne and dust borne infections were considered, also the transmission of infections from hands of the attendants revived. From thence supply of clean air for operation theatres, procedures of wound dressing and provision of isolation unit and various other infection control methods has emerged till date (Smith and Easmon, 1990).

Infection Control as a formal discipline in the United States developed in the late 1950s primarily to address the problem of staphylococcal nosocomial infection. In 1970's Centers for Disease Control and Prevention (CDC, Atlanta) conducted extensive studies and found that nosocomial infection rate fell dramatically in hospitals with organized surveillance and control activities; a trained, effectual infection control physician; and one infection control practitioner per 250 beds (Braunwald *et al,* 2001). Over the ensuing years, the field of infection control, through the incorporation of epidemiologic principles and the application of statistical analysis, emerged as one facet of the broader discipline of hospital epidemiology.

According to WHO, urinary tract infection is the most common nosocomial infection; 80.0% of these infections are associated with the use of an indwelling bladder catheter. The genitourinary tract is the most common site of nosocomial infection in the intensive care unit, accounting for 20.0–40.0% of all hospital-acquired infection. Plowman *et al* (2001) has

reported the prevalence of nosocomial UTI to be between 21.0% to 45.0% of all HAIs. Catheterization and instrumentation of the urinary tract are predisposing factors in approximately 60.0–80.0% of the cases and caused by bacteraemia in 1.0–10.0% of the cases (Manley and Bellman, 2000; Arunodaya, 2001; Özinel, 2003). In the study done Saint determined that bacteriuria would occur in 26.0% of hospitalized patients who have an indwelling catheter for 2–10 days (Eggimann and Pittet, 2001). Similarly in another study it has been reported that the incidence of catheter-related bloodstream infection ranges from 2 to 14 episodes per 1000 catheter-days (Eggimann and Pittet, 2002).

Similarly surgical site infections are also frequent: the incidence varies from 0.5 to 15.0% depending on the type of operation and underlying patient status (WHO, 2002). Surgical wound infections may be seen within 48 hours after operation. Deficient surgical technique, host immunity, lack of antimicrobial prophylactic measures, and the factors which may be controlled by very simple measures, have been shown to have a significant impact on surgical wound infections (Celik and Aksoy, 2001; Eggimann and Pittet, 2001).

Nosocomial pneumonia is another important type of nosocomial infections: the most important are patients on ventilators in intensive care units, where the rate of pneumonia is 3.0% per day. There is a high case fatality rate associated with ventilator-associated pneumonia, although the attributable risk is difficult to determine because patient co-morbidity is so high (CDC, 2000). Pneumonia is the second most common nosocomial infection in the United States and is associated with substantial morbidity and mortality. Most patients who have nosocomial pneumonia are infants, young children, and persons greater than 65 years of age; persons who have severe underlying disease, immunosuppression, depressed sensorium, and/or cardiopulmonary disease; and persons who have had thoracoabdominal surgery. Although patients receiving mechanically assisted ventilation do not represent a major proportion of patients who have nosocomial pneumonia, they are at highest risk for acquiring the infection. Most bacterial nosocomial pneumonias occur by aspiration of bacteria colonizing the oropharynx or upper gastrointestinal tract of the patient (CDC, 1997). Nosocomial pneumonia is seen in 5 to 10 cases per 1000 hospitalized patients. This rate may rise to 25.0–45.0% by continuous aspiration of contaminated subglottic

secretions into the lower airway in intubated patients (Tasota *et al,* 1998). Other risk factors contributing to the development of nosocomial pneumonia are include altered consciousness, cardiopulmonary diseases, age >70 years, use of H_2 blockers or antacids, chronic lung disease, surgical procedures, antibacterial therapy, obesity, diagnostic procedures or techniques, diabetes mellitus, and conditions favoring aspiration (endotracheal intubation, nasogastric intubation and supine positioning) (Tasota *et al*, 1998; Aktafl, 2000; Arunodaya, 2001; Eggimann and Pittet, 2001). Early postintubation pneumonia (within 72 hours) is most often due to *Haemophilus influenzae*, methicilin sensitive *S. aureus* or *S. pneumoniae* in the critically ill patients. Late onset ventilator associated pneumonia (after than 72 hours) is frequently due to *P. aeruginosa*, methicilin resistant *S. aureus* or *Enterobacter* spp (Aktafl, 2000; Arunodaya, 2001). Non-invasive ventilation was shown to reduce significantly the risk of infection. Nosocomial infection is reported to be reduced in critically ill patients receiving non-invasive ventillation (Eggimann and Pittet, 2001).

Nosocomial bacteraemia represent a small proportion of nosocomial infections (approximately 5.0%) but case fatality rates are high — more than 50.0% for some microorganisms. The incidence is increasing; particularly for certain organisms such as multiresistant coagulase-negative *Staphylococcus* and *C*and*ida* spp. Infection may occur at the skin entry site of the intravascular device, or in the subcutaneous path of the catheter (tunnel infection). Organisms colonizing the catheter within the vessel may produce bacteraemia without visible external infection. The resident or transient cutaneous flora is the source of infection. The main risk factors are the length of catheterization, level of asepsis at insertion, and continuing catheter care (WHO, 2002). Intravascular catheters may cause 40.0% bacteraemia. As stated earlier, intravascular devices known to be associated with infections are peripheral lines, central intravenous lines, total parenteral nutrition catheters, arterial lines and catheters used to provide long-term central venous access. Many primary infections that are related to intravascular devices are known to originate from the patient's own flora or from microorganism transmitted from the hands of person inserting the device. These patients show fever, leucocytosis, and homodynamic equlibriumness (Şahinoğlu, 1992; Tasota *et al,* 1998; Arunodaya, 2001; Yang and Li, 2003). Nosocomial bacteraemia infections are most often due to coagulase negative staphylococci, *S. epidermidis*, *S. aureus*

(Arunodaya, 2001). In another study 704 episodes of pedriatric bacteremia were reviewed during six year period. The predominant isolates causing neonatal nosocomial bacteraemia was *S. aureus* (23.9%), followed by *P. aeruginosa* (15.5%), *K. pneumoniae* (12.5%) and *Enterobacter* spp. (11.1%).The remaining isolates each accounted for less than 10.0% of the total isolates. The mortality rate was found to be highest for *P. aeruginosa*, *S. pneumoniae* and *E. coli* (Orrett and Changoor, 2006).

Other common type of nosocomial infections are infections of skin and soft tissues, gastroenteritis, sinusitis and other enteric infections, infections of the eye and conjunctiva, and endometritis and other infections of reproductive organs following child birth (WHO, 2002). Nosocomial gastrointestinal infection usually manifests as diarrhoea. It is most commonly due to *Clostridium defficile*. Diarrhoea may take place due to the medical treatment and enteral nutrition. Unhygienic handling of feeding systems is one of the most important exogenous routes for bacterial contamination of enteral tube feeding. Diarrhoea may cause colitis, mucus or blood in stool, fever, abdominal tenderness, and leukococytosis. To cure this disease oral metronidazole or vancomycin is used (Arunodaya, 2001; Eggimann and Pittet, 2001). Strategies such as hand hygiene, barrier cautions, reduction of environmental contamination with sterilization, and disinfecting can be used to prevent these infections (Tucker *et al*, 1996; Arunodaya, 2001; Eggimann and Pittet, 2001).

Nosocomial meningitis is a common but serious complication of modern hospital care. Although most cases follow neurosurgery, other neuroinvasive procedures, such as lumbar puncture or placement of an epidural catheter, can rarely infect the menings or the subarachnoid space. The National Nosocomial Infection Surveillance (NNIS), which consisted of 163 United States hospital, documented nosocomial central nervous system infection for every 100,000 patients discharged between 1986 and 1993. Meningitis accounted for 91.0% of all infections followed by intracranial and spinal abscesses. Meningitis is the second most common infection after craniotomy, accounting for 22.0% of cases, and is the most frequent infection after ventricular shunt placement. (Celik, 2004).

Incidence of shunt infections ranges from 2.0 to 33.0%. Infection rate is likely to be high

when the procedure is done by an inexperienced surgeon, duration of ventricular catheterization >5 days, intracerebral or intraventricular haemorrhage, increased intracranial pressure, leak of drainage systems (Celik, 2004).

From the study done in West Indies, it was found that NIs rate was 67.0% in ICU, 30.0% in urology, 29.5% in neurosurgery and 28.4% in newborn nursery (Orrett *et al*, 1998). In a study of prevalence of nosocomial and community acquired infections in Australian hospitals, the overall adjusted prevalence of NIs was found to be 6.3%. The rate of infections was respiratory tract (35.4%), urinary tract (15.1%), surgical wounds (34.0%), gastrointestinal tract (3.4%), skin infections (4.4%), abscesses (0.9%), traumatic wounds (0.9%), bacteraemia (1.6%), and other infections (13.4%). From the same study it was found that there was significant association between hospital size and infection rates. It has been reported that the nosocomial infection prevalence rate increased from 4.2% in hospitals with 50-99 beds to 7.6% in hospitals with 500 or more beds. Public hospitals had significantly higher prevalence of NIs (6.7%) than did private hospitals (4.8%) (Mclaws *et al*, 1998).

In a study performed to assess the magnitude of NI in general hospitals of Belo Horizonte, Brazil, it was found that of the 2,339 patients surveyed, 267 patients had nosocomial infections. The global prevalence rate of NI was 14.0%, ranging from 4.6% to 27.3% in the hospitals surveyed. The most prevalent infections were found to be pneumonia and surgical wound infections, representing 19.5% and 19.2%, respectively of the total infections. The highest prevalence of NI was observed in the cardiac surgery (31.9%), pedriatric (27.2% and orthopedic services (20.7%). The most frequently isolated microorganisms associated with the infections were *S. aureus*, *E. coli*, *Pseudomonas* spp, and *Klebsiella* spp (Rezende *et al*, 1998).

CDC has reported that after establishment of National Nosocomial Infection Surveillance (NNIS) there has been marked decrease in the occurrence of nosocomial infection in United States. It has reported that patients in intensive care units (ICUs) are at high risk for nosocomial infections. By ICU type, these patients have been monitored using site-specific, risk-adjusted infection rates. During 1990-1999, risk-adjusted infection rates decreased for all

three body sites (i.e., respiratory tract, urinary tract, and bloodstream) monitored in ICUs. Bloodstream infection rates decreased substantially in medical (nonsurgical) ICUs (44.0%), coronary ICUs (43.0%), pediatric ICUs (32.0%), and surgical ICUs (31.0%). Device use ratios, the proportion of days spent in the ICU in which the patient's treatment included invasive devices, also were calculated. Urinary catheter-associated urinary tract infection (UTI) rates were highest in medical (nonsurgical) ICUs (6.5 UTIs per 1000 days a catheter was used) and lowest in pediatric ICUs (5.6 UTIs per 1000 days a catheter was used). Central line-associated bloodstream infection (BSI) rates were highest in pediatric ICUs (7.7 BSIs per 1000 days a central line was used) and lowest in coronary ICUs (4.3 BSIs per 1000 days a central line was used). Ventilator-associated pneumonia (VAP) rates were highest in surgical ICUs (13.0 cases of pneumonia per 1000 days a ventilator was used) and were lowest in pediatric ICUs (5.0 cases of pneumonia per 1000 days a ventilator was used) (CDC, 2000).

The largest prevalence study of nosocomial infections is the European Prevalence of Infections in Intensive Care study. This study addressed the prevalence of nosocomial infections in 10,038 patients. The prevalence was 20.6%. Pneumonia was the most nosocomial infection (46.9%), followed by lower respiratory tract infections (17.8%), urinary tract infections (17.6%), and bloodstream infections (12.0%) (Celik, 2004).

Different pathogens may cause nosocomial infections. The infecting organisms vary among different patient populations, different health care settings, different facilities, and different countries. In Semmelweis' 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 pathogens 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 *Enterobacteriaceae*, became 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 34.0% of nosocomial infections, and the four most common gram-negative pathogens—*E. coli*, *P. aeruginosa*, *Enterobacter* spp., and *K. pneumoniae* accounted for 32.0% (Weinestein, 1998).

Despite the advances in hospital care and the introduction of a wide variety of antimicrobial agents, *P. aeruginosa* continues to be a major nosocomial pathogen particularly in patients who suffer from immunosuppression (Zenone and Souillet, 1984; Morrison and Wenzel 1994]. It also involves a broad spectrum of infections including the respiratory, gastrointestinal, and urinary tracts as well as wound infections, sepsis and other infections. Jones *et al* (2000) have reported that *P. aeruginosa* accounts for 10.0% of all hospital acquired infections, a site specific prevalence which may vary from one unit to another and from study to study. Bacteraemia caused by *P. aeruginosa* is clinically indistinguishable from other gram negative infections although mortality rate is higher. This bacterium is commonly resistant to common antibiotics.

S. aureus is another very important pathogen associated with nosocomial infection. It is one of most resistant non-sporing bacteria and survives well in the environment under both moist and dry condition (Forbes *et al*, 2002). Especially multidrug resistant strains are an important cause of hospital acquired infection. The emergence of *S. aureus* strains resistant to methicillin and other antimicrobial agents has become a major concern, especially in the hospital environment, because of higher mortality due to hospital acquired systemic MRSA infection. Infections associated with such organism is particularly a threat to vulnerable patients such as neonates, cancer patients and those who are immunocompromised, debilitated or elderly, or patients from ICU, burn units, high dependency units and infectious disease care centers. Methicillin resistance *S. aureus* (MRSA) has become an important hospital pathogen. Multidrug-resistant bacteria, such as Methicillin resistant *S. aureus* (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* in the United States emphasizes the need for the better control for the MRSA and other resistant bacteria within healthcare settings

(Henderson, 2006). In a study done at St. Thomas hospital, London, UK 267 cases of MRSA bacterimia were detected during two year period (2001-2003) giving a rate of 0.37 per 1000 occupied bed days (Jevaratnam *et al,* 2006). *S. aureus* bloodstream infections are common and serious causes of morbidity and mortality that incur considerable health care costs and are potentially preventable (Collignon *et al,* 2006). The rise in the occurrence of MRSA worldwide is due to the increase of nasal colonization with MRSA (Neurmaier *et al,* 2006). Colonization increases the risk of infection. In addition patient-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 cultures, as indicated (Henderson, 2006). Zhang *et al* has reported that there could be cross infection of *S. aureus* between the medical staff, inpatients and the infected patients.

In 1990 to 1996, the three most common pathogens, *S. aureus,* coagulase negative staphylococci, and enterococci, accounted for 24.0% of nosocomial infections and the four other pathogens, *E. coli, P. aeruginosa*, *Enterobacter* spp., and *K. pneumoniae*, accounted for 22.0% (Weinstein, 1998).

In a study 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 e*t al,* 2006).

Occurrence of *S. aureus* among carriers (anterior nare carrier) and occurrence of multi drug resistant strains has made control of this organism very difficult. 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 and Climo, 2002).

The pool of circulating MSSA strains is an important parameter with regard to the epidemiology of hospital and community acquired MRSA clones and their potential virulence. It is because MRSA strains carry the readily transmissible *mec* cassette which may be the cause of rapid dissemination of MRSA in a hospital with a pool of MSSA (Layer *et al,* 2006).

Other gram positive bacteria like coagulase negative staphylococci, *S. pyogenes, S. agalactiae, S. pneumoniae,* enterococci and other streptococci are also important pathogens causing nosocomial infection. Among these coagulase negative staphylococci has been an important one as it may cause endogenous infection or device associated infections.

Although classically considered a commensal of the gastrointestinal tracts of humans and animals rather than a specialized human pathogen, enterococci have become extremely relevant in hospital acquired infections. Their ability to acquire specific genetic traits such as virulence and antibiotic resistance determinants that could increase their fitness in such a complex ecosystem has been recognized. The paradigm of this evolutionary development is the emergence and spread of vancomycin-resistant enterococci (Ruiz-Garbajosa *et al,* 2006).

Micrococci are free living gram positive cocci arranged in cluster and are often similar in morphology to CoNS. They are found on both skin and environment and are occasionally recognized as a causative agent of infection, particularly in immunocompromised patients (Forbes *et al,* 2000).

Members of family Enterobacteriacae are also organisms commonly associated with hospital acquired infection. *E coli* is one of the frequently encountered bacteria in urinary tract infection, intra-abdominal and gut-related wound infection and bacteraemia, but the infection is always endogenous and sporadic; even resistant strains seldom appear to spread among

hospital patients .. In one study it has been reported that the most commonly isolated microorganisms were *E. coli* (37.2%) in urine, S. aureus (50.0%) in blood, *P. aeruginosa* (25.7%) in tracheal aspirates, and *Acinetobacter* spp. (37.5%) in wounds. Considering all specimens, MRSA (22.0%) was the most common microorganism (Akcam *et al*, 2006). The members of family *Enterobacteriaceae* have become more important cause of nosocomial infection due to their ability to produce beta-lactamase enzymes. These organisms have marked capacity to produce extended spectrum beta-lactamase (ESBL) which makes them resistant to newer beta lactams. It has been reported that about 25.0% of *E. coli* isolated from blood and CSF are now multi drug resistant. 20.0-40.0% of Enterobacter and Citrobacter are resistant to all beta lactams except carbapenems (imipenem and meropenem) and tomocillin. ESBL producing *K. pneumoniae* have caused major outbreaks of infection in the hospitals of UK (Hernandez *et al*, 2005).

S. marcescens is a well known pathogen that is responsible for endemic and epidemic nosocomial infection and is associated with a high morbidity and mortality. Epidemic outbreak of nosocomial infections caused by *S. marcescens* particularly in ICU and NICU are well reported. Outbreaks of infection due to *S. marcescens* are particularly serious owing to the tendency of these microorganisms to infect severely compromised patients as a result of their resistance to antibiotics (Smith and Easmon, 1990).

Acinetobacter spp. has emerged as important nosocomial pathogens. They are ubiquitous in nature and are highly resistant to commonly used antibiotics. In a study blood samples from 400 suspected neonatal septicemia cases were cultured from Feb 2003 - Dec 2004. One hundred and eighty seven (46.8%) neonates were positive in blood culture and *Acinetobacter* spp. were isolated from 23 (12.3%) of which 13 were *A. baumanni* and 10 were *A. luioffii* . It was also found that babies born in hospital had higher isolation of *Acinetobacter* spp (Arora *et al*, 2006). Barbara W. has reported that *A. baumanni*, a gram-negative coccobacillus, has emerged as a significant pathogen in the hospital setting. The organism is fairly stable in the environment and multidrug-resistant strains limit therapeutic options. Before 1997, only one hospital reported a nosocomial outbreak of *Acinetobacter* spp to the New York State Department of Health. Since 1997, twelve hospitals have reported significant

outbreaks of *Acinetobacter* infection and a number of other smaller outbreaks are believed to have occurred. Preliminary epidemiologic information provided by the hospitals revealed that most of the patients who developed infections were in critical care units and Patients on mechanical ventilation were affected most often. Common infections due to *Acinetobacter* spp were bacteremia, pneumonia, urinary tract and surgical site infections. It was also reported that during the course of the outbreaks, the organism tended to become increasingly resistant to antibiotics.

Waterborne pathogens are also important cause of infections in health-care facilities. Despite guidelines addressing these pathogens, outbreaks and pseudo-outbreaks continue to occur. We reviewed recent reports of infections caused by *P. aeruginosa, Stenotrophomonas maltophilia, Chryseobacterium* species, nontuberculous mycobacteria, and *Legionella* species. *Mycobacterium avium* complex (MAC) infection in HIV patients has been linked to hospital water distribution systems; molecular subtyping showed that MAC isolates in patients and hospital water were identical. In immunosuppressed patients, *Fusarium* infection has been linked to the hospital water distribution system; again molecular subtyping showed that isolates from patients and the water supply were identical. Parasites, especially *Cryptosporidium*, and viruses have also been implicated in nosocomial infection. Transmission occurs via contact, ingestion, aspiration, or aerosolization of potable water, or via the hands of health-care workers. Interventions designed to interrupt transmission of waterborne pathogens have included the use of antimicrobial handwashes, targeted disinfection of the water supply, and, in high-risk populations, restricting the use of tap water (Cheryl *et al,* 2000).

Candida spp. is one of the potential pathogen causing NIs. *C*and*ida* species commonly cause hospital-acquired bloodstream infections (BSIs) among patients in the intensive care unit (ICU), and these infections are associated with high rates of morbidity and mortality. In a study done by NNIS from 1st January 1989 through 31st December 1999, there was a significant decrease in the incidence of *C. albicans* BSI ($P<0.001$) and a significant increase in the incidence of *C. glabrata* BSI ($P=0.05$) (Trick *et al,* 2000).

Some parasites (e.g. *Giardia lamblia*) are transmitted easily via food or water among adults or children. Many fungi and other parasites are opportunistic organisms and cause infections during extended antibiotic treatment and severe immunosuppression (*C*and*ida albicans*, *Aspergillus* spp., *Cryptococcus neoformans*, *Cryptosporidium*). These are a major cause of systemic infections among immunocompromised patients. Environmental contamination by airborne organisms such as *Aspergillus* spp. which originate in dust and soil is also a concern, especially during hospital construction. The species of *Aspergillus* documented to cause infection in the setting of nosocomial infection include *A fumigatus*, *A flavus* and *A terreus*. Unsurprisingly, given the ubiquitous nature of Aspergillus spores in the external environment, numerous reservoirs have been identified in hospitals: unfiltered air; ventilation systems; contaminated dust dislodged during hospital construction; carpeting (and a ward vacuum cleaner); food, and ornamental plants (Vandenbergh *et al,* 1999). Fungi like *Aspergillus* spp., and *Fusarium* spp., cause nosocomial infections in immunosuppressed (particularly neutropenic) patients and creates a risk of pulmonary and /or paranasal sinus infection and disseminated aspergillosis. So, to lower the risk, hospitals should inspect and clean air-handling equipment on a routine schedule, review all planned hospital renovations with infection-control personnel and subsequently construct appropriate barriers, remove immunosuppressed patients from renovation sites, and consider the use of high-efficiency particulate air filters for rooms housing immunosuppressed patients (Braunwald *et al,* 2001).

Sarcoptes scabies (scabies) is an ectoparasite which has repeatedly caused outbreaks in health care facilities (WHO, 2002). Kachi *et al* has reported the outbreak of NI due to *Nocardia farcinica* in the same ward within six months period in a hospital at Japan (Kachi *et al,* 2006).

Epidemiologically healthy carriers serve as more important source of infection than are the potentially apparent individuals in the disease transmission chain. Especially occurrence organisms like *S. aureus* in nasal fossa, beta haemolytic streptococci i.e. *S. pyogenes* in throat of healthy carriers are dangerous as they are important nosocomial pathogens causing a wide range of nosocomial infections.

Nasal carriage of *S. aureus* has been identified as a risk factor for community-acquired and nosocomial infections (Cole *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). Or 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 patients, 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 (M *et al,* 2006). 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.

It is unclear whether the levels of *S. aureus* colonization of hospital personnel with patient exposure are increased or whether personnel become colonized with more antibiotic-resistant strains. 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 and Climo, 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 Wertheim, 2005; Wernitz *et al*, 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* septicemia (Holtfreter *et al*, 2006). Similarly occurrence of individuals as carrier for *S. pyogenes* increases the risk of nosocomial infection as these are important group of pathogens causing pyogenic wound infections and a number of other nosocomial infections.

Thus surveillance for the determination of carriers and their subsequent treatment for the eradication of the carriers help to significantly decrease the incidence of nosocomial infections.

Epidemiology

Global scenario

Nosocomial infections occur worldwide and affect both developed and resource-poor countries. Infections acquired in health care settings are among the major causes of death and increased morbidity among hospitalized patients (WHO, 2002). At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital (Tikhomirov, 1987). Hospital-acquired infections are adverse patient events that affect approximately 2 million persons annually (CDC, 1992). It has been reported that in United States 5.0% to 10.0% of the patients admitted to acute care hospitals develop nosocomial infections during their hospital stay (Forbes *et al*, 2000).

The National Nosocomial Infection Study (NNIS) carried out by the Centre for Disease Control (CDC) indicates that 5.0 to 6.0% of hospitalized patient develop nosocomial infection. A prevalence survey conducted under the auspices of WHO in 55 hospitals of 14 countries representing 4 WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) showed an average of 8.7% of hospital patients had nosocomial infections.

Despite rapid advances in therapeutics, diagnostics and a better understanding of the disease process, the problem of hospital acquired infections is constantly rising and its occurrence varies from hospital to hospital; it is estimated to be around 8.7% of hospital admission. 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 (WHO, 2002). The prevalence of nosocomial infection in hospitalized patients was approximately 6.0%, a disproportionate 20.0% of these occur in critically ill patients. The infection rate was higher than 30.0% in those patients staying longer than 48 hours in the intensive care unit (Tasota *et al*, 1998; Sablotzkl *et al*, 2000). It has been reported that the global prevalence rate of NI was 14.0%, ranging from 4.6% to 27.2% in the hospitals surveyed. The most prevalent infections were found to be pneumonia and surgical-wound infections, representing 19.5% and 19.2%, respectively of the total infections. The most frequently isolated microorganisms were *S. aureus, E. coli, Pseudomonas* spp., and *Klebsiella* spp. (Rezende *et al*, 1998).

Nosocomial infection causes increased length of stay at hospital. One study showed that the overall increase in the duration of hospitalization for patients with surgical wound infections was 8.2 days ranging from 3 days for gynecology to 9.9days for general surgery. This increased length of hospital stay for infected patients is the greatest contributor to cost (WHO, 2002).Treatment of nosocomial infections is estimated to add between $4.5 and $15 billion annually to the cost of health care and represent as enormous economic problem in today's environment of cost containment. The Institute of Medicine reports that preventable adverse patient events, including hospital-acquired infections, are responsible for 44,000-98,000 deaths annually at a cost of $17-$29 billion (CDC, 2000). In a study it has been reported that NIs have contributed to more than 88,000 deaths—1 death every 6 minutes. These numbers have grown with each passing year (Weinstein, 1998). In another study done in West Indies it has been reported that the cost to the government for nosocomial infections was estimated as $697,000 annually (Orrett *et al*, 1998). The increased use of drugs, the need for isolation, and the use of additional laboratory and other diagnostic studies also contribute to costs (WHO, 2002). In addition many of these infections lead to death of hospitalized patients or at minimum, lead to additional complications (patient morbidity) and

antimicrobial chemotherapy (Forbes *et al,* 2000) Hospital acquired infections add to the imbalance between resource allocation for primary and secondary health care by diverting scarce funds to the management of potentially preventable conditions(WHO, 2002).

According to a four year (2001-2004) study performed at France, the overall point prevalence of infection was found to be 6.1% and was found to vary according to the category of patient from 1.9% (no risk factors) to 15.2% (three risk factors). The frequency of nosocomial .infection related to invasive procedures and to cross contamination with multidrug resistant (MDR) bacteria were 30.9% and 12.3%, respectively. These percentages were found to be independent to the type of patient. The study also suggested that 30.0% of NIs were potentially avoidable (Floret *et al,* 2006).

In a retrospective review done from 1992 to 1995 at a rural government hospital in West Indies 7,158 nosocomial infection was reported from total 72,532 patients i.e. 10.5% (Orrett *et al,* 1998).

As mentioned previously, 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 (WHO, 2002). On the other hand there is lack of effective notification system in South Asian regions due to which it is difficult to know the exact situation in these regions (WHO, 2002).

In a retrospective study done on January to December 2000, in Beijing Hospital on 12,418 inpatients it was found that three hundred and seventy-eight i.e. 3.0% of the cases were infected due to nosocomial infection. In the study it was found that infection rates were higher in males than in females and the difference was significant. The infection rate was higher in old age people. Nosocomial infection occurred mainly in the lower respiratory tract (36.0%), upper respiratory tract (22.5%), urinary tract (17.6%) and gastrointestinal tract (10.4%). The main pathogenic organisms of the nosocomial infection were *C. albicans* (15.8%), *Pseudomonas* spp. (13.2%) and *Enterococcus* spp. (11.9%) with gram negative

bacilli (46.0%), gram positive cocci (23.7%) and fungus (15.8%). The overall death rate due to NIs was 14.3% (Wang *et al,* 2001).

In a study done in a tertiary care centre in India in ICU it was found that intoxication was most common, followed by respiratory insufficiency due to severe pneumonia and/or chronic obstructive respiratory disease, then trauma, postoperative conditions, and cerebro-vascular problems. The mean number of culture studies per patient was 5.36+/-3.27. Cultures were most commonly obtained from patients with respiratory insufficiency and trauma. According to clinical specimens, the most commonly isolated microorganisms were *E. coli* (37.2%) in urine, *S. aureus* (50.0%) in blood, *P. aeruginosa* (25.7%) in tracheal aspirates, and *Acinetobacter* spp. (37.5%) in wounds. Considering all specimens, MRSA (22.0%) was the most common microorganism isolated (Ackam *et al,* 2006).

Nepalese scenario

In a study done at Tribhuvan University Teaching Hospital in 1989 it was found that the rate of occurrence of NIs was 10.5% (Tuladhar *et al,* 1990). In another study performed at the same hospital in Nepal, the overall point prevalence of nosocomial infection has been reported to be 2.4% (Lamichhane and Shrestha, 2001).

In a study done in TUTH various bacterial isolates from air were *S. aureus,* coagulase negative *Staphylococcus, Streptococcus* spp., *Bacillus* spp, Micrococci, *P. aeruginosa and K. pneumoniae.* In the same study done to study the wound infection *S. aureus* was found to be the most predominant bacteria causing wound infection followed by *P. aeruginosa, E. coli, K. pneumoniae, Acinetobacter* spp. *C. fruendii, Proteus mirabilis, C. diverges, Pr. vulgaris, S. epidermidis, S. faecalis, K. oxytoca,* and *A. hydrophila* (Banjara *et al,* 2002). In similar study done to assess the microbial flora of ICU environment, *S. aureus,* CoNS, *Micrococcus* spp., *Bacillus, Streptococcus* spp., and *Serratia* were found to be the predominant microorganisms (Sharma *et al,* 2002).

In a random bacteriological study conduted at Tribhuvan University Teaching hospital, to assess the level of medically important organisms prevailing in the hospital it was found that

the predominant organisms in the order of frequency were *S. aureus*, Micrococcus spp., coagulase negative *Staphylococcus* and other gram negative bacteria. About 23.5% of the *S. aureus* isolates were reported to be methicillin resistant strains (Pokhrel *et al*, 1993).

CHAPTER-IV

4. MATERIALS AND METHODS

4.1 Materials

A list of materials used during the study is given in the appendix.

4.2 Methods

A. This study was carried out from September 2005 to July 2006 in joint collaboration of Nepal Medical College Teaching Hospital and Central Department of Microbiology, Tribhuvan University, Kirtipur.

B. The study site for this study was Nepal Medical College Teaching Hospital, Jorpati.

C. Air (indoor) and Surface samples (floor, bed bar handle, table, door handle, tray, instruments like hemodialysis machine etc) were collected from different wards for the study of environmental microflora of Nepal Medical College Teaching Hospital. Similarly Nasal swabs, Hand Swabs and Throat Swabs were also collected from staffs working at different wards for the study of carrier pattern among the staffs working at the hospital.

D. The Isolates were then processed for identification and antibiotic susceptibility testing by Kirby Bauer method.

4.2.1 Indoor air sample

86 air samples (43 for bacteria and 43 for fungi) were collected. Air Samples were collected from different wards by plate exposure for 5 minutes for unsterilized wards and for 20 minutes in the wards which were sampled immediately after sterilization. The plates were then incubated at 37^0C for 24 hours for nutrient agar and 25^0-28^0C for 3- 5 days for SDA plates. The bacterial isolates were then identified by standard diagnostic procedure as

described in the appendix. Fungal isolates were identified on the basis of colony morphology and microscopic observation. Antibiotic susceptibility of the isolates was done by NCCLS recommended Kirby- Bauer disc diffusion method.

Flow- Chart-1

Collection of air sample

Sample collection

↓

Plate exposure (Nutrient Agar/ SDA) for 5 min / 20 min

↓

Incubate at 37^0C for 24 hours/ 25^0-28^0C for 3-5 days

↓

Enumeration and observation

↓

Subculture

↓

Isolation and Identification

↓

Antibiotic Susceptibility test

4.2.2 Surface sample

117 surface samples were collected from different wards. The surface samples include samples from floor, table, bed, instruments and door. Surface samples were collected from different wards by using sterile cotton swabs soaked in Brain Heart Infusion (BHI) broth. The swabs were then immediately plated on to NA plates. The plates were then incubated at 37^0C for 24 hours. The bacterial isolates were then identified by standard diagnostic procedure as described in the appendix. Antibiotic susceptibility of the isolates was done by NCCLS recommended Kirby- Bauer disc diffusion method.

Flow-chart-2

Collection of surface sample

Preparation of Sterile cotton swabs soaked on BHI broth

↓

Surface swabs collected by swabbing at different sites

↓

Surface swabs inoculated onto NA plates

↓

Plates incubated at 37^0C for 24 hours

↓

Observation

↓

Subculture

↓

Isolation and Identification

↓

Antibiotic Susceptibility test

4.2.3 Carrier

A total of 150 samples were collected from human subjects (staffs) for the detection of carriers. Out of 150 carrier samples, 54 were for hand carrier, 48 nasal carrier and 48 for throat carrier.

4.2.3.1 Hand carrier

To determine hand carrier, hand samples were collected from 54 staffs from different wards. Hand swabs were collected from 40 staffs while for the remaining 14, the samples were collected by requesting them to insert their hand into gloves with sterile BHI broth and then the broth was swabbed over onto nutrient agar media. Similarly for swabs, the swabs were immediately transferred to the laboratory and plated on to nutrient agar plates. The plates

were then incubated at 37^0 C for 24 hours and the isolates were then processed for the identification.

Flow-chart-3

Detection of hand carrier

Preparation of Sterile cotton swabs soaked on BHI broth/ preparation of sterile gloves with added BHI broth

↓

Hand swabs collected by swabbing at both hands/ inserting hands into the gloves with added BHI broth

↓

Hand swabs inoculated onto NA plates/ BHI broth from the gloves swabbed over NA

↓

Plates incubated at 37^0C for 24 hours

↓

Observation

↓

Subculture into appropriate media

↓

Isolation and Identification

↓

Antibiotic Susceptibility test

4.2.3.2 Nasal carrier

To detect for the nasal carrier for *S. aureus* 48 nasal samples were collected from anterior nares of the staffs by using dry, sterile cotton swabs and the swabs were then plated on to mannitol salt agar (MSA) plates. The plates were then incubated at 37^0C for 24 hours. Suspected yellow colonies on MSA were then processed for further identification such as gram staining, catalase test, oxidase test, Mannitol fermentation and coagulase test. Confirmed *S. aureus* were then tested for antibiotic susceptibility test for various antibiotics.

4.2.3.3 Throat carrier

To determine for the presence of throat carrier for group A streptococci, throat swabs were collected from posterior pharyngeal wall from 48 individuals using sterile, dry cotton swabs. The swabs were then plated on to blood agar (BA) plates. The beta-hemolytic colonies sensitive to bacitracin were then processed for antibiotic susceptibility test.

Flow-chart-4

Detection of nasal carrier

Preparation of dry sterile cotton swabs

Nasal swabs collected by swabbing at anterior nares of the nose

Nasal swabs inoculated onto Mannitol Salt Agar (MSA) plates

Plates incubated at 37^0C for 24 hours

Observation for yellow colonies on MSA

Subculture

Isolation and Identification

Antibiotic Susceptibility test

Flow-chart-5

Detection of throat carrier

Preparation of dry sterile cotton swabs

↓

Throat swabs collected by swabbing at Posterior pharyngeal wall

↓

Throat swabs inoculated onto Blood Agar (BA) plates

↓

Plates incubated at 37^0C for 24 hours

↓

Observation for group A streptococci sensitive to bacitracin

↓

Subculture

↓

Isolation and Identification

↓

Antibiotic Susceptibility test

4.2 Quality control

Quality control of each tests were maintained by using standard procedures. The qualities of each agar plates prepared were tested by incubating one plate of each lot on the incubator as the test plates were incubated. For performance testing, ATCC control strains were streaked on to the prepared media plates and observed for significant growth.

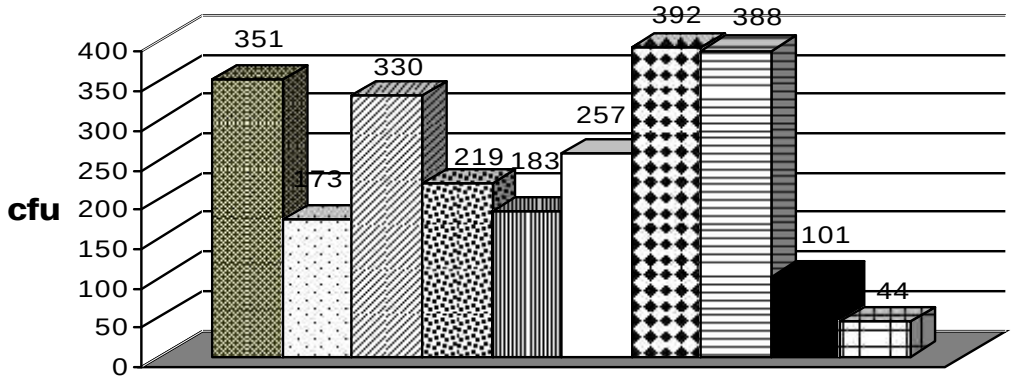
CHAPTER-V

5. RESULTS

The study was conducted from September 2005 to July 2006 in joint collaboration of Nepal Medical College Teaching Hospital and Central Department of Microbiology, Tribhuvan University, Kirtipur. During the study period different environmental samples and carrier samples from staffs were analyzed from different wards of Nepal Medical College Teaching Hospital, Jorpati.

5.1 Microbial quality of hospital environment:

Altogether 203 environment samples were collected: 86 Air samples and 117 Surface samples from different wards. Among the wards surgical ward showed the highest density of microorganism followed by orthopedic ward, post operative and ICU (POW &ICU), antenatal and postnatal care (ANC &PNC), medical ward, neonatal ICU and neonatal ICU mothers (NICU &NICUM), labour in and labour waiting (LI & LW) and operation theatre (OT) as shown in figure 1.



Legend: A-POW &ICU; B-OT; C-ANC & PNC; D-NICU & NICUM;
E- LI & LW; F- Medical G-Surgical H- Orthopedic
I- Female & Child J- Haemodialysis [Total No. of isolates (n) =2439cfu]

Figure 1 Wardwise occurrence of microorganisms in environment of the hospital

Among the isolates gram positive cocci were the most predominant microbes isolated followed by yeast, gram positive rods and gram negative rods as demonstrated in figure 2.

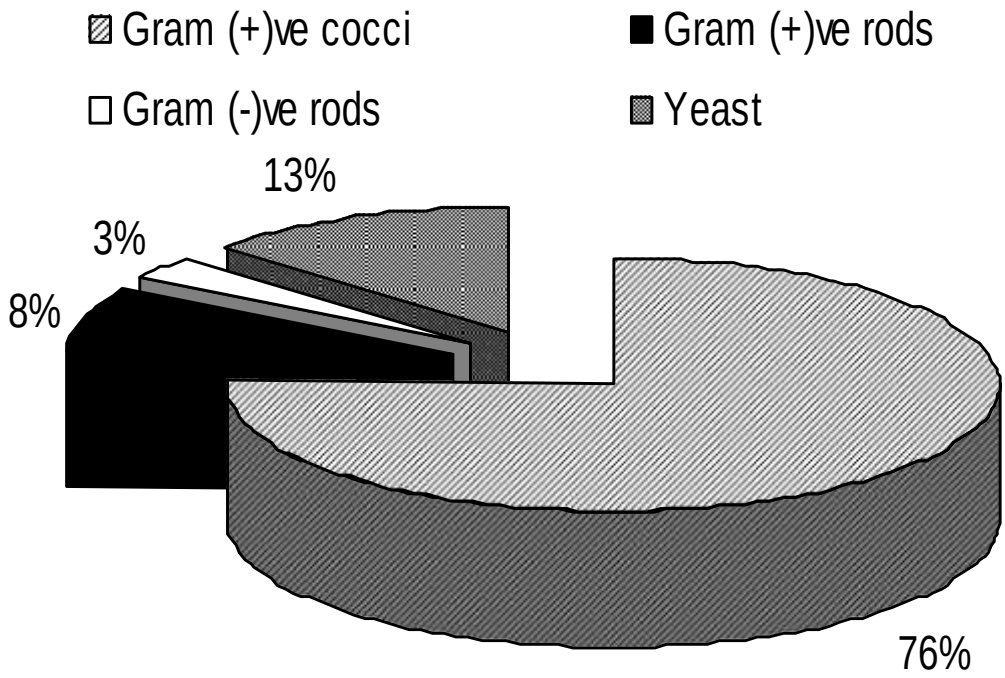


Figure 2 Occurrence of different microorganisms in environment of different wards

Coagulase negative staphylococci were the most predominant gram positive cocci followed by *S. aureus*, Micrococci, and *Streptococcus* spp. Among gram negative isolates *P. aeruginosa* was the most predominant followed by *E. coli*, *Klebsiella* spp. and *Citrobacter* spp. The occurrence of different microorganisms in different wards is shown in table 1 and 2.

Table 1 Occurrence of gram +ve isolates in the environment of different wards in the hospital

Ward	Gram-positive cocci				Gram-positive rods
	S. aureus	**CoNS**	**Micrococci**	***Streptococcus* spp.**	
POW and ICU	100	80	55	42	14
OT	43	55	26	27	20
ANC and PNC	81	86	29	42	18
NICU and NICUM	40	54	41	23	21
LI and LW	56	77	12	0	13
Medical	70	52	44	24	29
Surgical	97	110	30	29	41
Orthopedic	77	113	79	37	23
Female and Child Ward	34	20	17	9	9
Haemodialysis	7	20	4	0	11
Total	**605**	**667**	**337**	**233**	**199**

Gram positive cocci were found to be the most predominating microorganism representing 75.5% of the total isolates. Out of total 2439 isolates 667 (27.4%) were coagulase negative staphylococci, 605 (24.8%) *S. aureus*, 337 (13.8%) Micrococci, 233 (9.6%) *Streptococcus* spp., 317 (13.0%), yeast 199 (8.2%) *Bacillus* spp., and 81 (3.3%) gram-negative rods. Among gram negative isolates were *P. aeruginosa* (39.0%), *E. coli* (37.0%), *Klebsiella* spp. (21.0%), and *Citrobacter* spp. (1.2%) of the total (81) gram negative isolates.

Table 2 Occurrence of gram -ve rods and yeast in environment of different wards

Ward	Gram-negative rods				Yeast
	P. aeruginosa	***E. coli***	***Klebsiella* spp.**	***Citrobacter* spp.**	
POW and ICU	19	0	0	0	41
OT	0	0	0	0	2
ANC and PNC	0	7	7	0	60
NICU and NICUM	0	7	6	0	27
LI and LW	0	0	0	1	24
Medical	0	4	4	0	30
Surgical	13	12	0	0	60
Orthopedic	0	0	0	0	59
Female and Child Ward	0	0	0	0	12
Haemodialysis	0	0	0	0	2
Total	**32**	**30**	**17**	**1**	**317**

5.1.1 Microbiology of air

Table 3 Number of NA plates showing positive growth after exposure in different wards

Wards	No. of plates exposed	Growth Positive
Post op. and ICU	4	3 (75%)
OT	12	8 (66.67%)
ANC and PNC	4	4 (100%)
NICU and NICU Mothers	4	4 (100%)
Labour in and Labour Waiting	4	4 (100%)
Medical ward	4	4 (100%)
Surgical ward	4	4 (100%)
Orthopedic ward	4	4 (100%)
Female and Child ward	2	2 (100%)
Haemodialysis ward	1	1 (100%)
TOTAL	**43**	**38 (88.4%)**

Out of 43 plates exposed in various wards, 38 (88.4%) showed positive growth for microorganisms as given in table 3.Average number of microorganism per plate was highest in Haemodialysis ward followed by ANC & PNC, NICU & NICUM, medical, LI & LW, surgical, female & child, orthopedic and OT. The average microbial load was found to be lowest for Operation theatre as shown in figure 3.

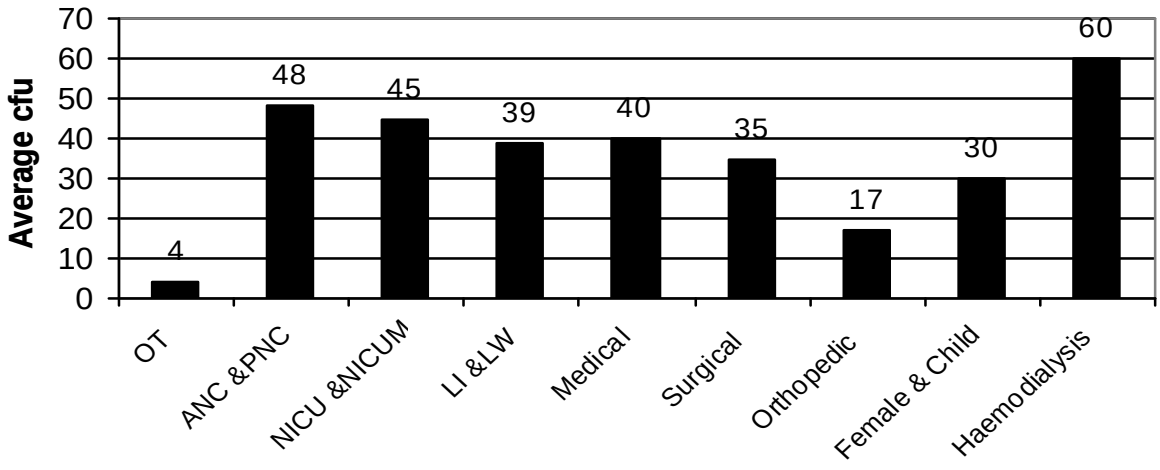


Figure 3 Average number of microorganisms per plate in different wards (Air)

Table 4 Growth of fungi on SDA plates after plate exposure in various wards

Wards	No. of plates exposed	Plates showing (+)ve growth	Fungi Isolated
POW & ICU	4	3 (75.0%)	*Aspergillus* spp., *Rhizopus* spp., *Scopulariopsis* spp.
OT	12	10 (83.3%)	*Aspergillus* spp., *Alternaria* spp., *Mucor* spp.
ANC & PNC	4	4 (100.0%)	*Aspergillus* spp., *Alternaria* spp., *Mucor* spp., *Penicillium* spp.
NICU & NICUM	4	4 (100.0%)	*Aspergillus* spp., *Alternaria* spp., *Mucor* spp., *Penicillium* spp.
LI & LW	4	4 (100.0%)	*Aspergillus* spp., *Alternaria* spp., *Penicillium* spp.
Medical	4	4 (100.0%)	*Aspergillus* spp., *Alternaria* spp., *Mucor* spp., *Fusarium* spp.
Surgical	4	4 (100.0%)	*Aspergillus* spp., *Alternaria* spp., *Fusarium* spp.
Orthopedic	4	4 (100.0%)	*Alternaria* spp.
Female & Child ward	2	2 (100.0%)	*Aspergillus* spp., *Alternaria* spp., *Fusarium* spp
Hemodialysis	1	1 (100.0%)	*Aspergillus* spp., *Penicillium* spp. *Fusarium* spp.

Thus out of 43 plates exposed, 40 showed positive growth for fungi that accounts for 93.0% of the total plates exposed. *Aspergillus* spp., was the most common fungi isolated in most of the wards, followed by *Alternaria* spp., *Penicillium* spp., *Fusarium* spp., *Mucor* spp., *Rhizopus* spp., and *Scopulariopsis* spp., could be isolated from only single ward as described in table 4. *A. niger* was the most common aspergilli followed by *A. fumigatus* while *A. flavus and A. terreus* were isolated from only two sites: POW and ICU.

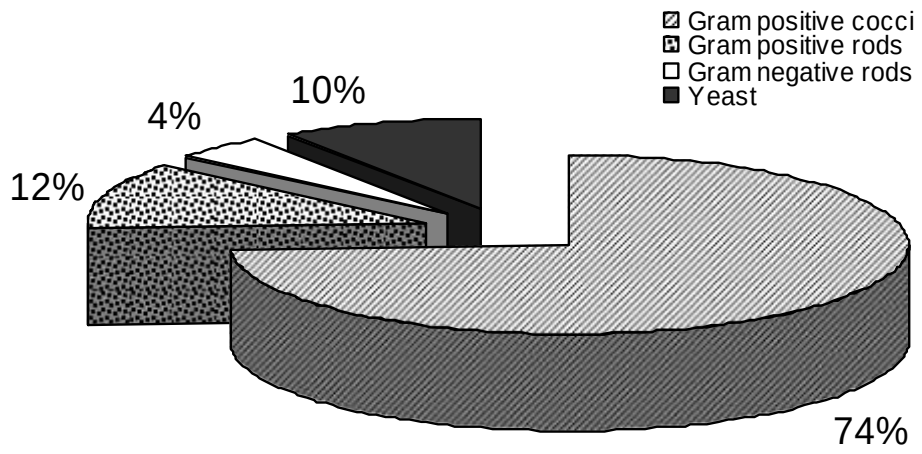


Figure 4 Percentage of different microorganisms in air sample

Out of total 1133 isolates from air, 860 (74.0%) was gram positive cocci, followed by gram positive rods 137 (12.0%), yeast (10.0%) and gram negative rods 49 (4.0%) as shown in figure 4. Among gram positive cocci *S. aureus* 291 (33.8%) was the most common isolate followed by coagulase negative staphylococci 264 (30.7%), Micrococci 177 (20.6%) and *Streptococcus* spp. 128 (14.9%). Among gram negative isolates, *E. coli* (21) is the most common isolates representing 42.9% of the gram negative isolates followed by *P. aeruginosa* 14 (34.7%) and *Klebsiella* spp. 6 (12.2%).

5.1.2 Surface

117 surface samples were collected from different wards. Among the isolates gram positive cocci were the most predominant representing 77% of the total isolates, followed by yeast (14%), gram positive rods (7%) and gram negative rods (2%) were the least predominant ones. The occurrence of different microorganisms in surface samples is shown in figure 5.

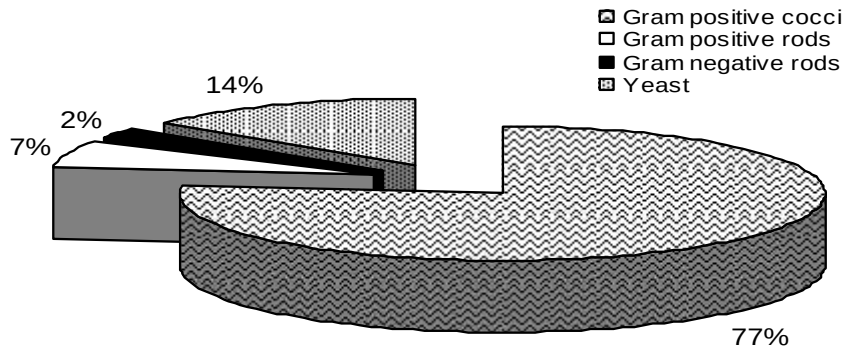


Figure 5 Percentage of different microorganisms in surface samples

Out of total 1312 cfu, coagulase negative staphylococci 397 (30.3%) were the most predominant microorganism followed by *S. aureus* 342 (26.1%), Yeast 183 (13.9%), Micrococci 180 (13.7%), Streptococci 95 (7.2%), gram positive bacilli 89 (6.8%), and gram negative bacilli (2.4%).

5.1.3 Antibiotic susceptibility pattern of the environmental isolates

Among the antibiotics tested Methicillin, Cloxacillin and Chloramphenicol were found to be most effective antibiotics for *S. aureus*. 3/182 (1.6%) were found to be Methicillin and multidrug resistant. Most of the *S. aureus* 74/182 (40.7%) isolated from environmental samples were resistant to Amoxicillin, followed by Erythromycin 28/182 (15.4%), Cotrimoxazole 23/182 (12.2%) and Gentamicin 5/182 (2.7%). Coagulase negative *Staphylococcus* were found 30/18 (16.7%), 31 (17.2%), 30/180 (16.7%), 22/182 (12.2%) resistant to Amoxicillin, Erythromycin, Cotrimoxazole and Chloramphenicol respetively. 10/180 (5.6%) were resistant to Methicillin, Gentamicin, and Cloxacillin. *Streptococcus* spp. (α-hemolytic) were found 25/123 (20.3%), 23/123 (18.7%), 20/123 (16.3%), 20/123 (16.3%), 20/123 (16.3%), 19/123 (15.4%), 19/123 (15.4%) resistant to Amoxicillin, Erythromycin, Chloramphenicol, Cotrimoxazole, Gentamicin, Methicillin, Cloxacillin. *E. coli* was found 24/30 (80%), 26/30 (86.7%), 2/30 (6.7%), 2/30 (6.7%), 1/30 (3.3%), 1/30 (3.3%) resistant to Amoxicillin, Ciprofloxacin, Amikacin, Cephalexin, Nitrofurantoin and Gentamicin respectively. *Klebsiella* spp. was found 16/17 (94.1%) resistant to Amoxicillin and Ciprofloxacillin, 2/17 (11.8%) resistant to Amikacin and Cephalexin, 6/17 (35.3%), 4/17 (23.5%) resistant to Nitrofurantoin and Gentamicin respectively. *Citrobacter* spp. was found

to be sensitive to all the antibiotics tested. *P. aeruginosa* isolates were 30/32 (93.8%) resistant to Amoxicillin, Ciprofloxacin and Cephalexin 6/32 (18.8%) and 2/32 (6.3%) resistant to Chloramphenicol and Gentamicin respetively.

Table 5 Percentage of environmental isolates resistant to antibiotics

Organism tested	**Total (n)**	**Antibiotic used (% resistance)**						
		Am	**E**	**C**	**Co**	**M**	**G**	**Cx**
S. aureus	182	40.7	15.4	1.6	12.2	1.6	2.7	1.6
CoNS	180	16.7	17.2	12.2	16.7	5.6	5.6	5.6
Streptococcus spp.	123	20.3	18.7	16.3	16.3	15.4	16.3	15.4
E. coli		**Am**	**Cp**	**Ak**	**Cf**	**Nf**	**G**	
	30	80	86.7	6.7	6.7	3.3	3.3	
Klebsiella spp	17	94.1	94.1	11.8	11.8	35.3	23.5	
Citrobacter spp	1	0	0	0	0	0	0	
		Am	**Cp**	**G**	**C**	**Cf**		
P. aeruginosa	32	93.8	93.8	6.3	18.8	93.8		

5.2 Carrier

Out of total 150 samples: 54 for hand carrier, 48 for nasal and 48 for throat carrier, 32 had *S. aureus* in their hands, similarly 21 out of 48 staffs had *S. aureus* in their anterior nares, and no one among the 48 staffs had group A streptococci in their throat.

Table 6 Number and percentage of carrier among staffs

Site	**n**	**Positive (%)**
Hand Carrier	54	32 (59.3%)
Nasal carrier	48	21 (43.8%)
Throat carrier	48	0 (0%)

5.2.1 Hand carrier

Hand swabs were collected from 54 individual volunteers. The organisms isolated from hand samples were *S. aureus*, coagulase negative *Staphylococcus*, Micrococci, *Strptococcus* spp., *Bacillus* spp., and *E. coli*. CoNS was the most common isolate from the hands of staff, followed by *S. aureus*, Micrococci, *Bacillus* spp., *Streptococcus* spp. and *E. coli* as shown in figure 5. CoNS was detected from the hands of all staffs (100.0%), *S. aureus* (60.0%), Micrococci (27.0%), *Bacillus* spp. (19.0%), *Streptococcus* spp. (15.0%) and *E. coli* (2.0%).

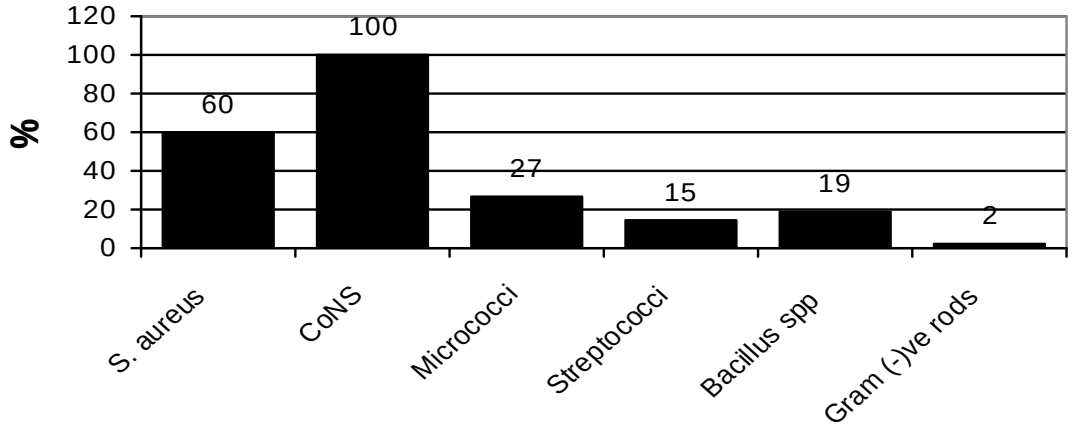


Figure 6 Occurrence of different microorganisms in the hands of staffs (by swab + gloves method)

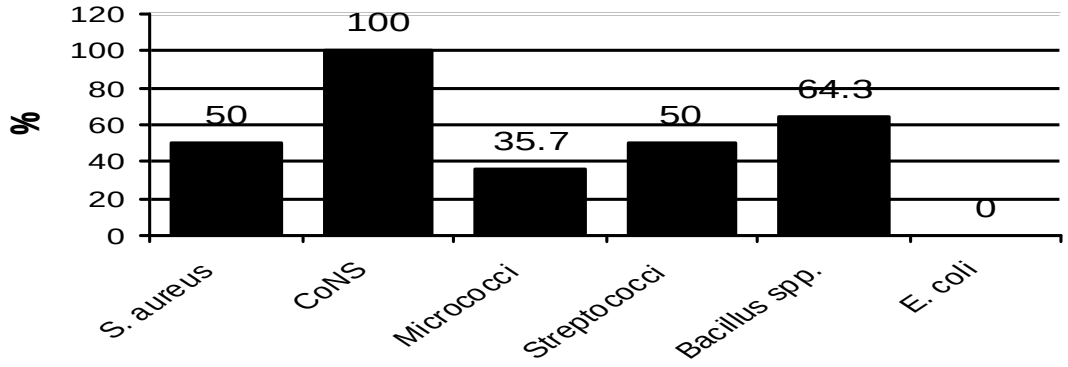


Figure 7 Detection of microorganism from hands of staffs by gloves method

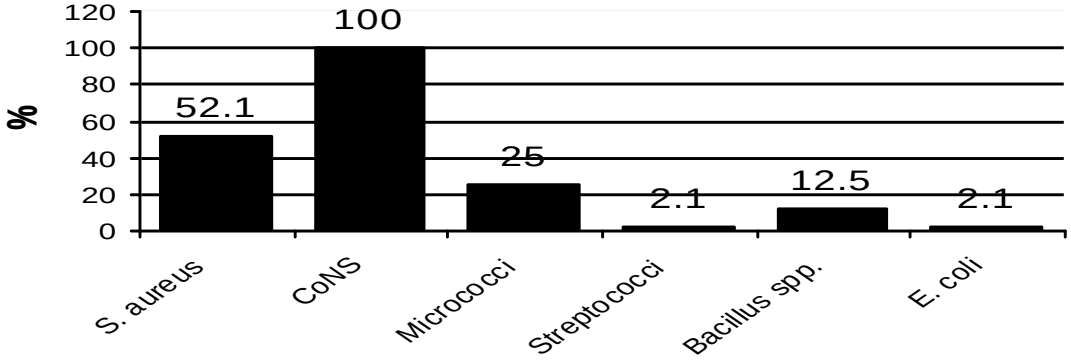


Figure 8 Detection of microorganisms from hands of staffs by swab method

As shown in figure 7 and 8 different microorganisms could be isolated from hands of staffs by using two different methods for the purpose of collection of sample. Though the microorganisms isolated by two methods were similar there was some difference in the

pattern of detection in the two methods. Though difference was seen in the pattern of detection of microorganisms from the hands of staffs by two methods (gloves and swab method) the difference is minimal. Since the size of sample is less in the case of gloves method than in the swab method, no inference can be drawn from the data. However, according to the pattern seen, if large sample size is taken gloves method may prove to be more effective than the swab method as larger suface is covered by the former than the later one.

5.2.2 Nasal carrier

Out of 48 nasal swabs collected from staffs from different wards in the hospital, *S. aureus* could be isolated from 21 samples i.e. 44.0% of the total sample. The graphical representation of the given fact is shown in figure 7.

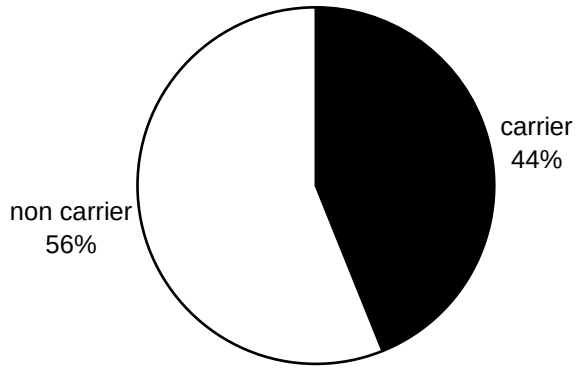


Figure 9 Percentage of nasal carrier and non carrier among staffs

Among 21, 14 (67.0%) of the nasal carriers were found to have *S. aureus* in their hands too. Presence of *S. aureus* in hands of the staff was not associated with the presence of the organism in the anterior nares of the individuals.

Table 7 Occurrence of *S. aureus* in the hands and the nose of staffs

Site	**+nce of *S. aureus***	**-nce of *S. aureus***	**P-value**
Hand (n=54)	32	22	<0.05
Nose (n=48)	21	27	
Total	**53**	**49**	

Out of 21 *S.* aureus isolated from the anterior nares of staffs working at different wards, 8/21(38.1%) were resistant to Amoxicillin, 3/21(14.3%) were resistant to Chloramphenicol and Gentamicin, 2/21(9.5%) were resistant to Methicillin, Cloxacillin and Gentamicin and 7/21 (33.3%) were resistant to Erythromycin which is shown clearly in table 7.

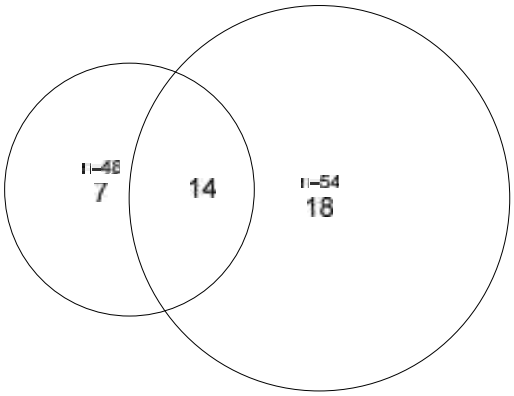


Figure 10 Diagrammatic representation of number of *S. aureus* from hand and nose of staffs

Similarly out of 32 *S. aureus* isolated from hands of staffs working at different wards, 10/32(31.3%) were resistant to Amoxicillin and Erythromycin, 3/32(9.4%) were resistant to Cotrimoxazole and Gentamicin, 2/32(6.3%) were resistant to Chloramphenicol and 1/32(3.1%) were resistant to Methicillin and Cloxacillin. Out of 53 *S. aureus* carrier isolates: 21 from nasal carriers and 32 from hand carriers, 17/53 (32.1%) were found to be resistant to Erythromycin, 18/53 (34.0%) to Amoxicillin, 6/53 (11.3%) to Cotrimoxazole, 5/53 (9.4 %) to Chloramphenicol and Gentamicin and 3/53 (5.7%) to Methicillin and Cloxacillin as given in table 7.

Table 7 Antibiotic susceptibility pattern of *S. aureus* isolates from hand and nose of staffs

Site	N	Antibiotics (% resistant)						
		Am	C	Co	G	E	M	Cx
Nasal	21	38.1	14.3	14.3	9.5	33.3	9.5	9.5
Hand	32	31.3	6.3	9.4	9.4	31.3	3.1	3.1
Total	53	34.0	9.4	11.3	9.4	32.1	5.7	5.7

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 Discussion

The health-care facility environment is rarely implicated in disease transmission, except among patients who are immunocompromised. Nonetheless, inadvertent exposures to environmental pathogens (e.g., *Aspergillus* spp. and *Legionella* spp.) or airborne pathogens (e.g., *M. tuberculosis* and varicella-zoster virus) can result in adverse patient outcomes and cause illness among health-care workers (CDC, 2003). In some other conditions, the environmental microorganisms in the hospital can infect the patients who are often weak and immunocompromised. Moreover microorganisms in the hospital environment are often resistant to antibiotics commonly used which make them even more dreadful. Environmental infection-control strategies and engineering controls can effectively prevent the infections arising in the hospital.

Attention to indoor air and environment quality is important in health care settings because many patients have infections that can spread through airborne exposure. The density of people in health care settings is relatively high causing easy spread of microorganisms via airborne route. The airborne route of infection is thought to account for 10.0% of all cases of hospital infection (Greenwood *et al*, 1997). Infection also spreads via inanimate objects and also through hands of health care workers. So this study was done to assess the microbial quality of air as well as surfaces in the environment of the hospital and also to assess the carrier pattern among the staffs and hence increase awareness to improve the environmental quality and thereby decrease the occurrence of NIs.

A total of 2439 cfu was isolated from environmental samples (air and surface samples) in the hospital. From the study surgical ward was found to have the highest density of microorganisms followed by orthopedic ward, POW & ICU, ANC & PNC, medical ward, NICU & NICUM, LI & LW and OT in the environmental samples. Surgical wound infections

have been one of the most frequently occurring nosocomial infections these days. The incidence of surgical wound infections has been reported to be in the range of 0.5 to 15.0% depending upon the type of operation and underlying patient status and has been one of the factors for lengthening hospital stay of a patient (WHO, 2002). In his study Banjara *et al* (2002) has reported the rate of surgical wound infection to be 4.7% and Tuladhar *et al* has reported it to be 10.2%.

Among the environmental isolates, gram positive cocci were found to be the most predominating microorganisms, followed by yeast, gram positive rods and gram negative rods were the least occurring microorganisms. Similar result was found by other researchers too where gram positive cocci were the most predominating microorganisms in the environment. In this study, CoNS were the most predominating microorganism in the environment followed by *S. aureus*, *Streptococcus* spp., *Micrococcus* spp., *Bacillus* spp., and gram negative bacilli were the least prevalent of all the isolates. Howevewr, in previous studies Pokhrel *et al* (1993) has reported that *S. aureus* were the most common isolate followed by *Micrococcus* spp., CoNS and other gram negative isolates in the order of frequency. Similarly Banjara *et al* has also reported *S. aureus* to be the most predominating isolate in the indoor air of the hospital. In her study Sharma *et al* has reported the similar study. Occurrence of higher percentage of CoNS in this study may be due to the difference in the study sites in these studies. Microbial flora of a hospital depends on a host of factors, which include the type of air control, the activity of the personnel, the nature of the area, and, above all, the excellence of housekeeping (Davidson and Henry, 1969). Another possible reason for the difference may be inclusion of large number of surface samples in our study, because CoNS have remarkable property of surface adhesiveness due its ability to form slime. Though CoNS were not considered as pathogens previously, they have been an emerging threat in the recent years due to their ability to cause infection in hospitalized patients (Nayak *et al*, 1990; Rai *et al*, 1987). In contrast to *S. aureus*, CoNS infections are almost entirely associated with hospital care. They are an important cause of nosocomial bacteremia and device associated nosocomial infections. Gram positive cocci are one of most resistant non-sporing bacteria and survive well in the environment under both moist and dry condition (Forbes *et al*, 2002). Hence gram positive cocci, derived from the body of the

hospital population, are found in the air, dust and on surfaces where they may survive along with fungal and bacterial spores and so are the major environmental inhabitants (Greenwood *et al*, 1997).

Gram positive cocci are associated with a number of NIs. In 1990 to 1996, the three most common pathogens, *S. aureus*, coagulase negative staphylococci, and enterococci, accounted for 24.0% of nosocomial infections and the four other pathogens, *E. coli, P. aeruginosa*, *Enterobacter* spp., and *K. pneumoniae*, accounted for 22.0% (Weinstein, 1998). Gram negative isolates though isolated in small number are significant as they are more pathogenic. Tuladhar *et al* (1990) has reported that the most common isolate causing nosocomial infection were *E. coli, Klebsiella, Pseudomonas* and *S. aureus* in the order of frequency.

Only 83.4% of the total NA plates exposed showed bacterial growth while 93.0% of the total SDA plates exposed for fungal isolation showed positive growth. This might be because fungal spores can spread easily which contribute to their ubiquitous nature. However, Pokhrel *et al* have reported in their study that only 45.0% of the specimens collected from the environment showed positive growth. This result alarms for the necessity of regular monitoring and more strict provision for the cleanliness in NMCTH.

All of the environmental isolates (bacteria) show high resistance to Amoxicillin followed by Erythromycin. A similar result was found in another study which reported Amoxicillin as the most commonly prescribed antibiotic and most of the clinical isolates were reported to be resistant to the drug. In the study out of 575 clinical isolates 12.4% were MRSA, 59.3% resistant to Amoxicillin, 24.0% resistant to Cephalexin and >90.0% were sensitive to Erythromycin (Rijal, 2003). However, in his study Banjara *et al* reported that most of the environmental isolates were resistant to Erythromycin. In the study he found all the environmental isolates of *S. aureus* to be sensitive to Gentamicin. But in this study most of the *S. aureus* isolates were resistant to Amoxicillin followed by Erythromycin, Cotrimoxazole and Gentamicin. Only 3 isolates out of 182 tested (1.6%) were found to be Methicillin resistant, this is less as compared to the previous findings by Rijal, 2003 which was 12.4% which might be due to the fact that the clinical isolates show higher degree of

drug resistance than the environmental isolates. Previous studies have shown that *S. aureus* strains from patients showed a much higher resistance to antibiotic than did those from the environment or the employees (Davidsohn and Henry, 1969). But in a similar study done in TUTH Pokhrel *et al* has found 4 isolates out of 30 (13.0%) to be Methicillin resistant which is much greater than our finding. Occurrence of smaller proportion of MRSA in this study may be due to the fact that TUTH is an older and larger hospital with greater patient inflow than NMCTH.

The occurrence of Methicillin resistant *S. aureus,* though small, indicates the need for further screening and eradication because these organisms can be the cause of NIs and patients infected with MRSA usually have poor prognosis. In addition the pool of circulating MSSA strains is an important parameter with regard to the epidemiology of hospital and community acquired MRSA clones and their potential virulence. It is because MRSA strains carry the readily transmissible *mec* cassette which may be the cause of rapid dissemination of MRSA in a hospital with a pool of MSSA (Layer *et al,* 2006).

Among the fungal isolates yeast was the most common fungal isolates. Similarly among mold, *Aspergillus* spp., was the most common fungi isolated in most of the wards, followed by *Alternaria* spp., *Penicillium* spp., *Fusarium* spp., *Mucor* spp., and *Rhizopus* spp. *Scopulariopsis* spp., could be isolated from only single ward i.e. ICU. *Aspergillus* spp. is one of the common causes of fungal nosocomial infection. The species of *Aspergillus* documented to cause infection in the setting of nosocomial infection include *A. fumigatus, A. flavus* and *A. terreus*. It may be due to the ubiquitous nature of the fungal spores due to which it is dispersed in the environment and is present in unfiltered air; ventilation systems; contaminated dust dislodged during hospital construction; carpeting; food, and ornamental plants (Vandenbergh *et al,* 1999). Hence detection and control of fungal spores in the environment is an important aspect in the control of fungal nosocomial infections as they can be an important cause of nosocomial infections especially in immuno-compromised patients.

The various isolates that could be isolated from the hands of staffs were *S. aureus,* CoNS, *Bacillus* spp., *Micrococcus* spp., *Streptococcus* spp. and *E. coli.* Among these isolates,

S. aureus, and *E. coli* are considered to be true pathogen associated with a number of nosocomial infections while the others especially CoNS and *Micrococcus* spp., are opportunistic pathogens. *S. pneumoniae* and other enterococci are also an important cause of nosocomial infections (Ayliffe, 2000). Occurrence of *S. aureus* and *E. coli* and *Streptococcus* spp. in the hands of staffs is significant as these organisms can be spread easily from one patient to another via hands of staffs and can cause a number of infections to patients during hospitalization (Greenwood *et al*, 1997). *S. aureus* could be isolated from the hands of 32 out of 54 staffs. 21 out of 48 staffs tested were found to be nasal carrier for *S. aureus* and only 14 of these nasal carriers were found to have the organism in their hands too. Thus it can be inferred that healthy staffs can play an important role in the dissemination of the organism to the patients via their hands. Hand hygiene of staffs working in a hospital has long been identified as an important factor for reducing nosocomial infection. Two different methods were used for the collection of sample from hands of the staffs and difference could be observed in the pattern of detection of microorganisms by these methods. Since the size of sample is less in the case of gloves method than in the swab method, no solid inference can be drawn from the data. However, according to the pattern seen, if large sample size is taken, gloves method may prove to be more effective than the swab method as larger suface is covered by the former than the later one.

The nasal carrier rate among the staffs was found to be significantly higher in this study (67.0%) than that reported by previous workers (10.0 to 40.0%) in healthy people (Cheesebrough, 2000). One possible reason may be that hospital staffs may get exposure to the bacterial flora by three times than others resulting in higher degree of colonization among hospital people (Cookson *et al,* 1989). The result of this study was slightly higher than that reported by Shah *et al* (2002). It may be due to different study site in the two studies. 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 organism (Chiang and Climo, 2002).

The antibiogram pattern of the MRSA isolates from hand, nose and environment were same. Thus it can be said on the basis of antibiotic profile that same clone of *S. aureus* might have

been distributed in these areas. However further proof using strong molecular epidemiological markers is essential.

In a study to detect healthy MRSA carrier in a hospital in Abidjan out of 269 *S. aureus* carriers 38.7% were MRSA carriers. However in the present study, only 9.5% out of 21 isolates were MRSA. The smaller percentage of MRSA isolates in this study might be due to small sample size. Another possible reason may be due to the difference in the study site. Since the result is in coordination with the result seen in the environmental isolates, it may be inferred that occurrence of MRSA in NMCTH is low. One possible reason may be that NMCTH is a recently established hospital with small number of patient inflow.

As in the case of environmental isolates, most of the *S. aureus*, isolated from the staffs were resistant to amoxicillin followed by erythromycin, cotrimoxazole, chloramphenicol, gentamicin, and methicillin. Thus on the basis of antibiogram pattern it may be inferred that same clones of *S. aureus* might have been distributed all around the hospital. However this needs to be further tested using stronger molecular tools such as Pulsed Field Gel Eletrophoresis (PFGE) or PCR.

Out of 48 healthy staffs tested for the presence of group A streptococci in their throat no one was found to be the positive carrier. A negative result might have occurred due to small size of the sample in the study. Other reasons may be that most of the people acquire immunity to the organism after being exposed to it during their childhood days. Further screening by similar study using larger sample size is suggested because occurrence of group A streptococci even in a single healthy carrier is epidemiologically significant.

6.2 Conclusion

From the study, it can be concluded that the indoor environment of Nepal Medical College Teaching Hospital is built up with a large number of bacterial and fungal microflora. Gram positive bacteria built up the major portion of hospital flora. Occurrence of bacterial and fungal flora in large quantities alarms for the need of effective house keeping and supervision. High prevalence of nasal carrier indicates the need for screening for *S. aureus*

nasal carriers and subsequent elimination. Similarly occurrence of various bacteria in the hands of staffs indicates the lack of effective hand washing and sanitation and indicates for the need of guidelines for hand washing and strict adherence to the guideline by effective monitoring and supervision. Prevalence of MRSA though low alarms for further screening of the organism and its subsequent eradication. Finally study of hospital environmental microflora and screening staffs especially for nasal carrier and hand hygiene can be helpful in the control of nosocomial infection.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATION

7.1 Summary

Nosocomial infections contribute significantly to morbidity and mortality as well as to excess costs for hospitalized patients. At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital. Inadvertent exposures to environmental pathogen (e.g. *Aspergillus* spp. and *Legionella* spp.) or airborne pathogens (e.g. *M. tuberculosis* and varicella-zoster virus) can result in adverse patient outcomes and cause illness among health-care worker. In some other conditions, the environmental microorganisms in the hospital can infect the patients who are often weak and immunocompromised. Moreover microorganisms in the hospital environment are often resistant to antibiotics commonly used which make them even more dreadful.

In the present study, out of total 2439 environmental isolates (hospital), gram positive cocci (76.0%) were the most predominant microorganisms followed by yeasts (13.0%), gram positive rods (8.0%) and gram negative rods (3.0%). The gram negative isolates were *P. aeruginosa, E. coli, Klebsiella* spp. and *Citrobacter* spp.

Coagulase negative staphylococci were the most predominant bacteria followed by *S. aureus, Streptococcus* spp., *Micrococcus* spp., *Bacillus* spp., and gram negative rods. Among molds *Aspergillus* spp. was the most common isolate followed by *Alternaria* spp., *Penicillium* spp., *Fusarium* spp., *Mucor* spp., and *Rhizopus* spp.while *Scopulariopsis* spp., could be isolated from only single ward i.e. ICU.

Most of the isolates were resistant to Amoxicillin and Erythromycin. Only 1.6% of the environmental isolates of *S. aureus* were found to be Methicillin resistant. 5.6% of the CoNS were resistant to Methicillin.

Out of 54 staffs 32 staffs had *S. aureus* in their hands. Other organisms isolated from the hands of the staffs were CoNS, *Micrococcus* spp., *Streptococcus* spp., *Bacillus* spp., and *E. coli*. 21 out of 48 i.e. 44% of the total samples were positive nasal carrier for *S. aureus*. Out of these 21, only 14 had *S. aureus* in their hands too. None of the 48 staffs tested were carrier for group A streptococci.

Out of 53 *S. aureus* carrier isolates: 21 from nasal carriers and 32 from hand carriers, 32.1% were found to be resistant to Erythromycin, 34.0% to Amoxicillin, 11.3% to Cotrimoxazole, 9.4 % to Chloramphenicol and Gentamicin and 5.7% to Methicillin.

7.2 Recommendations

1. Disinfection of indoor air and environmental surfaces in the hospital and proper monitoring for maintenance.

2. Since routine microbilogical sampling of air and environmental surfaces is not recommended by CDC, sampling should be done whenever suspected of nosocomial infection outbreaks that may spread via air or the source of infection is environment.

3. Development of guidelines for Hand-washing and Hospital Environmental control and strict adherence to the guideline.

4. Conduction of workshops and seminar to make the staffs aware about the nosocomial infection and their role in the control of it.

5. Screening for methicillin resistant *S. aureus*, and its proper management since such screening programs have been found to be effective in the control of infection due to the organism.

6. Regular screening of staffs for nasal carriage of *S. aureus* and MRSA and their proper management for the eradication of the organism.

7. Screening of high risk patients such as hemodialysis or chronic ambulatory peritoneal dialysis (CAPD) or surgical patients for nasal carriage.

8. Establishment of an effective hospital acquired infection control program with guidelines for antimicrobial use which should improve the use of prophylactic antibiotics.

CHAPTER-VIII

8. REFERENCES

Akcam FZ, Karaaslan D, Dogan M and Yayli G (2006) Microbiological surveillance in the intensive care unit: a tertiary hospital experience. Med Sci Monit. 12: CR81-5

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

Arunodaya GR (2001) Infections in neurology and neurosurgery intensive care unit. Neurology India June (Suppl.)**:** 51–9

Ayliffe GAJ (1992) Control of hospital infection. A practical handbook, 3rd ed. London. Chapman and Hall. pp. 850-61

Ayliffe GAJ (2000) Recommendations for the control of methicillin-resistant *Staphylococcus aureus* (MRSA).WHO/EMC/LTS/96.1

Arora U and Jaitwani J (2006) *Acinetobacter* spp. - An emerging pathogen in Neonatal Septicemia in Amritsar. Indian J Med Microbiol. 24: 81

Banjara MR (2002) Study of air, water and wound infection in different wards of T.U Teaching Hospital. A dissertation submitted to the central department of Microbiology. T.U. Kirtipur

Boyce JM (1996) Preventing Staphylococcal Infections by Eradicating Nasal Carriage of *Staphylococcus aureus.* Infect Control Hosp Epidemiol. 18: 622-7

Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL and Jameson JL, (2001) Harrison's principles of internal medicine 15th edition, 1: 853-60

CDC (1992) Public health focus: surveillance, prevention and control of nosocomial infections. MMWR. 41: 783-7

CDC (1995) CDC recommendations for preventing the spread of vancomycin-resistance: Recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC). MMWR. 44: 1–12

CDC (1997) Guidelines for the prevention of nosocomial pneumonia. MMWR. 46: 1-79

CDC (2000) Monitoring Hospital-Acquired Infections to Promote Patient Safety - United States, 1990-1999. MMWR. 49: 149-53

CDC (2004) National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004, AJIC special article

Celik SA (2004) Nosocomial Infections in Neurosurgery Intensive Care Units. J Clin Nurs. 13: 741–7

Çelik SA and Aksoy G (2001) Infection control in the intensive care units. J Inten Care Nurs. 1: 16–24

Chakraborty P (2000) A Text Book of Microbiology. 1st edition. New Central Book Agency Pvt. Ltd., Calcutta pp. 609-13

Cheryl LS, Victor L and Janet SE (2000) Waterborn Nosocomial Infections. Current Infectious Disease Reports. 2: 490-96

Cheesbrough M (2000) District Laboratory Practice in Tropical countries. Cambridge University Press, London. 2: 196-274

Chiang FY and Climo M (2002) *Staphylococcus aureus* Carriage and Health Care-acquired Infection, Curr Infect Disease Reports. 4: 498-504

Cole AM, Tahk S and Oren A (2001) Determinants of *Staphylococcus aureus* Nasal Carriage. Clin Diag Lab Immun 8:164-9

Cookson B, Peters B, Webster M, Philips I, Rahman M and Noble W (1989) Staff Carriage of Epidemic Methicillin Resistant *Stahpylococcus aureus*. J Clin Microbiol. 27: 1471-6

Davidsohn I and Henry JB (1969) Todd-Sanford clinical diagnosis by laboratory methods (14th ed.). Philadelphia. London. Toronto: W.B. Saunders pp. 1074-88

Eggimann P and Pittet D (2001) Infection control in the ICU. Chest 120: 2059–93

Eggimann P and Pittet D (2002) Overview of catheter-related infections with special emphasis on prevention based on educational programs. Clin Microbiol Infect. 8: 295

Floret N, Bailly P, Bertrand X, Louis-Martinet C, Picard A, Tueffert N and Talon D (2006) Results from a four- year study on the prevalence of nosocomial infections in Franche-Comte: attempt to rank the risk of nosocomial infection. J Hosp Infect: Epub ahead of print

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

Geoffrey RS and Easmon CSF (1990) Topley and Wilson's Principles of bacteriology, virology and immunity 8th edition. 3: 141-67

Greenwood D, Slack RCB and Peutherer JF (1997) Medical Microbiology, 14th edition. ELBS pp. 781-9

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

Horan TC and Gaynes RP (2004) Surveillance of nosocomial infections. In: Hospital Epidemiology and Infection Control, 3rd ed. Mayhall CG Philadelphia: Lippincott Williams and Wilkins pp. 1659-1702

Hernandez JR, Martinez-Martinez L, Canton R, Coque TM, Pascual A and Spanish Group for Nosocomial Infections (GEIH) (2005) Nationwide Study of *Escherichia coli* and *Klebsiella pneumoniae* Producing Extended-Spectrum {beta}-Lactamases in Spain. Antimicrob Agent Chemother. 49: 2122-5

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

Jones RN, Croco MA, Kugler KC, Pfaller MA and Beach ML (2000) Respiratory tract pathogens isolated from patients hospitalized with suspected pneumonia: frequency of occurrence and susceptibility patterns from the Sentry Antimicrobial Surveillance Program. Diagn Microbiol Infect Dis. 37: 115-25

Kachi S, Okazaki M, Takeda H, Igarashi H, Kobayashi O, Watanabe H, Nakata K, Kawai S, Aoshima M, Watanabe T and Goto H (2006) Outbreak of *Nocardia farcinica* infection with the same pattern in randomly amplified polymorphic DNA analysis. J Hosp Infect. 62: 502-6

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

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

Layer F, Ghebremedhin B, Konig W and Konig B (2006) Heterogeneity of Methicillin-Susceptible *Staphylococcus aureus* Strains at a German University Hospital Implicates the Circulating-Strain Pool as a Potential Source of Emerging Methicillin-Resistant Strains *S. aureus* Clones. J Clin Microbiol. 44: 2179-85

Manley K and Bellman L (2000) Surgical Nursing Advancing Practice, 1st edition. Churchill Livingstone, London: 250–61

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

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

Morrison AJ and Wenzel RP (1984) Epidemiology of infections due *to Pseudomonas aeruginosa.* Rev Infect Dis. 6: S627-42

Nakanishi M, Shrestha HG and Rai SK (1996) Text Book of Medical Laboratory Technology, 1st Edition. Medical Education Project JICA, TUTH, Kathmandu Nepal: pp. 359

Nayak N, Pal N, Ayyagari N (1990) Slime production as a marker of coagulase negative staphylococcal infections with/without artificial devices. Indian J Med Microbiol. 8: 92-6

Neumaier M, Kappstein I and Scherer MA (2006) Positive screening for MRSA- clinical consequences? Unfallchirurg. 109: 499-504

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

Orrett FA, Brooks PJ and Richardson EG. (1998) Feb, Nosocomial infections in a rural regional hospital in a developing country: infection rates by site, service, cost and infection control practices. Infect Control Hosp Epidemiol. 19: 136-40

Orrett FA and Changoor E (2006) Bacteremia in Children at a regional hospital in Trinidad. Int J Infect Dis. 18: Epub ahead of print

Özinel MA (2003) Guideline for preventing of urinary catheter infection. Hospital Infections Education Program, Bursa: 1–20

Plowman R, Esquivel J and Roberts JA (2001) An economic model to assess the cost and benefits of the routine use of silver alloy coated urinary catheters to reduce the risk of urinary tract infections in catheterized patients. J Hosp Infect. 48: 33-42

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

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

Rai SK, Tuladhar NR and Shrestha HG (1990) Methicillin resistant *Staphylococcus aureus* in a tertiary medical care center, Nepal (short communication). Ind J Med Microbiol. 8: 108-9

Rijal B (2003) Study on antimicrobial resistance and its correlation with antimicrobial use. A report submitted to Nepal Health Research Council.

Ruiz-Garbajosa P, Bonten MJM and Robinson DA (2006) Multilocus Sequence Typing Scheme for *Enterococcus fecalis* Reveals Hospital-Adapted Genetic Complexes in a Background of High Rates of Recombination. J Clin Microbiol. 44: 2220-8

Rezende EM, Couto BR, Starling CE and Modena CM (1998). Prevalence of nosocomial infections in general hospitals in Belo Horizonte. Infect Control Hosp Epidemiol. 19: 872-6

Sablotzkl A, Ebel H, Mühling J, Dehne MG, Nopens H, Giesselman H. and Hempelmann G (2000) Dysregulation of immune response following neurosurgical operations. Acta Anasthesiologica Scandinavica. 41: 82–7

Şahinoğlu H (1992) Intensive Care Problems and Treatment. Turkey Clinics Publishers. Ankara: 605–9, 729–40

Shah KB (2002) Study on nasal carriage of *Staphylococcus aureus* among the post operative ward visitors, staff and patients of TU teaching hospital with drug sensitivity pattern. A dissertation submitted to Central Department of Microbiology T.U. Kirtipur

Sharma S (2002) Pattern of microbial flora among the visitors and the environment of intensive care unit (ICU) Tribhuvan University Teaching Hospital. A dissertation submitted to Central Department of Microbiology T.U. Kirtipur

Smith GR and Easmon CSF (1990) Topley and Wilson's Principles of Bacteriology, Virology and Immunity, Vol. 3, Bacterial Diseases. B.C. Decker Inc. Philadelphia Hamilton. pp. 142-67

Tasota FJ, Fisher EM, Coulson CF and Hoffman LA (1998) Protecting ICU patients from nosocomial infections: practical measures for favorable outcomes. Critical Care Nurse. 1: 54–67

Tikhomirov E (1987) WHO Programme for the Control of Hospital Infections. Chemiotherapia. 3: 148–51

Trick WE, Fridkin SK, Edwards JR, Hajjeh RA, Gaynes RP, and the National Nosocomial Infections Surveillance System Hospitals (2000) Secular Trend of Hospital-Acquired Candidemia among Intensive Care Unit Patients in the United States during 1989–1999. Candida NNIS. WHO

Tuladhar NR, Nakanishi M, Shrestha HG, Duffy J, Tomiyoshi Y, Pandey G and Sharma SP (1990) Nosocomial Infection in Surgical patient at Tribhuvan University Teaching Hospital. J Nep Med Ass. 28: 152-6

Vandenbergh MFG, Verweij PE and Voss A (1999) Epidemiology of Nosocomial Fungal Infections: Invasive Aspergillosis and the Environment. Diagn Microbiol Infect Dis. 34: 221-7

Wallace B (1999) Nosocomial *Acinetobacter* Infection. Guidelines for Infection Control (Letter)

Wang X, Zhou H and Wang X (2001) A study on nosocomial infection among inpatients in Beijing Hospital for elderly. Zhonghuaha Liu Xing Bing Xue Za Zhi. 22: 212-4

Wernitz MH, Swidsinski S, Weist K, Sohr D, Witte W, Franke KP, Roloff D, Ruden H and Veit SK (2005) Effectiveness of a hospital-wide selective screening program for methicillin-resistant *Staphylococcus aureus* (MRSA) carriers at hospital admission to prevent hospital-acquired MRSA infections. Clin Microbiol Infect. 11: 457-65

Weinstein RA (1998). Nosocomial Infection Update. Emerging Infectious Diseases. Special Issue Vol.4, No. 3

Yang ZM and Li YS (2003) The risk of nosocomial infection in severe craniocerebral trauma. Chinese J Traumatology. 1: 28–31

Yano K, Sasagawa K, Yamano T, Bijukche SM, Gupta MP, Terasaki Y and Upadhyaya RP (1992) Reduced Air Pollution at the Outpatient Area of TUTH by Improving of Cleaning Procedure and Setting up Riddle-path for Dust Removal. J Inst Med. 14: 20-8

Zhang YL, Zhou HW, MAL Wang YY and Wang NP (2006) Homology Analysis of the epidemiological strains of methicillin-resistant Staphylococcus aureus and the strains isolated from the nasal fossa of the medical staff and inpatients. Nan Fang Yi Ke Da Xue Bao. 26: 635-7

Zenone T and Souillet G (1996) X linked Agammaglobulinemia presenting as *Pseudomonas aeruginosa* septicemia. Scand J Infect Dis. 28: 417-8

CHAPTER-IX

9. APPENDICES

APPENDIX: I

List of Materials

1. Equipments

Autoclave (Sakura)
Burner
Compound Microscope (Olympus)
Hot Air Oven (Sakura)
Incubator (Memmert)
Safety cabinet
Refrigerator (Philips)
D-freeze

2. Microbiological media (Hi-Media)

Nutrient Agar
Brain-Heart Infusion Broth
Mannitol salt Agar
Chocolate Agar
MR-VP Broth
TSI Agar
Nutrient broth
MacConkey Agar
Blood Agar
Mueller-Hinton Agar
Simmons Citrate Agar
Urease Agar

3. Chemicals/ Reagents

3% hydrogen peroxide
Acetone-alcohol
Safranine
Sodium Chloride
Blood plasma
Methyl red
Mineral oil
NNNN-tetramethyl paraphenyl diamine dihydrochloride
Crystal violet
Gram's iodine
Barium chloride
Sulphuric acid
Barrit's reagent
Kovac's reagent
Glycerol

4. Antibiotics Discs (Hi-Media)

Amoxycillin (10 mcg)
Ciprofloxacin (5 mcg)
Cloxacillin (5mcg)
Chloramphenicol (30 mcg)
Nitrofurantoin (300 mcg)
Cephalexin (30 mcg)
Bacitracin (10 units)
Erythromycin (15 mcg)
Gentamicin (10 mcg)
Methicillin (5mcg)
Cotrimoxazole (1.25 mcg)
Amikacin (30 mcg)
Optochin

5. Miscellaneous

Glasswares
Blotting paper, cotton, Tissue paper
Distilled water
Lysol
Inoculating loop, forceps, droppers
Immersion oil
Sticker

APPENDIX : II

Identification of bacteria

1. Typical Gram stain morphologies of gram(+)ve and gram variable genera

1. Most gram positive species can appear gram variable or even gram negative due to over decolorization, phagocytosis, antibiotic effect, age etc.
2. *Mycobacterium* spp. May stain gram neutral and appear as “ghost” forms; they may also appear beaded resembling chains of streptococci.
3. Individual cells in chain vary in size.

2. Identification chart of some gram negative bacteria:

S.N	Bacteria	Characteristic
1.	*Pseudomonas aeruginosa*	Gram -ve rod; oxiadse +ve; TSI: R/R No gas, No H_2S citrate positive; bluish green pigmented colonies on nutrient agar

2. Aeromonas hydrophilia Gram-ve rod; oxiadse +ve; VP+ve; small β-hemolytic colonies on blood agar, fermentive.
3. Actinobacter spp. Gram-ve coccobacilli; TSI inert; Oxidase -ve; Catalase +ve; Indole-ve

3. Typical Gram stain morphologies of Gram-negative genera

4. Flow chart for identification of Gram-positive cocci

5. Biochemical reactions of some enteric bacteria

Species	Urea	VP	ONPG	Lact	Man	Glu	Suc	Ox	Cit	Mot	Ind	LDC	KIA			
													Slope	Butt	H_2S	Gas
E. coli	-	-	+	+	+	+	D	-	-	+[5]	+[2]	+	Y[6]	Y	-	+[2]
***Shigella* spp.**	-	-	-[7]	-	d	+	-[1]	-	-	-	d	-	R	Y	-	-[3]
Salmonella typhi	-	-	-	-	+	+	-	-	-	+	-	+	R	Y	+weak	-
***Salmonella paratyphi* A**	-	-	-	-	+	+	-	-	-	+	-	-	R	Y	-	+
Other *Salmonella*	-	-	-	-	+	+	-	-	+	+	-	+	R	Y	+[2]	d
Citrobacter fruendii	D	-	+	+late	+	+	D	-	+	+	-[3]	-	R or Y	Y	d	+
Klebsiella pneumoniae	+slow	+	+	+	+	+	+	-	+	-	-[3]	+	Y	Y	-	+
***Enterobacter* spp.**	-	+	+	+	+	+	D	-	+[2]	+	-	d	Y	Y	-	+
Serratia macescens	D	+	+	d	+	+	+	-	+	+	-	+	R or Y	Y	-	d
Proteus vulgaris	+	-	-	-	-	+	+	-	d	+	+	-	R	Y	+	d
Proteus mirabilis	+	D	-	-	-	+	D	-	+[2]	+	-	-	R	Y	+	+
Morganella morganii	+	-	-	-	-	+	-	-	-	+[5]	+	-	R	Y	-	d
***Yersinia enterocolitica*[4]**	+slow	-	+	-	+	+	+	-	-	+	d	-	R	Y	-	-
Vibrio cholerae	-	D	+	-24h	+	+	+	+	d	+	+	+	R	Y	-	-

Key: LDC = Lysine decarboxylase, VP =Voges-Proskauer, ONPG = beta-galactosidase, Lact = Lactose, Man = Mannitol, Glu = Glucose, Suc = Sucrose, Ox = Oxidase, Cit = Citrate test, Mot = Motility, Ind = Indole test, Urea = Urease test, H_2S = Hydrogen sulphide test (blackening), R = Red-Pink (alkaline reaction), Y = Yellow (acidic reaction), d = different strains give different results

Note:

1. ***S. sonnie* ferments sucrose slowly.**
2. **A minority of strains give a negative result.**
3. **A minority of strains give a positive result.**
4. **Tests should be incubated at 20-28^0 C.**
5. **A few strains are non-motile.**
6. **A few strains give positive reactions similar to *Shigella* sps.**
7. ***S. sonnie* is ONPG positive.**

(Source: Cheesbrough M, 2000)

APPENDIX: III

Composition/ Preparation/ Procedure of Media Used in Isolation and Identification of Bacteria

(Note: All compositions are given in grams per litre and at 25^0 C temperature.)

1. **Nutrient Agar (NA)**

Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Agar	15.0
Final pH	7.4 ± 0.2

2.8 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121^0C for 15 mins.

2. Nutrient Broth (NB)

Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Final pH	7.4 ± 0.2

1.3 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121^0C for 15 mins.

3. MacConkey Agar (MA)

Pancreatic digest of gelatin	17.0
Peptone	3.0
Lactose	10.0
Sodium Chloride	5.0
Bile salt	1.5
Agar	13.5
Neutral red	0.03
Crystal Violet	0.001
Final pH	6.9 – 7.3

5.5 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121^0C for 15 mins.

4. Blood Agar

Composition of Blood Agar base

Proteose peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	15.0
pH	7.4

Blood Agar base medium is prepared and autoclaved at 121^0C for 10 mins. t is then cooled down to 48^0C and blood (7-10%) is added aseptically and mixed thoroughly. About 18-20 ml. of the media is then poured on Petri-plates. If bubbles appear in the poured plates, a flame is passed over the bubbles before the media sets.

5. Chocolate Agar Medium

Chocolate agar is prepared by heating blood agar until the medium becomes chocolate coloured. Blood agar base is prepared, autoclaved and cooled down to 75^0 C. At this temperature, blood (approx. 8%) is added. After waiting for 10-15 mins, the colour is chocolate brown. It is then cooled to 50^0C and poured aseptically onto the3 sterile petridishes.

In the case of a prepared blood agar plate, it is kept at about 75^0C for 30 mins. By this time the colour has changed to chocolate brown.

6. Mueller Hinton Agar

Beef infusion Broth	300.0
Casein Acid Hydrolysate	17.0
Starch	1.0
Agar	17.0
Final pH	7.0 ± 0.2

3.8 gms of media was suspended in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121^0C for 15 mins. It was then poured while at 45-48^0C into sterile petriplates in 25 ml quantity.

7. MR-VP media

Buffered peptone	7.0
Dextrose	5.0
Dipotassium Phosphate	5.0
Final pH	6.9 ± 0

1.7 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was distributed in the amount of 5ml each into several test tubes and sterilized by autoclaving at 15 lbs pressure at 121^0C for 15 mins.

8. Simmons Citrate Agar

Magnesium Sulphate	0.2
Mono-ammonium Phosphate	1.0
Dipotassium Phosphate	1.0
Sodium Citrate	2.0
Sodium Chloride	5.0
Bromothymol Blue	0.08
Agar	15.0
Final pH	6.8 ± 0.2

2.42 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was distributed in the amount of 5ml each into several test tubes and sterilized by autoclaving at 15 lbs pressure at 121^0C for 15 mins. It was then allowed to cool in slanting position to prepare slant.

9. Triple Sugar Iron (TSI) Agar

Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Beef Extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous Sulphate	0.2
Sodium chloride	5.0
Sodium Thiosulphate	0.3
Phenol red	0.024
Agar	12.0
Final pH	7.4 ± 0.2

6.5 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was distributed in the amount of 5ml each into several test tubes and sterilized by autoclaving at 15 lbs pressure at 121^0C for 15 mins. The media was allowed to set in slope form to form a slant with butt about 1 inch long.

10. Urea Broth Base

Monopotassium Phosphate	9.1
Dipotassium Phosphate	9.5
Yeast Extract	.1
Phenol red	.01
Final pH	6.8 ± 0.2

1.85 gms of media was dissolved in 95 ml of distilled water and heated to dissolve the media. The media was sterilized by autoclaving at 15 lbs pressure at 121^0C for 15 mins. It was then cooled to 55^0C, and 5ml of sterile 40% urea solution was aseptically added. The contents were then mixed well and distributed into sterile test tube.

Appendix: IV

Statistical Tools

Occurrence of *S. aureus* in the hand and nose of staffs

Site	**Presence of *S. aureus***	**Absence of *S. aureus***	**Total**
Hand	**32**	**22**	**54**
Nasal	**21**	**27**	**48**
Total	**53**	**49**	**102**

Here, the null hypothesis (H_0) is that, the occurrence of *S. aureus* in hand and anterior nares are not associated with each other.

Calculation of χ^2 Value:

Expectation of 32= 53×54/105 = 28.05
So, the table of expected frequencies will be as follows:

Observed value (O)	Expected value (E)	$(O-E)^2$	$\chi^2_{cal} = \frac{(O-E)^2}{E}$
32	28.05	15.60	0.56
22	25.94	15.52	0.60
21	24.94	15.52	0.62
27	23.05	15.60	0.68
			$\Sigma\chi^2_{cal} = \frac{(O-E)^2}{E} = 2.46$

Calculated value of χ^2 (χ^2_{cal}) = 2.46
Here, the degree of freedom (d.f.) = (r-1) × (c-1) = (2-1) × (2-1) = 1
According to the table of χ^2 (χ^2_{tab}) at 5% level of significance (P> 0.05) for 1 d.f. = 3.84

Since the calculated value of χ^2 (χ^2_{cal}) is lesser than the corresponding tabulated value of χ^2 (χ^2_{tab}), the null hypothesis is accepted. This implies that it is not important that the *S. aureus* isolate, isolated from the hand of an individual be derived from his/her own nasal cavity. These isolates may be from other exogenous sources.