CHAPTER - I

1. INTRODUCTION

A WHO Expert Committee in 1963 proposed the following definition of a hospital: “A hospital is a residential establishment which provides short term and long-term medical care consisting of observational, diagnostic, therapeutic and rehabilitative services for persons suffering or suspected to be suffering from a diseases or injury and for parturient. It may or may not also provide services for ambulatory patients on an outpatient basis” (Park, 2002).

Although hospitals primarily offer services aimed at alleviating the sufferings of the patients, one can not ignore the fact that hospital environment and its apparatus contribute to the spread of a wide range of diseases if no proper sanitation or disinfection measures are employed. Hospital attending patients are usually debilitated and susceptible to infections from environmental microbes which are left over by the attending patients or attending persons during patients check-up.

Hospital acquired infection, also known as “Nosocomial Infection” is applied to any clinical infection, that is to say, infection causing illness, that was neither present nor in its incubation period when the subject entered hospital (Speller and Humphreys, 1998). Nosocomial infection is very much susceptible to inpatients, discharged inpatients, outpatients and even to the staffs, volunteers, visitors, workmen and delivery personnel. It may spill over into the community necessitating investigation and its control.

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 patients and hospital. Although a great deal of attention is being directed towards the control and prevention of these infections, old problems continue to occur and new problem are constantly arising (Brachman, 1981). Nosocomial infection adds functional disability, and emotional stress of the patient and may in some cases, lead to disabling conditions that reduce the quality of life.

The prevalence of hospital infection reported by WHO (1992) is 3-21% with a mean of 8.4%. So on an average 8.4% of all hospital patients will develop an infection as a result of their stay in hospital (Sharma, 2002). The estimated direct annual cost of those infections is in excess of $ 1 billion. Additionally 3% of nosocomial infections probably result directly in the death of the patient (Bennett and Brachman, 1979).

The microbiological investigation is responsible for the special support activities related to the surveillance, prevention and control of nosocomial infection. The inanimate environment present throughout the hospital is closely related to nosocomial infection and it may contribute to sporadic cases of disease or outbreaks in institutions by providing sources for contact, common vehicle, and air borne transmissions. The inanimate environment is in constant contact with the animate environment, patients and staffs. Prevention of nosocomial infection is partly directed at controlling this contact in order to achieve the desired relationships and prevent the transmission of microorganisms (Banjara, 2002).

The immediate environment of a patient readily becomes contaminated with the bacteria he/she carries. Infection from this source has been invoked to explain clustering of hospital infection (Speller and Humphreys, 1998). The air as a part of the inanimate environment serves as the means through which infectious microorganisms from animate or inanimate source are transmitted by droplet nuclei or dust (Banjara, 2002).

The clothing of personnel can be shown to become contaminated with potential pathogens, such as *Staphylococcus aureus* and less frequently, Gram-negative bacilli, particularly after the handling of heavily colonized patients (Byers *et al.*, 1998).

Transmission of nosocomial infections by medical equipments is frequent in hospital settings. But some of these equipments have escaped attention because the risks associated with them appeared to be low or simply had not been perceived. Others are pieces of equipment that are difficult to clean and disinfect adequately or are expensive and in short supply (Speller and Humphreys, 1998).

It has long been recognized that there is an urgent need to carry out studies that can help improve the quality of care, as well as lower the rate of nosocomial infections and the costs of hospitalization (de Andrade *et al.*, 2000).

Antibiotic treatment and hospital infection control are intimately entwined. The widespread use and misuse of antibiotic therapy has led to the problems like establishment of reservoir of virulent and antibiotic resistant bacteria concentrated in the hospital environment. Organisms causing nosocomial infection can be transmitted to the community through discharged patients, staffs and visitors. If organisms are multi-resistant, they may cause significant diseases in the community.

Tribhuvan University Teaching Hospital is a tertiary hospital of Nepal. Hundreds of patients under go treatment and routine check up in various outpatient departments of the hospital daily, where they encounter with various equipments and the inanimate environment around. So the fact that patients are prone to hospital infection via hospital environment and its equipments can not be ignored. The reason of this kind of study is to support health care of hospital attending patients and avoidance of cross or horizontal infections.

CHAPTER - II

2. OBJECTIVES

2.1 General

To examine the pattern of bacterial flora in various out patient departments of the hospital.

2.2 Specific

- To isolate and identify microorganisms from new and used bed sheets.
- To isolate and identify microorganisms from aprons of health care personnel (Doctors, nurses, health assistants and interns).
- To isolate and identify microorganisms from various equipments used in different out patient departments of the hospital.
- To examine air microorganisms of various outpatient departments.
- To examine the antibiotic sensitivity pattern of pathogenic microorganisms isolated.

CHAPTER-III

3. LITERATURE REVIEW

3.1 Hospital and its outpatient department

Hospitals primarily offer services aimed at alleviating the sufferings of the patients but the hospital environment and its apparatus offers excellent conditions for the propagation of microorganisms, in spite of disinfectants, antibiotics, and chemotherapeutics. In addition, with their immune systems weakened by illness, surgery, or accidents, patients generally make good hosts for microorganisms (de Andrade *et al.*, 2000).

Ambulatory care is fundamental arm of health care, as it is provided at all levels of health care system i.e., sub-center to tertiary care hospital. Ambulatory care or health care provided in outpatient department is defined as the care provided to patients, who are not confined to bed and care can be provided at a clinic, health center or a hospital (Rangrez *et al.,* 2005).

The outpatient facilities should be considered as the part of the inpatient facilities of the hospital so far as infection control activities are concerned. It may be necessary, however, to give some special consideration to certain aspects of an efficient and sensitive surveillance program and to establish effective cleaning procedures in some areas of the department because of the rapidity and frequency of patient movement through such areas (Banjara, 2002).

Increased provisions of health care in outpatient settings and concerns about occupational transmission of infections have focused attention on the risk of transmission of infectious diseases in ambulatory health care settings. In contrast to inpatient nosocomial infections, infections transmitted in out patient settings are neither systematically monitored nor likely to be detected by routine surveillance. To better define the spectrum of such events, we reviewed the literature to identify cases and

clusters of infections associated with outpatient health care. In this review, we identified and epidemiologically characterized 53 such reports that occurred from 1961 through 1990. Transmission occurred in general medical offices, clinics, and emergency departments (23); ophthalmologists' offices and clinics (11); dental offices (13); and alternative-care settings (6). Our findings suggest that inpatient infection control practices should be extended to outpatient health care settings by assigning specific responsibility for infection control and by adapting surveillance methods and prevention measures (Goodman and Solomon, 1991).

Hospital outpatient departments straddle an uncomfortable position, teetering between inpatient departments and freestanding ambulatory centers. Because they are more "open" to the community, they may find it more of a challenge to meet cleaning, disinfection and sterilization standards. That is not to say that these departments are deficient in any way - simply due to their high traffic and their rapid turnover, more people and therefore pathogens make their way into the facility.

Preventing disease transmission in a hospital setting is hard enough. But filling the space with the sick- and allowing them to return to the community after just enough time to be exposed to whatever is in the air and on the doorknobs-can create challenges for outpatient departments (Middleton, 2006).

There are many challenges for these departments-community-acquired antibiotic-resistant pathogens, a large number of reusable instruments that require sterilization or high-level disinfection, and a sterilization department that may be located miles away. The high traffic and quick turnover is the biggest challenge in ambulatory areas. Cleaning is difficult in outpatient departments as hours are more conductive to thorough cleaning.

Vancomycin resistant enterococci (VRE) and methicillin-resistant *S. aureus* (MRSA) have become a concern for hospitals, but outpatient departments see so many patients that there may be more of an opportunity for these organisms to be introduced from the community.

There have not really been good studies in outpatients departments in terms of how the cleaning/disinfection/ sterilization actually contributes to transmission of illnesses in the outpatient setting. Severe acute respiratory syndrome (SARS) has changed the equation a lot in the outpatient setting. One transmission in Toronto occurred from a patient who came from China and transmitted it in the waiting room (Dettenkofer, 2005).

3.2 Nosocomial infection

3.2.1 Definition

Nosocomial infections are those which are a result of treatment in a hospital or hospital-like setting, but secondary to the patient's original condition. Infections are considered nosocomial if they first appear 48 hours or more after hospital admission. Nosocomial comes from the Greek word ‘nosokomeion’ meaning hospital (*nosos* = disease, *komeo* = to take care of) (Wikipedia, 2006).

A nosocomial infection, also called “hospital-acquired infection” can be defined as: An infection acquired in hospital by a patient who was admitted for a reason other than that infection. An infection occurring in a patient in a hospital or other health care 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 (Ducel, 2002).

The hospital acquired infection (syn nosocomial infection) is applied to any infection causing illness that was not present of in its incubation period when the subject entered hospital or received treatment in an outpatient or accident and emergency department.

Hospital acquired infection may also affect discharged inpatients, outpatients and staff, and an episode of hospital infection may be initiated by the admission of a patient infected in the general population. Hospital infection may spill over into the community, necessitating investigation and control in both populations. Most surveys of hospital infection are restricted to inpatients (Speller and Humphreys, 1998).

“no-so-co-mi-al” [from the Greek *noso-* (disease) + *komeion* (to take care of)] pertaining to or originating in the hospital; said of an infection not present or incubating prior to admittance to the hospital, but generally occurring 72 hours after admittance; the term is usually used to refer to patient disease, but hospital personnel may also acquire nosocomial infection (Larson**,** 1995).

Infections acquired by a patient while he is admitted in a hospital or while using the services in a healthcare institution e.g diagnostic services such as laboratory investigations, preventive services, vaccination, etc are referred as 'nosocomial infections' or 'iatrogenic infections'. Initially, the term nosocomial infections was used to describe the infection acquired in the hospitals only, but later it was realized that patients who have utilized the services of a healthcare institution were also found to develop certain nosocomial infections. Nosocomial infections cause major problems in healthcare facilities, resulting in prolonged hospital stay and substantial morbidity and mortality (NNIS, 2004).

Nosocomial infections are diseases that we, as healthcare professionals, give to our clients. Monitoring agencies such as the National Nosocomial Infections Surveillance (NNIS) now recognize that the term nosocomial infection should not be limited to the hospital setting. Clients who have been served in outpatient settings or who reside in chronic care facilities such as nursing homes have also been found to develop infections across the same spectrum of pathogens (Mc Kibben *et al*., 2005).

Nosocomial infections may involve not only patients, but also anyone else who has contact with a hospital, including members of the staff, volunteers, visitors, workmen and delivery personnel. The inanimate environment present throughout the hospital is closely related to nosocomial infection and it may contribute to sporadic cases of disease or outbreaks in institutions by providing sources for contact common vehicle, air borne or vector borne transmission. Environment microbial contamination plays a prominent role as a source and a means for transmitting such infections (Sharma, 2002).

3.2.2 History

Modern understanding of nosocomial infection predates the infancy of microbiology as a discipline. The entire concept of infection control is grounded in the work of Ignaz Semmelweis, who demonstrated the importance of hand hygiene for controlling transmission of infection in hospitals. The work of Semmelweis (1861) on puerperal sepsis, now well known, was largely disregarded at the time. Having observed its association with the attendance on patients by medical officers and students who also performed autopsies, he deduced the spread of the disease from morbid matter- from cadavers or other affected patients- on their hands. A dramatic reduction in infection rates was achieved by the introduction of hand washing with chlorinated lime.

Florence Nightingale established important principles of nursing and hospital design and hygiene, after experiencing sepsis at army medical centre. About the same time the introduction of aseptic surgery by Lord Lister (1867) with extensive use of carbolic acid for packing wounds, sterilizing instruments and sutures, decontaminating his hands, and finally as air spray. And its replacement by Bergman's 'asepsis' with the introduction of gloves made contribution in infection control.

The concept of asepsis and control of hospital infection became more explicit with the discovery of pathogenic bacteria in the early 20^{th} century, such as the importance of *Streptococcus pyogenes* infection in burns and post operative wound infection (Forbes *et al.,* 2002).

3.2.3 Problem statement

Hospital acquired infections are considered as a major cause of mortality, emotional stress and enhanced morbidity in hospitalized patients. These also account for significant economic loss and additional burden on health care institutions. In a study conducted by WHO, the highest frequencies of HAI were reported from hospitals in the Eastern Mediterranean Region (11.8%) followed by South- East Asia, where it was 10

%. It has also been estimated that these infections cost more than US$ 40 million every year in Thailand alone (Ducel, 2002).

Patients are in increased risk for acquiring infection merely by being hospitalized. More than two million people (5% to 6% of all hospitalized patients) acquire a nosocomial infection each year. The cost of increased antibiotics, increased length of hospital stay and loss of work caused by nosocomial infections is staggering (Forbes *et al.,* 2002).

In the United States it has been estimated that as many as one hospital patient in ten acquires a nosocomial infection, or 2 million patients a year. Estimates of the annual cost range from $4.5 billion to $11 billion and up. Nosocomial infections contributed to 88,000 deaths in USA in 1995. One third of nosocomial infections are considered preventable. The most common nosocomial infections are of the urinary tract. The second most common are pneumonias (Wikipedia, 2005).

A recent Chicago Tribune investigative report alleges that in the USA in 2000, an estimated 103,000 patients' deaths were linked to nosocomial infections and that the causes of 75 percent of these deadly infections (unsanitary facilities, unwashed hands and unsanitary instruments) were preventable. The Tribune also cites a USA Centers for disease control and prevention (CDC) report that deaths linked to hospital infections represent the fourth-leading cause for mortality among Americans (Patterson, 2003).

Infections contracted in hospitals are the fourth largest killer in the USA, causing as many deaths as AIDS, breast cancer and auto accidents combined. One out of every twenty hospital patients gets an infection. That's two million Americans a year, and an estimated 103,000 of them die (Cassandro and Barry, 2003).

Over the past twenty-five years, the CDC's National Nosocomial Infections Surveillance (NNIS) system has received monthly reports of nosocomial infections from a nonrandom sampling of more than 270 hospitals in the USA. They have found that the nosocomial infection rate has remained remarkably stable, with approximately 5 to 6 hospital acquired infections for every 100 admissions.

However, the end rate of nosocomial infections per 1,000 client days has actually increased from 7.2 in 1975 to 9.8 in 1995, a 36% growth. As of 1995 nosocomial infections cost $4.5 billion and have contributed to more than 88,000 deaths—1 death every 6 minutes. These numbers have grown with each passing year (Weinstein, 1998).

It is believed that the majority, perhaps as many as 80% of nosocomial infections, are caused by the microbial flora that clients bring with them upon admission to the hospital. This “stay-at-home” flora appears to be opportunistic to the new environment and is able to take advantage of new routes that medical procedures offer.

Other nosocomial infections, perhaps 10% to 20%, develop following contamination with microbial organisms found within the hospital environment, often via the hands or instruments of healthcare workers or through contact with contaminated hospital materials. Examples of this include transfer of *S. aureus* or *Streptococcus pneumoniae* from one client to another via the hands of a hospital worker with successful colonization on the new host, followed by development of symptomatic illness later in the hospitalization period (Mc Kibben *et al.,* 2005).

A historical cohort study conducted in Brazil in a large general hospital between March 1992 and May 1993 concluded that in total the incidence rate of all HAIs for all sites combined was 20.20% (Wagner *et al.,* 1997).

In India, hospital infections estimates vary from10 to 30%, the least being about 3% in the best of hospitals (Sharma, 2002).

A study on nosocomial infection was done at Tribhuvan University Teaching Hospital, Kathmandu in 1989 and was found to be 10.5% (Tuladhar, 1990).

3.2.4 Chain of infection

Infection results from the interaction between an infectious agent and a susceptible host. The transmission of nosocomial infections requires three elements: a source of infecting microorganisms, a susceptible host and a mode of transmission.

3.2.4.1 Source of infection

The first link in the chain of infection is the microbial agent. The entire spectrum of microbes from bacteria to viruses, fungi and protozoa has been incriminated in Nosocomial infection. Nearly 25 to 50 percent of nosocomial infections have been found to be due to Gram-negative organisms and 10 percent of infections are due to gram-positives. Most frequently encountered microorganisms in nosocomial infection are *Escherichia coli, Staphylococcus* spp., *Pseudomonas aeruginosa,* Enterococci, Streptococci etc (Sharma, 2002).

Microorganisms causing nosocomial infections have a reservoir and a source. The organisms in the natural environment may provide a reservoir from which they may be passed to other patients and cause infections. However, there are many reservoirs; the one from which infections arise is usually called the source. Identification of the correct source is essential to arrest the spread from this source. Organisms that cause nosocomial infections come from either endogenous source (autogenous) or exogenous sources.

Endogenous infection (self infection) are caused by patients' own flora, which become opportunistic to the patients during their diseased or immune suppressed condition, the infected organisms being derived from the patients' own skin, gastrointestinal or upper respiratory flora or from the microbes that are carried by the patient himself . For example, Gram-negative bacteria in the digestive tract frequently cause surgical site infections after abdominal surgery or urinary tract infection in catheterized patients. A high proportion of clinically apparent hospital infections are endogenous.

Exogenous infection results from the transmission of organisms from a source other than patient. Exogenous source may be another person in the hospital (cross infection) or a contaminated item of equipment or building service (environmental infection). Bacteria are transmitted between patients: (a) through direct contact between patients (hands, saliva droplets or other body fluids), (b) in the air (droplets or dust contaminated by a patient's bacteria), (c) via staff contaminated through patient care (hands, clothes,

nose and throat) who become transient or permanent carriers, subsequently transmitting bacteria to other patients by direct contact during care, (d) via objects contaminated by the patient (including equipment), the staff's hands, visitors or other environmental sources (e.g. water, other fluids, food).

Inanimate environment as source of infection

Environment significantly influences the multiple factors in the chain of infection. The transmission of the agent from the source to the host occurs in an environment that represent the transmission of many individual factors; changes in any of these can have an impact on any link in the chain of infection.

The inanimate environment is an area of concern in infection transmission because of the prominent role it plays as a source and a means for transmission of nosocomial infection. The inanimate environment is in constant contact with the animate environment, patients and staff.

The most common route of transmission of nosocomial infections are by direct contact spread from person to person or by indirect contact spread via contaminated hands or equipment. The HCW come in regular contact with patients and the resident and transient microorganism may gain access to the susceptible host. Hands and to a lesser extent, clothing of hospital staff serve as vector of Gram-negative and Gram-positive infection around a busy region (Greenwood *et al.*, 2000). The surgical, therapeutic and diagnostic equipment also serves in the emergence of hospital acquired infection. Inoculation through blood transfusion, accidental injury from contaminated sharp instrument, contaminated needle, blood and contaminated infusion fluid are also responsible (Speller and Humphreys, 1998).

Equipment and materials in use in hospitals often become contaminated with microorganisms, which may subsequently be transferred to susceptible sites on patients.

Many nosocomial infections are easily transferable from patient-to-patient, either via the hands of healthcare workers, or through the contamination of inanimate objects, including clothing and equipment (Emmerson *et al.*, 1996)

In a healthcare setting, we are surrounded by thousands of non-living objects that can nonetheless harbor pathogens. Admitting there is a problem is just the first step. There are so many places where pathogens can set up camp that it's nearly impossible to list them all.

In all of these areas are doors and door handles or knobs, floors and walls. As healthcare workers (HCWs) move throughout the healthcare facility, they see risks specific to the setting (Dettenkofer and Block, 2005).

A fomite is defined as an inanimate object that serves to transmit an infectious agent from person to person. Fomites serve as a reservoir for pathogens, which are spread from the inanimate object to an animate object (person) via hands. Although most nosocomial infections usually result from patient contact; poor hand hygiene and person to person transmission, contaminated surfaces also have been linked to infection spread. Micro organisms can come from hospital environment and the inanimate objects like air, dust, IV fluids and catheters, washbowls, bedpans, endoscopes, ventilators and respiratory equipments, water and disinfectants and facilitate the nosocomial infection (Patterson, 2003).

Nosocomial infections (perhaps 10% to 20%) develop following cross-colonization with microbial organisms, often via the hands or instruments of health care workers or contact with the hospital environment (Larson**,** 1995).

Contaminated objects such as floors, bed linens, patients' gowns, bedside tables, blood pressure cuffs, IV fluid pumps and stethoscopes, among other items, have been reported to be reservoirs for nosocomial pathogens (e.g., methicillin-resistant *S. aureus*, vancomycin-resistant enterococci) (Garner, 1996).

The stethoscope is a universal tool in the hospital that is in direct contact with many patients and can therefore be a vector in the dissemination of bacterial infections Stethoscope may harbor pathogenic microorganisms through various processes; while examining the patients the stethoscope come in contact with patients, linen, other equipments and may also get the organisms from the health care workers. Thus stethoscope may also transmit the microorganisms.

A recent study from Brazil has confirmed previous findings that potentially dangerous bacteria can be cultured from most stethoscopes in clinical use. The commonest germ isolated is *S. aureus*, including the methicillin resistant (MRSA) variety (The Lawyers Weekly, 2002).

Similarly, another study among the 300 swab samples of stethoscope, 85% showed the positive growth with predominance of Gram-positive cocci *S. aureus*, and CoNS, yeasts, fungi and Gram-positive and negative bacilli. Thus stethoscopes presented a high rate of contamination and their use without precautions can spread nosocomial infections (Zuliani *et al.*, 2002).

Many nosocomial infections are easily transferable from patient-to-patient, either via the hands of healthcare workers, or through the contamination of inanimate objects, including clothing and equipments. Simply wiping stethoscopes with 70% alcohol between patients would probably make a material difference to cross-infection but the precaution has not yet been adequately tested or instituted as a routine (Emmerson *et al.*, 1996).

Hospital beds, linen, side-tables, and nurse's gowns and uniforms have been shown to be readily and regularly contaminated by *S. aureus* and other potentially dangerous germs, including those that can cause epidemic diarrhea and vomiting.

Blood-pressure cuffs, including those that are not visibly contaminated by blood or other body fluids, readily demonstrate colonization with potentially harmful bacteria (The lawyers weekly, 2002).

The review reports on the transmission of infections by flexible gastrointestinal endoscopy and bronchoscopy in order to determine common infecting microorganisms, circumstances of transmission, and methods of risk reduction revealed that two hundred and eighty-one infections were transmitted by gastrointestinal endoscopy, and 96 were transmitted by bronchoscopy. The clinical spectrum of these infections ranged from asymptomatic colonization to death. *Salmonella* spp. and *P. aeruginosa* were repeatedly identified as the causative agents of infections transmitted by gastrointestinal endoscopy, and *Mycobacterium tuberculosis*, atypical mycobacteria, and *P. aeruginosa* were the most common causes of infections transmitted by bronchoscopy. One case of hepatitis B virus transmission via gastrointestinal endoscopy was documented. Major reasons for transmission were improper cleaning and disinfection procedures; the contamination of endoscopes by automatic washers; and an inability to decontaminate endoscopes, despite the use of standard disinfection techniques, because of their complex channel and valve systems (Spach *et al.,* 1993).

A study reported that Broomfield hospital in England reduced infections in its orthopedic unit by two thirds and totally eradicated methicillin resistant *S. aureus* (MRSA) infections in one year. By methodical hand cleaning, rigorous adherence to hygiene, putting doctors in freshly laundered coats whenever they approached patients' bedsides, barring caregivers from wearing jewelry, restricting the movement of wheelchairs and other equipment, and other steps all designed to reduce the transmission of bacteria from infected patients to inanimate objects and then to other patients (Plowman *et al.,* 1999).

The United States lags behind several other countries in the prevention of one of the most deadly hospital infections, MRSA. It remains a major threat in the United States. MRSA is spread primarily by unclean hands and contaminated equipment (Cassandro and Barry, 2003).

Nosocomial transmission of agents that cause gastrointestinal infections usually results from contact with infected individuals, from consumption of contaminated food, water,

or other beverages, or from exposure to contaminated objects or environmental surfaces. Airborne transmission of small round structured viruses (Norwalk-like viruses) has been postulated but not proved. Inadequate hand washing by health care personnel and inadequate sterilization or disinfection of patient care equipment and environmental surfaces increases the likelihood of transmission of agents that cause gastrointestinal infections.

Gram-positive nosocomial pathogens may persist in the inanimate hospital environment, but they do not usually multiply there. Gram-negative organisms and fungi may persist as well as multiply in a moist or wet environment and these are more frequently associated with environmentally transmitted infections than Gram-positive organisms or other micro organisms.

Computer key boards and faucet handles may serve as a reservoir of nosocomial pathogens and vectors for cross transmission in the Intensive care unit settings (Byers *et al.,* 1998).

Gram-positive cocci, derived from the body flora of the hospital population, are found in air, dust and on surfaces where they may survive for along with fungal and bacterial spores of the environment origin. Gram-negative aerobic bacilli are common in moist situation and in fluids; where they often survive for long periods, and may even multiply in the presence of minimal nutrients.

The inanimate surfaces near affected patients commonly become contaminated with MRSA and that the frequency of contamination is affected by the body site at which patients are colonized or infected. The personnel may contaminate their gloves (or possibly their hands) by touching such surfaces suggests that contaminated environmental surfaces may serve as a reservoir of MRSA in hospitals (Boyce *et al.,* 2004).

3.2.4.2 Modes of transmission

There are five main modes of transmission: contact, droplet, airborne, common vehicle, and vector borne.

a. Contact transmission is the most important and frequent mode of transmission of nosocomial infections. It is divided into two subgroups:

- Direct-contact transmission involves a direct body surface to body surface contact and physical transfer of microorganisms between a susceptible host and an infected or colonized person, such as occurs when a person turns a patient, gives a patient a bath, or performs other patient-care activities that require direct personal contact. Direct-contact transmission also can occur between two patients, with one serving as the source of the infectious microorganisms and the other as a susceptible host.
- Indirect-contact transmission involves contact of a susceptible host with a contaminated intermediate object, usually inanimate, such as contaminated instruments, needles, or dressings, or contaminated hands that are not washed and gloves that are not changed between patients.

b. Droplet transmission involves the formation of droplets, which are generated from the source person primarily during coughing, sneezing, and talking, and during the performance of certain procedures such as suctioning and bronchoscopy.

- Transmission occurs when droplets containing microorganisms generated from the infected person are propelled a short distance through the air and deposited on the host's conjunctiva, nasal mucosa, or mouth.
- Because droplets do not remain suspended in the air, special air handling and ventilation are not required to prevent droplet transmission; that is, droplet transmission must not be confused with airborne transmission.

c. **Airborne transmission** occurs by dissemination of either airborne droplet nuclei (small-particle residue, 5 µm or smaller in size, of evaporated droplets containing microorganisms that remain suspended in the air for long periods of time) or dust particles containing the infectious agent. The air borne route of transmission is thought to account for 10% of all cases of nosocomial infection.
    - Microorganisms carried in this manner can be dispersed widely by air currents and may become inhaled by a susceptible host within the same room or over a longer distance from the source patient, depending on environmental factors.
    - Special air handling and ventilation are required to prevent airborne transmission because many patients have infections that can spread through air borne exposure and the density of people in health care settings is relatively high.

d. **Common vehicle transmission** applies to microorganisms transmitted by contaminated items such as food, water, medications, devices, and equipment.

e. **Vector borne transmission** occurs when vectors such as mosquitoes, flies, rats, and other vermin transmit microorganisms. This route of transmission is of less significance in hospitals in the USA than in other regions of the world.

3.2.4.3 Host

The third link in the chain of infection is host or victim. Disease does not always follow upon the transmission of infectious agent to a host; various agent factors play a part. Host factor that influence the development of infection are site of deposition of the agent and the host's defense mechanism. Natural resistance to infection is lower in infants and the elderly, who often comprise the majority of hospital patients.

Pre-existing disease, such as diabetes and other conditions, the medical or surgical treatment, including immunosuppressive drugs, radiotherapy or splenectomy, may also reduce the patient's natural resistance to disease. Moreover, the natural defence

mechanisms of the body surfaces may be bypassed either by injury, surgery, and insertion of indwelling catheter, tracheostomy or venitlatory support.

3.2.5 Microorganisms causing hospital infection

Every person (patients, members of the staff, volunteers, visitors, workmen and delivery personnel) exposed in hospital environment comes in direct contact with various micro organisms like bacteria, viruses, fungi and parasites which can lead to nosocomial infection.

Almost any pathogen can, on occasion, cause hospital infection but those that are able to survive in the hospital environment for long periods and develop resistance to antibiotics and disinfectants are particularly important.

One of the important sources of these organisms is inanimate environment of the hospital. Various bacterial floras can be isolated from the inanimate environment of the hospitals and health care settings. A distinction may be made as:

Commensal bacteria found in normal flora of healthy humans. These have a significant protective role by preventing colonization by pathogenic microorganisms. Some commensal bacteria may cause infection if the natural host is compromised. For example, cutaneous CoNS cause intravascular line infection and intestinal *E. coli* are the most common cause of urinary infection.

Pathogenic bacteria have greater virulence, and cause infections (sporadic or epidemic) regardless of host status. For example:

a. Anaerobic Gram-positive rods (e.g. *Clostridium*) cause gangrene
b. Gram-positive bacteria: *S. aureus* (cutaneous bacteria that colonize the skin and nose of both hospital staff and patients) cause a wide variety of lung, bone, heart and bloodstream infections and are frequently resistant to antibiotics; beta-hemolytic streptococci are also important.

c. Gram-negative bacteria: Enterobacteriaceae (e.g. *E. coli*, *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., *S. marcescens*) may colonize sites when the host defences are compromised (catheter insertion, bladder catheter, cannula insertion) and cause serious infections (surgical site, lung, bacteremia, peritoneum infection). They may also be highly resistant.
d. Gram-negative organisms such as *Pseudomonas* spp. and *Acinetobacter* spp. are often isolated in water and damp areas. They may colonize the digestive tract of hospitalized patients.
e. Selected other bacteria are a unique risk in hospitals. For instance, *Legionella* spp. may cause pneumonia (sporadic or endemic) through inhalation of aerosols containing contaminated water (air conditioning, showers, and therapeutic aerosols).

3.2.5.1 Gram-positive cocci

Gram-positive cocci are included among some of the most significant human bacterial pathogens. These are not in the category of communicable diseases, but rather in the category of opportunistic pathogens, for the immune compromised, which are instrumented in every orifice of their bodies; those organisms find a portal of entry into the body so easily.

Gram-positive cocci which have significance in nosocomial infection are primary pathogens such as *S. aureus, S. pyogens,* and *S. pneumomiae,* along with species of lower virulence such as *Staphylococcus epidermidis, Staphylococcus saprophyticus* and *Enterococcus faecalis.*

a. Staphylococci

Staphylococci are spherical cocci, arranged characteristically in grape-like clusters, tetrads, single or short chains. They are catalase positive, facultative anaerobe, non-motile, non-spore forming and non-capsulated.

Staphylococci are classified into two groups on the basis of the production of enzyme coagulase, which catalyses the conversion of fibrinogen to fibrin. coagulase positive staphylococci, which gives a positive coagulase test, and coagulase negative staphylococci. There is an association between virulence and production of the enzyme.

The three main species of clinical importance are *S. aureus, S. epidermidis* and *S. saprophyticus*. *S. aureus* is coagulase positive, which differentiates it from other species.

Staphylococci survive well in the environment on skin squames and in dust and are readily transmitted in hospitals on the hands of medical and nursing staff and by the air borne route (Baron and Finegold, 1994).

Staphylococci are ubiquitous human parasites. The chief sources of infection are shedding human lesions, fomites contaminated from such lesions, and the human respiratory tract and skin. Contact spread of infection has assumed added importance in hospitals, where a large proportion of the staff and patients carry antibiotic resistant staphylococci in the nose or on the skin (Chambers, 2001).

They are non-sporulating but are resistant to drying and are readily dispersed in dust particles through the air and on surfaces (Madigan *et al.,* 2003). Dried on threads, they retain their viability for 3-6 months. They have been isolated from dried pus after 2-3 months. They may withstand 60^0c for 30 mins (Anantanarayan and Paniker, 2000).

Staphylococci are widespread in nature, their normal habitats being the skin and mucous membranes of mammals and birds (Collee *et al.,* 1999).

Staphylococci are primary parasites of human beings and animals, colonizing the skin, skin glands and mucous membrane. The most common sources of infection are human patients and carriers; animals and inanimate objects. Patients with superficial infections and respiratory infections disseminate large number of staphylococci into the

environment. The cocci shed by patients and carriers contaminate fomites such as handkerchiefs, bed linen and blankets and may persist on them for days or weeks.

Staphylococcal disease may follow endogenous or exogenous infection. The modes of transmission may be by contact, direct of through fomites, by dust or by airborne droplets.

Hospital infections by staphylococci deserve special attention because of their frequency and because they are caused by strains resistant to various antibiotics. Staphylococci are a common cause of postoperative wound infection and other hospital cross infections. Most of these are due to certain strains of staphylococci that are present in the hospital environment, the so-called 'hospital strains' (Anantanarayan and Paniker, 2000).

Coagulase positive staphylococci

In medical microbiology the term coagulase positive *Staphylococcus* is synonymous with *S. aureus*, which differentiates it from other members of the genus. *S. aureus* produces two forms of coagulase enzyme, bound and free.

S. *aureus* is approximately 1µm in diameter. Colonies are smooth, low convex and densely opaque. It is tolerant to concentrations of sodium chloride the inhibit most of other bacteria and on mannitol salt agar(MSA) it produce mannitol fermenting 1mm diameter colonies, surrounded by yellow medium due to acid formation (Collee *et al.*, 1999).

S. aureus has been recognized historically as a virulent and important human pathogen, its capacity to produce human disease has not diminished with the introduction of antibiotics. It is among the hardiest of non-sporing bacteria and survives well in the environment under both moist and dry conditions (Forbes *et al.*, 2002).

One of the most important and widespread hospital pathogens is *S. aureus*. It is the most common cause of pneumonia and the third most common cause of blood infections. *S.*

aureus is also particularly problematic in nurseries. Many strains are usually virulent and are also resistant to common antibiotics making their treatment very difficult. In addition to *S. aureus*, other *Staphylococcus* species are now the largest collective cause of hospital-acquired blood infections and are also very prevalent as the causal agents of wound infections (Madigan *et al.,* 2003).

Coagulase negative staphylococci (CoNS)

The term coagulase negative staphylococci embrace all species other than *S. aureus*. They often form smaller colonies in solid media than *S. aureus* and some may be slightly pigmented. On mannitol salt agar they form small orange colonies surrounded by red medium (Collee *et al.,* 1999).

Hospital acquired infections are due mostly to *S. epidermidis*. Studies have shown that the patients admitted to wards such as cardiac, orthopaedic or neonatal intensive care unit acquire CoNS endemic in these units, carried by staff and other patients, and it is these resistant strains that give rise to hospital acquired infections (Spencer, 1996).

Hospital acquired infections are due mostly to *S. epidermidis* and usually result from the colonization of prosthetic materials in patients with vascular catheters or implanted prostheses (Collee *et al.,* 1999).

The coagulase negative staphylococci are skin commensals. Coagulase negative staphylococci, especially *S. epidermidis* are increasingly important nosocomial pathogens, particularly in critically ill neonates. Results confirmed that specific strains of *S. epidermidis* may be an important cause of nosocomial catheter related sepsis resulting from cross infection (Mermel *et al.*, 2001).

In the St. Thomas series coagulase negative staphylococci accounted for 6.8% of hospital acquired bacteraemia (Speller and Humphreys, 1998).

b. Micrococci

Micrococci are somewhat larger than staphylococci, and are arranged in pairs, tetrads and cubical packets. Colonies of micrococci are domed and often brightly pigmented; mostly yellow (Collee *et al.,* 1999).

They are catalase positive and oxidase positive. They are aerobic with a strictly respiratory metabolism. They resemble staphylococci but in stained smears the cells are generally larger and more Gram variable than staphylococci. The common laboratory test used to differentiate between micrococci and staphylococci is the Hugh and Leifson's oxidative-fermentative test in which micrococci show oxidative and staphylococci show fermentative patterns. They are parasitic on mammalian skin and are ordinarily non-pathogenic (Ananthanarayan and Paniker, 2000).

Micrococcus spp. are opportunistic pathogens usually seen only in immunocompromised patients (Forbes *et al.,* 2002).

Colonies of micrococci are usually white but some strains produce yellow, orange or pink colonies due to production of carotenoid pigments. They have little pathogenic potential although it has been implicate as a cause of urinary tract infection (Chakraborty, 1998).

c. Streptococci

Streptococci are Gram-positive cocci arranged in chains of varying length or in pairs. They are typically non-motile, non sporing, facultative anaerobes, catalase negative and oxidase negative. They are part of the normal flora of humans and animals. Some of them are human pathogens. The most important of them is *S. pyogenes* causing pyogenic infections. Other pathogenic streptococci include *S. agalactiae*, an important causative agent of neonatal infection and *S. pneumoniae*.

There are evidences for spread of streptococci though air. They are readily shed from the upper respiratory tract, by coughing, sneezing and singing. Streptococci can also be

spread by hand contact. Sources of infection are cases and throat, nose, skin, rectal carriers (Speller and Humphreys, 1998). Throat carriers outnumber nasal carriers but nasal carriers are the most dangerous source of the organisms (Chakraborty, 1998).

The major source of *S. pyogenes* is the human upper respiratory tract- throat, nasopharynx or nose- of patients and carriers. Transmission of infection is either by direct contact or through contaminated fingers, dust or fomites. Crowding is an important factor in the transmission of infection (Ananthanarayan and Paniker, 2000).

Use of antibiotics has banished *S. pyogenes* as prevailing pathogen in hospital infection. *S. pyogenes* -once a cause of serious trouble in burns units-now colonizes comparatively few patients (Speller and Humphreys, 1998).

S. pneumoniae is Gram-positive lanceolate diplococci and are chiefly involved in the infection of upper and lower respiratory tracts. The pneumococcus in low numbers is part of normal nasopharyngeal and oropharyngeal flora of many healthy persons and also children. Pneumococci may be primary pathogen in immune compromised people (Collee *et al.*, 1999).

3.2.5.2 Gram-negative rods

Gram-negative bacteria less frequently survive in the dry and nutrientless environment. Few Gram-negatives such as *P. aeruginosa*, *Acinetobacter* spp. can survive in such adverse conditions.

a. Pseudomonas aeruginosa

P. aeruginosa is slender Gram-negative, motile, obligate aerobic rods. It is non-sporing and non-capsulated, but many strains have mucoid slime layer. *P. aeruginosa* produces a number of pigments, the best known pigment being pyocyanin and fluorescin (pyoverdin) which diffuses into the media.

The pathogenic importance of the bacillus was not adequately recognized till recently, when it has established itself as one of the most troublesome agents causing nosocomial infection. *P. aeruginosa* has become a very important cause of hospital infections.

In the hospitals, it may cause localized or generalized infections. Localized lesions are commonly infections of wounds and bed-sores, eye infections and urinary infections following catheterization. *P. aeruginosa* is the most common and most serious cause of infection in burns.

The bacillus exhibits a high degree of resistance to chemical agents. It is resistant to the common antiseptics and disinfectants such as quaternary ammonium compounds, chloroxylenol and hexachlorophene and may even grow profusely in bottles of such antiseptic lotions kept for use in hospitals.

The pre-eminent role of *P. aeruginosa* in hospital infection is due to its resistance to common antibiotics and antiseptics, and its ability to establish itself widely in hospitals. Being an extremely adaptable organism it can survive and multiply even with minimal nutrients, if moisture is available. Equipment such as respirators and endoscopes, articles such as bed pans and medicines such as lotions, ointments and eye drops and even stocks of distilled water or plants and flowers may be frequently contaminated (Ananthanarayan and Paniker, 2000).

P. aeruginosa is a classic opportunistic pathogen with innate resistance to many antibiotics and disinfectants. It is physiologically versatile and flourishes as a saprophyte in warm moist situations in the human environment, including sinks, drains, respirators, humidifiers and disinfectant solutions (Collee *et al.,* 1999).

The organism is widely distributed in the moist environment of the hospital and grows in fluids with minimal nutrients. Nosocomial outbreaks of *P. aeruginosa* have been linked to many sources, including contaminated respiratory, endoscopic, urodynamic, and pressure monitoring equipment; contaminated whirlpools, mattresses, antiseptics, and tap water (Bouza *et al.,* 1999).

In the last two decades *P. aeruginosa* has become increasingly recognized as the etiological agent in a variety of serious infection in hospitalized patients with impaired immune defense (Obritsch *et al.*, 2004).

b. Acinetobacter

Acinetobacter spp. are Gram-negative, aerobic bacteria. They are usually cocco bacillary or coccal in appearance. They are widely distributed in soil and water and can occasionally be cultured from skin, mucous membranes, secretions, and the hospital environment.

Acinetobacters often are commensals but occasionally cause nosocomial infection. *Acinetobacter baumannii* is the species most commonly isolated. *A. baumannii* has been isolated from blood, sputum, skin, pleural fluid, and urine, usually in device-associated infections.

Acinetobacter spp. encountered in nosocomial pneumonia often originates in the water or room humidifiers or vaporizer. In patients with *Acinetobacter* spp. bacteremia, intravenous catheters are almost always the source of infection. In patients with burns or with immune deficiencies, *Acinetobacter* spp. acts as an opportunistic pathogen and can produce sepsis.

Acinetobacter spp. strains are often resistant to antimicrobial agents, and therapy of infection can be difficult (Wang, 2003).

In the hospital setting, *Acinetobacter* spp. species are an important cause of nosocomial infection. Nosocomial infections caused by *Acinetobacter* spp. species include pneumonia, meningitis, bloodstream, urinary tract, surgical wound, and soft tissue infections. Such infections are challenging to treat because of extensive anti-microbial drug resistance.

War wound infection and osteomyelitis caused by multidrug-resistant (MDR) *Acinetobacter* spp. species has been prevalent during the 2003–2005 military operations in Iraq **(**Kepler *et al.,* 2005).

Acinetobacter spp. is ubiquitous bacteria that have been isolated from patients, the environment, soil, and water. Members of the genus *Acinetobacter* are strictly aerobic, nonmotile, non-spore-forming, non-fermentative, Gram-negative coccobacilli.

Acinetobacter spp. are important nosocomial pathogens reported with increasing frequency in outbreaks of cross-infection during the past 2 decades. The majority of such outbreaks are caused by *A. baumannii*.

Acinetobacter spp. is ubiquitous Gram-negative bacteria that can be isolated from soil, water, human skin, and the environment.

Acinetobacter spp. was ranked among the top ten bacteria causing septicemia in 18 of 44 large European hospitals. It has been shown that most clinical isolates are strains of *A. baumannii.*

It is unusual for Gram-negative bacteria to survive in the environment after exposure to dry conditions, but *Acinetobacter* spp. can survive in the environment and cause nosocomial infections.

Compared with other genera of Gram-negative bacilli, *Acinetobacter* spp. is found to survive much better on fingertips or on dry surfaces when tested under simulated hospital environmental conditions. The skin of patients and medical personnel is thought to be involved in the transmission of strains, and in some outbreaks.

Allen and Green were the first to report that airborne spread may also serve as a mode of transmission. Contaminated reusable medical equipment, such as ventilator tubing, respirometers, and arterial pressure monitoring devices, used for the management of severely ill patients has also been implicated as a route of transmission to patients. In addition, a wide variety of dry environmental objects such as bed mattresses, pillows, a

tape recorder, a television set, and a fan have been found to be contaminated with *Acinetobacter* spp. and may serve as reservoirs during nosocomial outbreaks (Jawad *et al.*, 1996).

Acinetobacter spp. are being reported with increasing frequency as a cause of nosocomial infection and have been isolated from the skin of healthy individuals, patients, hospital staffs, dry non-biotic objects, and different pieces of medical equipment.

Freshly isolated strains of *Acinetobacter* spp. belonging to the clinically important *A. calcoaceticus-A. baumannii* complex were found to be more resistant to drying conditions (e.g., 30 days for *A. baumannii* 16/49) than American Type Culture Collection strains (e.g., 2 days for *A. baumannii* ATCC 9955). The majority of strains belonging to the *Acb* complex had survival times similar to those observed for the gram-positive organism *S. aureus* tested in the experiment.

These findings are consistent with the observed tendency of *Acinetobacter* spp. to survive on dry surfaces, and they can be transferred not only by moist vectors but also under dry conditions in a hospital environment during nosocomial infection outbreaks.

The increasing clinical interest in the genus *Acinetobacter* is mainly attributed to its capability to cause a wide range of nosocomial infections (Jawad *et al.*, 1998).

3.2.6 Antibiotics and hospital infections

In the 1940s, the advent of antibiotics gave clinicians weapons against infections that once had wiped out entire populations. Mankind has been so pleased with its ability to conquer diseases. But today, we face a generation of microbes so resistant to antibiotics that they might again threaten us all.

Microorganisms strive to stay alive by making themselves resistant to the effects of antibiotics and passing along the ability to their offspring and sometimes to other

species through resistant genes. Able to adapt to nearly every part of the planet, bacteria have begun adjusting to a world ruled by antibiotics.

Since bacteria readily exchange genetic information, they can transfer drug resistance. New resistant genes can spread between species. The basis of resistance to antibiotics is genetic and lies in both chromosomal and extra chromosomal changes, which can lead to drug destruction (e.g., beta-lactamases destroy penicillin and cephalosporin derivatives) or an alteration of drug-receptor/target sites (e.g., methicillin resistance in *S. aureus*). It is also possible to see decreased drug permeability (e.g., imipenem resistance in *P. aeruginosa*). Extra chromosomal drug resistance is potentially more serious and enables microorganisms to distribute genetic material more rapidly.

Resistance is an emerging problem in human medicine and its effects are being noted on an ever-increasing scale. Multi-resistant organisms are diminishing our ability to control the spread of infectious diseases (Shenold, 2001).

Acquired antimicrobial resistance is a growing worldwide problem. Resistance emerges from following reasons:

- In many countries, antimicrobials can be obtained outside of recognized treatment centers, and taken without medical authorization or supervision. This leads to the inappropriate use of antimicrobials and their being taken at sub-optimal dosages and for an insufficient length of time. Often the high cost of an antibiotic results in an incomplete course being purchased, sufficient only to alleviate symptoms.
- Guidelines regarding the selection of drugs, correct prescribing, and information about drug resistance and how to minimize its spread are not communicated to those purchasing and prescribing antimicrobials.
- Antibiotics are often prescribed when they are not needed or for self-limiting infections, e.g. diarrhoeal disease and viral respiratory infections.
- Broad spectrum antibiotics are frequently used prophylactically, e.g. tetracycline.

- Laboratory facilities for accurate diagnosis and isolation of pathogens are often not available, resulting in an overuse and inappropriate use of antibiotics.
- Overcrowding and poor hygiene and sanitation facilitate the spread of resistant organisms, e.g. bacteria that cause tuberculosis, typhoid, and pneumonia.
- Infection control procedures in hospitals are often inadequate in the spread of infectious diseases and resistant strains of organisms such as *S. aureus* (MRSA), *P. aeruginosa, E. coli, Klebsiella* spp., *Proteus* spp., *Enterococcus* spp., *and Salmonella* spp.
- Developing countries are often unable to afford costly second-line antibiotics to treat infections due to resistant organisms. This results in prolonged illness with longer periods of infectivity and the further spread of resistant strains.

Resistant and multi-resistant microbes are an important cause of nosocomial infections. Infections associated with such microorganisms can pose a serious threat to vulnerable patients such as neonates, cancer patients and those who are immune compromised, debilitated or elderly.

Examples of antibiotic resistance found in hospitals in the past 50 years include:

- Penicillinase-producing *S. aureus*, which first appeared in the late 1950s
- Methicillin-resistant *S. aureus*, appearing in the 1960s
- Aminoglycoside (gentamicin, tobramycin) resistance among Gram-negative bacilli (1970s)
- Methicillin-resistant *S. aureus* resistance to fluoroquinolones (1980s)
- Vancomycin resistance among enterococci 1990s

(Shenold, 2001)

A year or two after penicillin went into widespread use, the first resistant strain of *Staphylococcu*s appeared. As other antibiotics came along, microbes found ways to resist them as well, through changes in genetic makeup

Methicillin resistant *S. aureus* (MRSA), *Enterococcus faecium, Enterococcus feacalis, E. coli, K. pneumoniae, Enterobacter* spp., *Citrobacter* spp., *P. aeruginosa* and *A.*

calcoaceticus have become important hospital pathogens. These pathogens can complicate treatment, increase morbidity and mortality, delay discharge and increase the cost (Banjara, 2002).

Staphylococcus spp. and *Streptococcus* spp. have acquired resistance to many standard antibiotics, making them much harder to treat. *S. aureus* has a resistant strain that can only be treated with an expensive antimicrobial such as vancomycin, which was the only choice for treatment of resistant organisms in the not-too-distant past.

Recently, *E. faeceium* and *E. faecalis* were identified to be vancomycin-resistant enterococci (VRE), leaving researchers searching for other pharmacologic options. Situation can be more complicated if VRE pass along its resistance to vancomycin, a last resort antibiotic, to other more common organisms. In a laboratory situation, VRE has been able to pass along its resistance, creating a vancomycin-resistant *S. aureus*.

Methicillin resistant *S. aureus* (MRSA) is a major nosocomial pathogen. Recently, there have been reports of increasing prevalence of MRSA in the community. We here report an outbreak of post operative wound sepsis by MRSA in the surgical ward of LN hospital. A surveillance study for MRSA was undertaken in the corresponding surgical ward, operation theaters and OPD and the source of this outbreak was traced to an outdoor patient with community acquired MRSA infection.

A study of antibiogram revealed that all the MRSA were uniformly resistant to penicillin, erythromycin, gentamicin, tobramycin and tetracycline and sensitive to vancomycin (Gupta *et al.*, 1999).

3.2.7 Prevention and control of hospital infection

Hospital infection may occur sporadically or as outbreaks. When an outbreak occurs, the source should be identified and eliminated. This requires the sampling of possible sources of infection such as hospital personnel, inanimate objects, water, air or food. Typing of isolate- phage, bacteriocin, antibiogram or biotyping- from cases and sites

may indicate a causal connection. Obvious examples of sources of hospital outbreaks are nasal carriage of staphylococci by surgeons or *Pseudomonas* spp. growing in hand lotions. Carriers should be suitably treated.

Sterilization techniques have to be tested. The cause of infection may be a defective autoclave or improper techniques such as boiling infusion sets in ward sterilizers. A careful analysis of the pattern of infection may often reveal the source but sometimes is eludes the most diligent search.

It must be emphasized that control of hospital infection should not merely be a spasmodic exercises to be employed when an outbreak occurs but rather a permanent ongoing activity in any large hospital (Ananthanarayan and Paniker, 2002).

Proper sterilization and disinfection of the inanimate objects in the hospital environments should be done. This helps to control the source or reservoir of infection.

Disinfection of excreta and infected material is necessary to control the exit point of infection.

The transmission route is to be controlled by regular washing of hands, disinfection of equipments and change of working clothes (Chakraborty, 1998).

A hospital infection surveillance program provides a mechanism to collect and analyze hospital infection information in an orderly manner, primarily for the use of those individuals charged with the prevention and control of such infections. Surveillance is required for determining baseline information about the frequency and type of endemic infections occurring in a hospital so endemic problems and upward deviations from the baseline can be recognized and investigated. Surveillance is recognized as an essential component of the prevention and control of infection in hospitals. Surveillance aims to reduce the risk of nosocomial infection by highlighting areas where changes need to be made, measuring the effects of change and leading to the development of guidelines for good practice (Weber and Rutala, 1997).

Nosocomial infections play a role in quality and cost control in health care. Surveillance of these infections is the only way to gain more insight into their frequency and causes. Since the results of surveillance may lead to changes in both patient and hospital management, which are sometimes major, it is necessary that all healthcare workers involved agree on the criteria used for the diagnosis and surveillance of these complications (Roberts *et al.*, 2003).

Describing the epidemiology of nosocomial infections in the hospital enabled to establish infection occurrence, distribution, and expected incidence, as well as to recognize trends and keep track of possible outbreaks. The knowledge acquired through surveillance allowed to target more specific and continuous quality improvement projects, to upgrade health care quality and to implement preventive strategies (Lopes and Tonelli, 2000).

CHAPTER-IV

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Equipments

i. Microscope
ii. Autoclave
iii. Hot air oven
iv. Incubator
v. Refrigerator
vi. Glassware: petri plates, culture tubes, glass slides

4.1.2 Media

i. Nutrient agar
ii. MacConkey agar
iii. Blood agar
iv. Mannitol salt agar
v. Biochemical media
vi. Hugh and Leifson media
vii. Sulphur indole motility media
viii. MR/VP broth
ix. Triple sugar iron agar
x. Urea agar
xi. Simmon's citrate agar

4.1.3 Reagents

i. Catalase reagent (3% H_2O_2)
ii. Oxidase reagent (1% Tetramethyl *p*-phenylene diamine dihydrochloride)
iii. Kovac's reagent
iv. Barritt's reagent (40% KOH, 5% α-naphthol in a ratio of 1:3)

4.2 Methods

The present research work was conducted in April to July, 2005 in the well equipped laboratory of Bacteriology section of Tribhuvan University Teaching Hospital, one of the major hospitals of Kathmandu valley.

4.2.1 Sample collection

Altogether 281 samples were collected from various sources from 10 out patient departments of TUTH and bacteriological investigations were carried out. Different samples were swab samples from new and used bed sheets, swab samples of aprons of health care personnel, swab samples of various equipments used for check up of patients and indoor air samples. Standard procedures were followed for the collection and processing of these samples.

4.2.1.1 Collection of sample from bed sheets

Sterilized plain cotton wool swab, dipped in normal saline, placed in a clean screw capped tubes were used for the sample collection. Swab samples were collected from used and new bed sheets. The swab was rubbed at various sites of the bed sheets and was replaced in its tube and delivered to the laboratory.

4.2.1.2 Collection of sample from apron

The swab samples were taken from the aprons of health care personnel (doctors, nurses, health assistants, interns). The sterilized swab was rubbed up and down over the chest, pocket and sleeve area of the apron and replaced into its tube and delivered to the laboratory.

4.2.1.3 Collection of sample from equipments

The swab samples were taken from those areas of the equipments which come in close contact with the patients and health care personnel. Then the swabs were replaced into its tube and delivered to the laboratory.

4.2.1.4 Collection of indoor air samples

For the collection of indoor air samples from various OPD, MHA plates were placed at different sites of the OPD. The plates were exposed for 5 minutes and the plates were transported to the laboratory.

4.2.2 Processing of the sample

The collected samples were immediately brought to the bacteriology section of the laboratory of TUTH. Different procedures were followed for the processing of different samples depending upon the nature and type of the sample.

4.2.3 Culture of the specimen

The swab samples from used and new bed sheets, apron and equipments, were rubbed at the side of the agar and was spread with the help of sterilized loop. Then the plates were incubated at 37^0C for 24 hours.

For air samples, the plates exposed for 5 minutes were brought into the laboratory and incubated at 37^0C for 24 hours.

4.2.4 Isolation of the organism

After 24 hours of incubation, the plates were observed for significant growth. Swab cultures were observed for the colonies. For the air sample, number of colony was counted and CFU per plate was noted.

To obtain pure culture, a single colony was picked up from the agar plate and sub cultured in nutrient agar for 24 hours at 37^0C. After incubation, the plates were observed for the colony morphology and other characteristics.

4.2.5 Identification of the organism

The isolates were identified by standard diagnostic procedure. Identification of the isolated organisms was carried out on the basis of microscopic examination by Gram's staining, morphological characteristic, colony characteristics and biochemical properties.

The organisms were inoculated on MacConkey agar and blood agar. After 24 hours incubation, the characteristics of colonies were observed for significant growth. In MacConkey agar, the lactose fermenting and non lactose fermenting organisms and in blood agar, haemolysis was observed.

The isolated pure colonies were inoculated into different biochemical media for different tests. The tests performed were as follows:

Table 1. Reagents and chemicals used for different tests

S.N	Media and Chemicals	Tests
1.	3% H_2O_2	Catalase production
2.	1% Tetramethyl p-phenylenediamine dihydrochloride	Oxidase production
3.	Plasma	Coagulase production
4.	Sulfide Indole Motility(SIM) medium	H_2S and indole production, motility
5.	MR/ VP broth	Acid or Acetoin production
6.	Simmon's citrate agar	Citrate utilization
7.	Triple sugar iron(TSI) agar	Fermentation of dextrose, lactose and sucrose, H_2S and gas production.
8.	Urea agar	Urease production
9.	Hugh and leifson medium	Aerobic or anaerobic utilization of carbohydrates

4.2.6 Antibiotic susceptibility testing

The antibiotic susceptibility testing was performed according to the National Committee for Clinical Laboratory Standard (NCCLS) recommended Kirby-Bauer sensitivity testing method.

1. Mueller Hinton agar was prepared and sterilized as instructed by the manufacturer.
2. The pH of the medium 7.2-7.4 and the depth of the medium at 4 mm (about 25 ml per plate) were maintained in Petri dish.
3. Using a sterile wire loop, a single isolated colony of which the sensitivity pattern is to be determined was touched and inoculated into a nutrient broth tube and incubated for 2-4 hrs.
4. After incubation in a good light source, the turbidity of the suspension was matched with the turbidity standard of McFarland 0.5 (Prepared by adding 0.6 ml of 1% w/v barium chloride solution to 99.4 ml of 1% v/v solution of sulphuric acid (Cheesbrough, 2000).

   Using a sterile swab, a plate of Mueller-Hinton agar was inoculated with the bacterial suspension using carpet culture technique. The plate was left for about 5 minutes to let the agar surface dry.
5. Using sterile forceps, appropriate antimicrobial discs (6 mm diameter) was placed, evenly distributed on the inoculated plates, not more than 6 discs were placed on a 90 mm diameter Petri plate.
6. Within 30 minutes of applying the discs, the plates were taken for incubation at 35^0C for 16-18 hrs.
7. After overnight incubation, the plates were examined to ensure confluent growth. Using a measuring scale, the diameter of each zone of inhibition in mm was measured and results interpreted accordingly.

4.2.7 Purity plate

The purity plate was used to ensure that the inoculation used for the biochemical tests was pure culture and also to see whether the biochemical tests were performed in an aseptic condition or not. Thus, while performing biochemical tests, the same inoculum was sub cultured in respective medium and incubated. The media were then checked for the appearance of pure growth of organisms.

4.2.8 Quality control for test

Quality of each test was maintained by using standard procedures. The quality of each agar plates prepared was tested by incubating one plate of each lot on the incubator. During identification of organism, for each test ATCC control positives and control negatives were taken simultaneously.

For stains and reagents, whenever a new batch of them were prepared, a control smear was stained to ensure correct staining reaction.

Quality of sensitivity tests was maintained by maintaining the thickness of Mueller-Hinton agar at 4 mm and the pH at 7.2-7.4. Similarly antibiotic discs containing the correct amount as indicated and beyond their expiry date were used.

Strict aseptic conditions were maintained while carrying out all the procedures.

CHAPTER-VIII

8. REFERENCES

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APPENDIX-I

QUESTIONNAIRE AND RECORD KEEPING

Code no: Date: _____

OPD:
Contact Person:
No. of beds:
No. of out patients per day:
When do you change bed sheets?

a. Daily	b. Once in 2 days	c. Once in a week	d. Others

At what interval do you change your apron?

S.N	Sample collected from	Time interval

==========

Record Keeping

Sample No:
Microbiological profile:
Microscopic characteristic:
Gram's stain:

_____

Cultural characteristics:
Nutrient Agar:_____

Mac Conkey: _____

Blood Agar: _____

Biochemical characteristic:

Sn	Test performed	Results	Inference
1	Catalase		
2	Oxidase		
3	Coagulase		
4	Indole		
5	MR		
6	VP		
7	TSI		
8	H_2S		

9	Motility		
10	Urease		
11	O/F		

Organism identified as:

Antibiotic susceptibility testing: Kirby-Bauer method

Antibiotics used	***Zone of inhibition (mm)***	***Interpretation***

Verified by

APPENDIX-II

I. Composition and Preparation of Different Culture Media

The culture media used were from two companies
a. Hi-Media Laboratories Pvt. Limited, Bombay, India.
b. Oxoid Unipath Ltd. Basingstoke, Hampshire, England

(All compositions are given in grams per liter and at 25^0C temperature)

1. Blood agar (BA)

Blood agar base (infusion agar) + 5-10% sheep blood

Ingredients	gm/liter
Beef heart infusion	500.0
Tryptose	10.0
Sodium Chloride	5.0
Agar	15.0

Final pH (at 25˚C) 7.3±0.2

42.5 grams of the blood agar base medium was suspended in 1000 ml distilled water and sterilized by autoclaving at 121˚C (15lbs pressure) for 15 minutes. After cooling to 40-50˚C, 50 ml sterile defibrinated sheep blood was added aseptically and mixed well before pouring.

2. MacConkey Agar (MA)

(Without sodium taurocholate, without salt and crystal violet)

Ingredients	gm/liter
Peptone	20.0
Lactose	10.0
Sodium taurocholate	5.0
Sodium chloride	5.0
Neutral Red	0.04
Agar	20.0

Final pH (at 25˚C) 7.4±0.2

55 grams of the medium was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121˚C (15 lbs pressure) for 15 minutes.

3. Mueller Hinton Agar (MHA)

Ingredients	gm/liter
Beef, Infusion form	300.0
Casein Acid Hyrolysate	17.5
Starch	1.5
Agar	17.0
Final pH (at 25˚C) 7.4±0.2	

38 grams of the medium was suspended in 1000 ml distilled water and the medium was warmed to dissolve. 10 ml was distributed in test tubes and sterilized by boiling in water bath for 10 minutes.

4. Nutrient Agar (NA)

Ingredients	gm/litre
Peptone	10.0
Sodium Chloride	5
Beef Extract	10.0
Yeast Extract	1.5
Agar	12.0
Final pH (at 25˚C) 7.4±0.2	

37 grams of the medium was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121˚C (15 lbs pressure) for 15 minutes.

5. Nutrient Broth (NB)

Ingredients	gm/litre
Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Final pH (at 25˚C) 7.4±0.2	

13 grams of the medium was dissolved in 1000 ml distilled water and autoclaved at 121˚C for 15 minutes.

II. Biochemical Test Media

1. MR-VP Medium

Ingredients	gm/litre
Buffered Peptone	7.0
Dextrose	5.0
Dipotassium Phosphate	5.0
Final pH (at 25˚C)	6.9±0.2

17 grams was dissolved in 1000 ml distilled water. 3 ml of medium was distributed in each test tube and autoclaved at 121˚C for 15 minutes.

2. Hugh and Leifson's Medium

Ingredients	gm/litre
Tryptone	2.0
Sodium Chloride	5.0
Dipotassium Phosphate	0.3
Bromothymol Blue	0.08
Agar	2.0
Final pH (at 25˚C)	6.8±0.2

9.4 grams of the medium was rehydrated in 1000 ml cold distilled water and then heated to boiling to dissolve completely. The medium was distributed in 100 ml amounts and sterilized in the autoclave for 15 minutes at 15 lbs pressure (121^0C). To 100 ml sterile medium aseptically added 10ml of sterile Dextrose and mixed thoroughly and dispensed in 5 ml quantities into sterile culture tubes.

3. Sulphide Indole Motility (SIM) medium

Ingredients	gm/litre
Beef Extract	3.0
Peptone	30.0
Peptonized Iron	0.2
Sodium Thiosulphate	0.025
Agar	3.0
Final pH (at 25˚C)	7.3±0.2

36 grams of the medium was suspended in 1000 ml distilled water and dissolved completely. Then it was distributed in tubes to a depth of about 3 inches and sterilized.

4. Simmon Citrate Agar

Ingredients	gm/litre
Magnesium Sulfate	0.2
Mono-ammonium Phosphate	1.0
Dipotassium Phosphate	1.0
Sodium Citrate	2.0
Sodium Chloride	5.0
Agar	15.0
Bromothymol Blue	0.08
Final pH (at 25˚C)	6.8±0.2

24.2 grams of the medium was dissolved in 1000ml distilled water. 3ml medium was distributed in test tubes and sterilized by autoclaving at 121˚C for 15 minutes. After autoclaving tubes containing medium were tilted to form slant.

5. Triple Sugar Iron (TSI) Agar

Ingredients	gm/litre
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 (at 25˚C)	7.4±0.2

65 grams of the medium was dissolved in 1000ml of distilled water and sterilized by autoclaving at 15 lbs (121˚C) pressure for 15 minutes. The medium was allowed to set in sloped form with a butt about 1 inch of thickness.

6. Christensen Urea Agar

Ingredients	gm/litre
Peptone	1.0
Dextrose	1.0
Sodium Chloride	5.0
Dipotassium Phosphate	1.2
Mono-potassium Phosphate	0.8
Phenol Red	0.012
Agar	15.0

Final pH (at 25˚C) 7.4±0.2

24 grams of the medium was suspended in 950 ml distilled water and sterilized by autoclaving at 121˚C for 15 minutes. After cooling to about 45˚C, 50 ml of 40% urea was added and mixed well. Then 5 ml was dispensed in test tube and set at slant position.

III. Staining and Test Reagents

1. For Gram's Stain

(a) Crystal Voilet solution

Crystal Voilet	20.0 g
Ammonium Oxalate	9.0 g
Ethanol or Methanol	95 ml

Distilled Water (D/W) to make 1 litre

Preparation: In a clean piece of paper, 20 gm of crystal violet was weighed and transferred to a clean brown bottle. Then, 95 ml of ethanol was added and mixed until the dye was completely dissolved. To the mixture, 9 gm of ammonium oxalate dissolved in 200 ml of D/W was added. Finally the volume was made 1 litre by adding D/W.

(b) Lugol's Iodine

Potassium Iodide	20.0 g
Iodine	10.0 g
Distilled Water	1000 ml

Preparation: To 250 ml of D/W, 20 gm of potassium iodide was dissolved. Then 10 gm of iodine was mixed to it until it was dissolved completely. Finally the volume was made 1 litre by adding D/W.

(c) Acetone-Alcohol Decoloriser

Acetone	500 ml
Ethanol (Absolute)	475 ml
Distilled Water	25 ml

Preparation: To 25 ml D/W, 475 ml of absolute alcohol was added, mixed and transferred into a clean bottle. Then immediately, 500 ml acetone was added to the bottle and mixed well.

(d) Safranin (Counter Stain)

Safranin	10.0 g
Distilled Water	1000 ml

Preparation: In a clean piece of paper, 10 gm of safranin was weighed and transferred to a clean bottle. Then 1 litre D/W was added to the bottle and mixed well until safranin dissolved completely.

3. Normal saline

Sodium Cholride	0.85 g
Distilled Water	100 ml

Preparation: The sodium chloride was weighed and transferred to a leak-proof bottle premarked to hold 100 ml. Distilled water was added to the 100 ml mark, and mixed until the salt was fully dissolved. The bottle was labeled and stored at room temperature.

4. Test Reagents

a. For Catalase test

Catalase Reagent (3% H_2O_2)

Hydrogen peroxide	3 ml
Distilled Water	97 ml

Preparation: To 97 ml of D/W, 3 ml of hydrogen peroxide was added and mixed well.

b. For Oxidase Test

Oxidase Reagent (impregnated in Whatman's No. 1 filter paper)

Tetramethyl *p*-phenylene diamine dihydrochloride (TPD)	1 gm
Distilled Water	100 ml

Preparation: This reagent solution was made by dissolving 1 gm of TPD in 100 ml D/W. To that solution strips of Whatman's No. 1 filter paper were soaked and drained for about 30 seconds. Then these strips were freeze dried and stored in a dark bottle tightly sealed with a screw cap.

c. For Indole Test

Kovac's Indole Reagent

Isoamyl alcohol	30 ml
p-dimethyl aminobenzaldehyde	2.0 g
Hydrochloric acid	10 ml

Preparation: In 30 ml of isoamylalcohol, 2 g of *p*-dimethyl aminobenzaldehyde was dissolved and transferred to a clean brown bottle. Then to that, 10 ml of conc. HCl was added and mixed well.

d. For Methyl Red Test

Methyl Red Solution

Methyl red	0.05 g
Ethyl alcohol (absolute)	28 ml
Distilled Water	22 ml

Preparation: To 28 ml ethanol, 0.05 gm of methyl red was dissolved and transferred to a clean brown bottle. Then 22 ml D/W was added to that bottle and mixed well.

e. For Voges-Proskauer Test (Barritt's Reagent)

Solution A

α-Napthol	5.0 g
Ethyl alcohol (absolute)	100 ml

Preparation: To 25 ml D/W, 5 g of α-Napthol was dissolved and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

Solution B

Potassium hydroxide	40.0 g
Distilled Water	1000 ml

Preparation: To 25 ml D/W, 40 gm of KOH was dissolved and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

APPENDIX-III

A. Gram-staining Procedure:

First devised by Hans Christian Gram during the late 19th century, the Gram-stain can be used effectively to divide all bacterial species into two large groups: those that take up the basic dye, crystal violet (Gram-positive) and those that allow the crystal dye to was out easily with the decolorizer alcohol or acetone (Gram-negative). The following steps are involved in Gram-stain:

1. A thin film of the material to be examined was prepared and dried.
2. The material on the slide was heat fixed and allowed to cool before staining.
3. The slide was flooded with crystal violet stain and allowed to remain without drying for 10-30 seconds.
4. The slide was rinsed with tap water, shaking off excess.
5. The slide was flooded with iodine solution and allowed to remain on the surface without drying for twice as long as the crystal violet was in contact with the slide surface.
6. The slide was rinsed with tap water, shaking off excess.
7. The slide was flooded with alcohol acetone decolorizer for 10 seconds and rinsed immediately with tap water until no further color flows from the slide with the decolorizer. Thicker smear requires more aggressive decolorizing.
8. The slide was flooded with counter stain (safranin) for 30 seconds and washed off with tap water.
9. The slide was blotted between two clean sheets of bibulous paper and examined microscopically under oil immersion at 1000X.

APPENDIX-IV

1. BIOCHEMICAL TESTS FOR IDENTIFICATION OF BACTERIA

A. Catalase test:

During aerobic respiration, in the presence of oxygen, microorganisms produce hydrogen peroxide, which is lethal to the cell itself. Catalase enzyme breaks down hydrogen peroxide into water and oxygen. The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria, the main exception being *Streptococcus* sp.

A small amount of a culture from Nutrient Agar plate was taken in a clean glass slide and about 2-3 drops of 3% H_2O_2 was put on the surface of the slide. The positive test is indicated by the formation of active bubbling of the oxygen gas. A false positive reaction may be obtained if the culture medium contains catalase (e.g., Blood Agar) or if an iron wire loop is used.

B. Oxidase test:

This test is performed for the detection of cytochrome oxidase in bacteria which catalyzes the transport of electrons between electron donors. In the presence of redox dye Tetramethyl-*p*-phenylene diamine dihydrochloride, the cytochrome oxidase oxidizes it into a deep purple colored end product Indophenol which is detected in the test.

A piece of filter paper was soaked with few drops of oxidase reagent. Then the colony of the test organism was smeared on the filter paper. The positive test is indicated by the appearance of blue-purple color within 10 seconds.

C. Oxidation-Fermentation test:

This test is done to determine the oxidative or fermentative metabolism of carbohydrate resulting in production of various organic acids as end product. Some bacteria are capable of metabolizing carbohydrates (as exhibited by acid

production) only under aerobic conditions, while others produce acid both aerobically and anaerobically. Most medical bacteria are facultative anaerobes.

The test organism was stabbed into the bottom of two sets of tubes with Hugh and Leifson's media, bromothymol blue being the pH indicator. The inoculated medium in one of the tubes was covered with a 10 mm deep layer of sterile paraffin oil. The tubes were then incubated at 37°C for 24 hours. After incubation the tubes were examined for carbohydrate utilization as shown by acid production. Fermentative organism utilizes the carbohydrate in both the open and sealed tubes as shown by a change in colour of the medium from green to yellow. Oxidative organisms, however, are able to use the carbohydrate only in the open tube.

D. Indole Production test:

This test detects the ability of the organism to produce an enzyme: 'tryptophanase' which oxidizes tryptophan to form indolic metabolites: indole, skatole (methyl indole) and indoleacetic acid.

A smooth bacterial colony was stabbed on SIM (Sulphide Indole Motility) medium by a sterile stab wire and the inoculated media was incubated at 37°C for 24 hours. After 24 hours incubation, 0.5 ml of Kovac's reagent was added. Appearance of red color on the top of media indicates indole positive. Indole if present combines with the aldehyde present in the reagent to give a red color in the alcohol layer. The color reaction is based on the presence of the pyrrole structure present in indole.

E. Methyl Red test:

This test is performed to test the ability of an organism to produce sufficient acid from the fermentation of glucose to give a red color with the indicator methyl red (denotes changes in degree of acidity by color reactions over a pH range of 4.4-6.0).

A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of methyl

red reagent was added and mixed well. The positive test was indicated by the development of bright red color, indicating acidity.

F. Voges Proskauer (VP) test:

This test is employed to detect the production of acetyl methyl carbinol (a neutral end product) or its reduction product 2, 3-butanidiol during fermentation of carbohydrates.

A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of Barritt's reagent was added and shaken well for maximum aeration and kept for 15 minutes, positive test is indicated by the development of pink red colour.

G. Citrate Utilization test:

This test is performed to detect whether an organism utilizes citrate as a sole source of carbon for metabolism with resulting alkalinity. Organisms capable of utilizing citrate as its sole carbon source also utilizes the ammonium salts present in the medium as its sole nitrogen source, the ammonium salts are broken down to ammonia with resulting alkalinity.

A loopful of test organism was streaked on the slant area of Simmon's Citrate Agar medium and incubated at 37°C for 24 hours. A positive test was indicated by the growth of organism and change of media by green to blue, due to alkaline reaction. The pH indicator bromothymol blue has a pH range of 6.0-7.6, i.e. above pH 7.6; a blue color develops due to alkalinity of the medium.

H. Motility test:

The motility media used for motility test are semisolid, making motility interpretations macroscopic. Motile organisms migrate from the stabline and diffuse into the medium causing turbidity. Whereas non-motile bacteria show the growth along the stabline, and the surrounding media remains colorless and clear.

I. Triple Sugar Iron (TSI) Agar:

The TSI agar is used to determine the ability of an organism to utilize specific carbohydrate incorporated in the medium (glucose, sucrose and lactose in concentrations of 0.1%, 1.0% and 1.0% respectively), with or without the production of gas (indicated by cracks in the media as well as an air gap at the bottom of the tube) along with determination of possible hydrogen sulfide production (detected by production of black color in the medium).

The test organism was streaked and stabbed on the surface of TSI and incubated at 37°C for 24 hours. Acid production limited only to the butt region of the tube is indicative of glucose utilization, while acid production in slant and butt indicates sucrose or lactose fermentation. Phenol red is the pH indicator which gives yellow reaction at acidic pH, and red reaction to indicate an alkaline surrounding.

J. Urea Hydrolysis test:

This test demonstrates the urease activity present in certain bacteria which decomposes urea, releasing ammonia and carbon dioxide. Ammonia thus produced changes the color of indicator incorporated in the medium.

The test organism was inoculated in a medium containing urea and the indicator phenol red. The inoculated medium was incubated at 37°C overnight. Positive organism shows pink red color due to the breakdown of urea to ammonia. With the release of ammonia the medium becomes alkaline as shown by a change in colour of the indicator to pink.

K. Coagulase test:

This test is used specifically to differentiate species within the genus *Staphylococcus*: *S aureus* (usually positive) from *S epidermidis* (negative). A positive coagulase test is usually the final diagnostic criterion for the identification of *Staphylococcus aureus.* Free coagulase and bound coagulase are the two types

of coagulase possessed by this organism; most strains possess both free and bound coagulase.

Slide Coagulase Test:

Bound coagulase (Clumping Factor) is detected by slide test. The bound coagulase is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in alteration of fibrinogen so that it precipitates on the staphylococcal cell, causing the cells to clump when a bacterial suspension is mixed with plasma.

For slide coagulase test, a drop of physiological saline was placed on three places of a slide, and then a colony of the test organism was emulsified in two of the drops to make thick suspensions. Later a drop of plasma was added to one of the suspensions and mixed gently. Then a clumping was observed within 10 seconds for the positive coagulase test. No plasma was added in second suspension. This was used for the differentiation of any granular appearance of the organism from true coagulase clumping. The third drop of saline was used for a known strain of coagulase positive staphylococci.

Tube Coagulase Test

This test is carried out to detect production of free coagulase. Plasma contains coagulase reacting factor (CRF) which activates free coagulase. The activated coagulase acts upon prothrombin thus converting it to thrombin. Thrombin converts fibrinogen into fibrin which is detected as a firm gel (clot) in the tube test. Tube test is performed when negative or doubtful results are obtained in slide coagulase test.

In the tube coagulase test, plasma was diluted 1 in 10 in physiological saline. Four small tubes were taken, one for test organism, one for positive control, one for negative control, and one to observe self clotting of plasma. Then 0.5 ml of the diluted plasma was pipetted into each tube and 0.5 ml of test organism, 0.5 ml of positive control (*Staphylococcus aureus* culture), and 0.5 ml negative control (*Staphylococcus epidermidis* culture) was added to three tubes, to the fourth tube, 0.5 ml sterile broth was added. After mixing gently, all tubes were incubated at

37^0C on a waterbath for 6 hours and observed for gel formation in every 30 minutes.

APPENDIX-V

LIST OF EQUIPMENTS AND MATERIALS USED DURING THE STUDY

A. Equipments

1.	Oven	Sakura (Japan)
2.	Incubator	Yamato (Japan)
3.	Autoclave	Sakura (Japan)
4.	Refrigerator	Hitachi (Japan)
5.	Microscope	Nikon (Japan)
6.	Centrifuge	Hitachi (Japan)
7.	Water bath	NSW (Japan)
8.	Weighing balance	Chyo MP 300 (Japan)
9.	Laminar Flow	Dalton (USA)
10.	Coagulator	Hirasawa (Japan)
11.	Distillation Plant	Yamato (Japan)

B. Antibiotic Discs

Different antibiotics discs used for the sensitivity tests were from different companies as:

1. Hi-Media Laboratories Pvt. Limited, Bombay, India.

2. Oxoid Unipath Ltd. Basingstoke, Hampshire, England.

APPENDIX-VI

ZONE SIZE INTERPRETATIVE CHART OF ANTIBIOTICS

Antimicrobial Agent	Symbol	Disc Content	Resistant (mm or less)	Intermediate (mm)	Sensitive (mm or more)
Amikacin	Ak	30 µg	14	15-16	17
Ampicillin					
When testing Gram-negative enteric organisms	A	10 µg	13	14-16	17
When testing Staphylococci			28	-	29
When testing *Haemophilus* sp.			18	19-21	22
Ceftazidime	Ca	30 µg	14	15-17	18
Ceftriaxone	Ci	30 µg	13	14-20	21
Cephalexin	Cp	30 µg	14	15-17	18
Chloramphenicol	C	30 µg	12	13-17	18
Ciprofloxacin	Cf	5 µg	15	16-20	21
Cloxacillin	OB	5 µg	11	12-13	14
Erythromycin					
When testing Staphylococci	E	15 µg	13	14-22	23
When testing Streptococci			15	16-20	21
Gentamicin	G	10 µg	12	13-14	15
Vancomycin					
When testing Staphylococci	Va	30 µg	-	-	15
When testing Streptococci			-	-	17

(Source: Hi-Media Laboratories Pvt. Limited, Bombay, India and Cheesbrough, 2000)

APPENDIX-VII

Distinguishing reactions of the commoner and pathogenic Enterobacteriaceae

Species	Test/ substrate[a]											
	lac	mot	gas	ind	VP	cit	PDA	ure	lys	H_2S	inos	ONPG
Escherichia coli	+	+	+	+	-	-	-	-	+	-	-	+
Shigella groups A, B, C	-	-	-	±	-	-	-	-	-	-	-	-
Sh. sonnei	-	-	-	-	-	-	-	-	-	-	-	+
Salmonella (most serotypes)	-	+	+	-	-	+	-	-	+	+	±	-
Salmonella typhi	-	+	-	-	-	-	-	-	+	+	-	-
Salmonella paratyphi A	-	+	+	-	-	-	-	-	-	-	-	-
Citrobacter fruendii	±	+	+	-	-	+	-	±	-	±	-	+
C. koseri	±	+	+	+	-	+	-	±	-	-	-	+
Klebsiella pneumoniae	+	-	++	-	+	+	-	+	+	-	+	+
K. oxytoca	+	-	++	+	+	+	-	+	+	-	+	+
Enterobacter aerogenes	+	+	++	-	+	+	-	-	+	-	+	+
Ent. cloacae	+	+	+	-	+	+	-	±	-	-	-	+
Hafnia alvei	-	+	+	-	+	-	-	-	+	-	-	+
Serratia marcescens[b]	-	+	±	-	+	+	-	-	+	-	±	+
Proteus mirabilis	-	+	+	-	±	±	+	++	-	+	-	-
P. vulagris	-	+	+	+	-	-	+	++	-	+	-	-
Morganella morganii	-	+	+	+	-	-	+	++	-	±	-	-
Providencia rettgeri	-	+	-	+	-	+	+	++	-	-	+	-
Prov. stuartii	-	+	-	+	-	+	+	±	-	-	+	-
Prov. alcalifaciens	-	+	+	+	-	+	+	-	-	-	-	-
Yersinia enterocolitica[c]	-	-	-	±	-	-	-	±	-	-	±	+
Y. pestis	-	-	-	-	-	-	-	-	-	-	-	±
Y. pseudotuberculosis	-	-	-	-	-	-	-	+	-	-	-	±

[a] lac, inos, fermentation of lactose, inositol; mot, motility; gas, gas from glucose; ind, indole production; VP, Voges-Proskauer; cit, Citrate utilization (Simmons'); PDA, phenylalanine deaminase; ure, urease; lys, lysine decarboxylase; H_2S, H_2S produced in TSI agar; ONPG, metabolism of *o*-nitrophenyl-β-D-galactopyranoside.

[b] Some strains of *Serratia* marcescens may produce a red pigment

[c] *Yersinia* are motile at 22°C.

{Key: +, ≥85% of strains positive; -, ≥ 85% of strains negative; 16-84% of strains are positive after 24-48 hour at 36°C}

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

APPENDIX- VII

Sample (No of isolates)	Sensitivity pattern	Antibiotics used					
		Amp(%)	Cip(%)	Cl(%)	OB(%)	E(%)	Co(%)
New bed	R	27(66.6%)	-	-	-	14(33.3%)	-

Antibiotic Susceptibility Pattern of *Staphylococcus aureus* in Various Samples

Sample (No of isolates)	Sensitivity pattern	Antibiotics used					
		Amp(%)	Cip(%)	Cl(%)	OB(%)	E(%)	Co(%)
sheet (N=42)	I	-	-	-	-	-	14(33.3%)
	S	14(33.3%)	41(100%)	41(100%)	41(100%)	27(66.6%)	27(66.6%)
Used bed sheet (N=62)	R	42(66.6%)	20(33.3%)	-	-	21(33.3%)	21(33.3%)
	I	-	-	-	-	-	-
	S	20(33.3%)	42(66.6%)	62(100%)	62(100%)	41(66.6%)	41(66.6%)
Aprons (N=64)	R	13(20.0%)	-	13(20.0%)	13(20.0%)	13(20.0%)	26(40.0%)
	I	13(100%)	13(100%)	13(100%)	13(20.0%)	13(100%)	13(100%)
	S	51(80.0%)	64(100%)	51(80.0%)	38(60.0%)	38(60.0%)	38(60.0%)
Dental (equipments) (N=13)	R	3(100%)	3(100%)	3(100%)	-	3(100%)	3(100%)
	I	-	-	-	-	-	-
	S	3(100%)	3(100%)	3(100%)	3(100%)	3(100%)	3(100%)
Endoscopy	R	5(50.0%)	3(30.0%)	3(30.0%)	-	5(50.0%)	5(50.0%)
	I	-	-	-	-	2(20.0%)	2(20.0%)
	S	5(50.0%)	7(70.0%)	7(70.0%)	10(100%)	3(30.0%)	3(30.0%)
Air (N=10)							

(equipments) (N=3)	I	-	-	-	-	-	-
	S	-	-	-	3(100%)	-	-
ENT (equipments) (N=5)	R	2(40.0%)	-	-	-	2(40.0%)	-
	I	-	-	-	-	-	3(60.0%)
	S	3(60.0%)	5(100%)	5(100%)	5(100%)	3(60.0%)	2(40.0%)
General Surgery (equipments) (N=7)	R	-	-	-	-	7(100%)	7(100%)
	I	-	-	-	-	-	-
	S	7(100%)	7(100%)	7(100%)	7(100%)	-	-
Gynaecology (equipments) (N=2)	R	2(100%)	2(100%)	-	-	-	-
	I	-	-	-	-	-	-
	S	-	-	2(100%)	2(100%)	2(100%)	2(100%)

Antibiotic Susceptibility Pattern of the *Acinetobacter* spp in various samples

Sample (No of isolates)	**Sensitivity pattern**	**Antibiotics used**			
		AMP(%)	**CIP(%)**	**CL(%)**	**CN(%)**
New bed sheet	R	8 (100%)	8 (100%)	-	-

(N=8)	I	-	-	-	-
	S	-	-	8 (100%)	8 (100%)
Used bed sheet (N=9)	R	7 (77.8%)	7 (77.8%)	1 (11.1%)	-
	I	1 (11.1%)	2 (22.2%)	2 (22.2%)	-
	S	1 (11.1%)	-	6 (66.6%)	9 (100%)
Apron (N=17)	R	8 (47.1%)	10 (58.8%)	1 (5.8%)	-
	I	1 (5.8%)	2 (11.7%)	1 (5.8%)	-
	S	8 (47.1%)	5 (29.4%)	15 (80.2%)	17 (100%)
Air (N=5)	R	2 (40%)	1 (20%)	1 (20%)	-
	I	1 (20%)	1 (20%)	-	-
	S	2 (40%)	3 (60%)	4 (80%)	5 (100%)
Dental (equipments) (N=5)	R	2 (40%)	3 (60%)	1 (20%)	-
	I	1 (20%)	1 (20%)	1 (20%)	-
	S	2 (40%)	1 (20%)	3 (60%)	5 (100%)
Endoscopy (equipments) (N=1)	R	1 (100%)	-	-	-
	I	-	1 (100%)	-	-
	S	-	-	1 (100%)	1 (100%)

CHAPTER-V

5. RESULTS

5.1 PATTERN OF SAMPLES COLLECTED

Altogether 281 samples were collected randomly from different sources of 10 out patient departments of Tribhuvan University Teaching Hospital. Out of 281 samples, 56 samples were collected from new bed sheets, 64 samples from used bed sheets, 76 from health care personnel (doctors, nurses, health assistants, interns), 10 samples were air samples and 75 samples were collected from different equipments used for the check up and treatment of out patients. The samples collected were all inanimate objects, which come in direct contact with patients and staffs working in the hospital.

Table 2. Pattern of samples collected

S.N	Sample	No. of samples	Percentage
1.	Bed sheets (new)	56	19.9%
2.	Bed sheets (used)	64	22.7%
3.	Apron	76	27.01%
4.	Air	10	3.5%
5.	Instruments	75	26.6%
Total		281	100%

5.2 PATTERN OF RESULTS

5.2.1 Pattern of Bacterial Isolates from New and Used Bed Sheets

Table 3. Pattern of bacterial isolates from 56 new bed sheets from 10 OPD

S. N	Name of OPD	No of samples	No. of bacterial isolates						
			Gram positives					Gram negatives	
			S. aureus	CoNS	*Bacillus* spp. (haemolytic)	*Bacillus* spp. (non haemolytic)	*Micrococcus* spp.	*Pseudomonas* spp.	*Acinetobacter* spp.
1.	Endoscopy	2	0	2	2	0	0	0	0
2.	Dermatology	6	1	6	6	6	1	0	2
3.	Dental	6	4	6	4	2	0	0	0
4.	General surgery	4	2	4	4	2	1	0	1
5.	Orthopedic	6	5	6	6	4	1	0	0
6.	Psychiatry	2	2	2	2	1	0	0	0
7.	Gynaecology	14	12	13	10	11	2	4	0
8.	General medicine	8	7	7	8	4	3	2	4
9.	General practice	6	4	5	3	4	2	0	0
10.	ENT	2	0	2	2	1	0	0	1
Total		56	41	53	47	35	10	6	8
Percentage		100%	73.2%	94.6%	83.9%	62.5%	17.8 %	10.7%	14.2%

Out of 56 samples of new bed sheets, the most predominant organism was CoNS (94.6%) and least predominant organism was *Pseudomonas* spp. (10.7%). *S. aureus* was present in 41 samples constituting of 73.2%.

Table 4. Pattern of bacterial isolates from 64 used bed sheets from 10 OPD

			No. of bacterial isolates

S.N	Name of OPD	No of samples	Gram positives					Gram negatives	
			S. aureus	CoNS	*Bacillus* spp. (haemolytic)	*Bacillus* spp. (non haemolytic)	*Micrococcus* spp.	*Pseudomonas* spp.	*Acinetobacter* spp.
1.	Endoscopy	2	2	2	1	2	2	0	0
2.	Dermatology	6	6	4	6	6	1	0	1
3.	Dental	6	6	6	5	6	2	0	0
4.	General surgery	10	10	8	6	6	6	0	2
5.	Orthopedic	7	6	6	7	6	2	1	2
6.	Psychiatry	4	4	3	3	4	2	0	0
7.	Gynaecology	11	10	11	5	6	3	1	1
8.	General medicine	10	10	10	10	6	6	2	3
9.	General practice	6	6	6	2	6	1	0	0
10.	ENT	2	2	2	2	1	1	0	0
Total		64	62	58	47	49	26	4	9
Percentage		100%	96.8%	90.6%	73.4%	76.5%	40.6%	6.2%	14 %

Out of 64 samples of used bed sheets, the most predominant organism was *S. aureus* (96.8%) and least predominant organism was *Pseudomonas* spp. (6.2%).

5.2.2 Pattern of Bacterial Isolates from Aprons

			No. of bacterial isolates	
			Gram positives	Gram

S.N	Name of OPD	No of samples						negatives	
			S. aureus	CoNS	*Bacillus* spp. (haemolytic)	*Bacillus* spp. (non haemolytic)	*Micrococcus* spp.	*Pseudomonas* spp.	*Acinetobacter* spp.
1.	Endoscopy	3	3	3	3	3	0	0	0
2.	Dermatology	7	2	7	3	5	7	0	4
3.	Dental	8	8	8	3	7	4	2	1
4.	General surgery	12	10	12	4	8	5	0	0
5.	Orthopedic	6	6	6	4	4	2	0	0
6.	Psychiatry	6	6	6	2	4	0	0	0
7.	Gynaecology	11	8	10	4	8	7	0	5
8.	General medicine	7	5	7	4	5	3	0	4
9.	General practice	2	2	2	1	1	0	0	0
10.	ENT	14	14	14	9	7	6	3	3
Total		76	64	75	37	52	34	5	17
Percentage		100%	84.2%	98.6%	48.6%	68.4%	44.7%	6.5%	22.3%

Table 5. Pattern of bacterial isolates from 76 apron samples from 10 OPD

Out of 76 apron samples collected from health care personnel, the most predominant organism was CoNS (98.6%) and the least predominant was *Pseudomonas* spp. (6.5%). *S. aureus* constitute 84.2%.

5.2.3 Pattern of Bacterial Isolates from Air Samples

Table 6: Pattern of bacterial isolates of 10 air samples from 10 OPD

S.N	OPD	No. of colony	Microorganisms isolated
1.	**Endoscopy**	113	*Staphylococcus aureus* CoNS *Bacillus* spp.*(*haemolytic*)* *Bacillus* spp.*(*non haemolytic*)* *Micrococcus* spp.

2.	**Dermatology**	120	*Staphylococcus aureus* CoNS *Bacillus* spp.*(*haemolytic*)* *Acinetobacter* spp.
3.	**Dental**	138	*Staphylococcus aureus* CoNS *Bacillus* spp.*(*haemolytic*)* *Bacillus* spp.(non haemolytic*)* *Micrococcus* spp.
4.	**General surgery**	152	*Staphylococcus aureus* CoNS *Bacillus* spp.*(*haemolytic*)* *Bacillus* spp.*(*non haemolytic*)* *Micrococcus* spp. *Acinetobacter* spp.
5.	**Orthopedic**	251	*Staphylococcus aureus* CoNS *Bacillus* spp.*(*haemolytic*)* *Bacillus* spp.*(*non haemolytic*)* *Micrococcus* spp. *Acinetobacter* spp.

6.	**Psychiatry**	151	*Staphylococcus aureus* CoNS *Bacillus* spp.*(*haemolytic*)* *Bacillus* spp.*(*non haemolytic*)*
7.	**Gynaecology**	158	*Staphylococcus aureus* CoNS *Bacillus* spp.*(*haemolytic*)* *Bacillus* spp.*(*non haemolytic*)* *Micrococcus* spp. *Acinetobacter* spp.
8.	**General Medicine**	237	*Staphylococcus aureus* CoNS *Bacillus* spp.*(*haemolytic*)* *Bacillus* spp.*(*non haemolytic*)* *Micrococcus* spp. *Acinetobacter* spp.

9.	**General Practice**	72	*Staphylococcus aureus* CoNS *Bacillus* spp.*(*haemolytic*)*
10.	**ENT**	124	*Staphylococcus aureus* CoNS *Bacillus* spp.*(*haemolytic*)* *Bacillus* spp.*(*non haemolytic*)*

Among 10 air samples collected by gravity settling method for 5 minutes, the CFU count of Orthopedic department was highest, which was 251 and the least CFU count was of General Medicine, which was 72. The types of bacteria isolated were *S. aureus*, CoNS, *Bacillus* spp. (haemolytic), *Bacillus* spp. (non haemolytic), *Micrococcus* spp. and *Acinetobacter* spp. The most predominant organisms were Gram positive cocci.

5.2.4 Pattern of Bacterial Isolates from Instruments

Table 7. Pattern of bacterial colony count of 23 equipment samples from dental department

Out of 23 equipments samples, different types of organisms were obtained, which were

S.N	Sample	Total No. of colony	No of colony					
			Gram positives					Gram negatives
			S. aureus	CoNS	*Bacillus* spp. (haemolytic)	*Bacillus* spp. (non haemolytic)	*Micrococcus* spp.	*Acinetobacter* spp.
1.	Dental hand stand	6	2	1	1	1	0	1
2.	Light care	8	2	3	0	2	1	0
3.	Dental light	3	0	0	0	2	1	0
4.	Hand piece	1	0	1	0	0	0	0
5.	Board switch	38	9	13	3	6	5	2
6.	Tray	30	6	11	4	7	2	0
7.	Tap switch	3	0	0	2	1	0	0
8.	Dental hand stand	11	2	3	2	4	0	0
9.	Compressor unit	6	0	2	1	3	0	0
10.	Dental light	22	6	9	2	3	2	0
11.	Hand piece	12	0	5	2	5	0	0
12.	Tray	46	11	17	6	9	2	1
13.	Tap	31	9	16	2	4	0	0
14.	Dental hand stand	48	10	18	3	8	7	2
15.	Light care	12	0	5	2	5	0	0
16.	Dental light	15	8	6	0	1	0	0
17.	Hand piece	6	2	2	2	0	0	0
18.	Compressor unit	10	0	4	3	3	2	1
19.	Tray	17	7	5	2	3	0	0
20.	Tap	24	0	9	4	6	5	0
21.	Suction switch	37	0	19	5	13	0	0
22.	Chital forceps	0	0	0	0	0	0	0
23.	X- ray developer	35	12	11	7	5	0	0

S. aureus, CoNS, haemolytic and non haemolytic *Bacillus* spp., *Micrococcus* spp. *Acinetobacter* spp.. Their densities are summarized in the table.

S.N	Bacterial isolates	Frequency	Percentage
1.	*Staphylococcus aureus*	13	56.5%
2.	CoNS	20	86.9%

3.	*Bacillus* spp.*(*haemolytic*)*	18	78.2%
4.	*Bacillus* spp.*(*non haemolytic*)*	20	86.9%
5.	*Micrococcus* spp.	9	39.1%
6.	*Acinetobacter* spp.	5	21.7%

Table 8. Pattern of bacterial isolates in 23 instrument samples from dental department

Among the 23 samples collected from Dental department, CoNS and non haemolytic *Bacillus* were present in 20 samples each, haemolytic *Bacillus* spp. in 18 samples, *S. aureus* in 13 samples, *Micrococcus* spp. in 9 samples and *Acinetobacter* spp. in 5 samples.

Table 9. Pattern of bacterial colony count of 15 equipment samples from endoscopy

S.N	**Sample**	**Total No.**	**No. of colony**	
			Gram positives	**Gram positives**

		of colony	***S. aureus***	CoNS	***Bacillus*** spp. (haemolytic)	***Bacillus*** spp. (non haemolytic)	Acinetobacter spp.
1.	Endoscope	2	2	0	0	0	0
2.	Endoscope	0	0	0	0	0	0
3.	Endoscope	0	0	0	0	0	0
4.	Endoscope	0	0	0	0	0	0
5.	Endoscope	2	1	0	0	1	0
6.	Colonoscopy	1	0	1	0	0	0
7.	Sigmoid	13	4	2	0	1	6
8.	ERCPscope	1	0	0	0	1	0
9.	Endoscope	0	0	0	0	0	0
10.	Endoscope	0	0	0	0	0	0
11.	Endoscope	0	0	0	0	0	0
12.	Endoscope	0	0	0	0	0	0
13.	Endoscope	0	0	0	0	0	0
14.	ERCPscope	6	0	3	1	2	0
15.	Colonoscopy	3	0	3	0	0	0

department

Out of 15 sterilized equipments samples, different types of organisms were obtained, which were *S. aureus*, CoNS, haemolytic and non haemolytic *Bacillus* spp. and *Acinetobacter* spp. Their densities are summarized in the table.

Table 10. Pattern of bacterial isolates in 15 instrument samples from endoscopy department

S.N	**Bacterial isolates**	**Frequency**	**Percentage**
1.	*Staphylococcus aureus*	3	20%
2.	CoNS	4	26.6%
3.	*Bacillus* spp.*(*haemolytic*)*	1	6.6%
4.	*Bacillus* spp.*(*non haemolytic*)*	4	26.6%
5.	*Acinetobacter* spp.	1	6.6%

Among the 15 sterilized samples collected from endoscopy department, CoNS and non

			No of colony

haemolytic *Bacillus* spp. were present in 4 samples each, *S. aureus* in 3 samples, haemolytic *Bacillus* spp. and *Acinetobacter* spp. in 1 sample each.

Table 11. Pattern of bacterial colony count of 12 equipment samples from ENT department

S.N	Sample	Total No. of colony	Gram positives					Gram negatives
			Frequency					
			S. aureus	CoNS	*Bacillus* spp. (haemolytic)	*Bacillus* spp. (non haemolytic)	*Micrococcus* spp.	*Pseudomonas* spp.
1.	IL mirror	1	0	0	1	0	0	0
2.	IL mirror	0	0	0	0	0	0	0
3.	IL mirror	2	0	0	0	0	2	0
4.	Tongue depressor	2	1	1	0	0	0	0
5.	Tongue depressor	0	0	0	0	0	0	0
6.	Tongue depressor	1	0	1	0	0	0	0
7.	Nose speculum	4	1	2	0	0	1	0
8.	Nose speculum	29	13	9	0	5	0	2
9..	Nose speculum	0	0	0	0	0	0	0
10.	Ear speculum	0	0	0	0	0	0	0
11.	Ear speculum	15	5	6	2	1	1	0
12.	Ear speculum	21	7	9	3	1	0	1

S.N	Bacterial isolates	Frequency	Percentage
1.	*Staphylococcus aureus*	5	41.6%
2.	CoNS	6	50%
3.	*Bacillus* spp.(haemolytic*)*	3	25%

Out of 12 sterilized equipments samples, different types of organisms were obtained, which were *S. aureus*, CoNS, haemolytic and non haemolytic *Bacillus* spp., *Micrococcus* spp. and *Pseudomonas* spp. Their densities are summarized in the table.

Table 12. Pattern of bacterial isolates in 12 instrument samples from ENT department.

4.	*Bacillus* spp.*(*non haemolytic*)*	3	25%
5.	*Micrococcus* spp.	3	25%
6.	*Pseudomonas* spp.	2	16.6%

Among the 12 sterilized samples collected from ENT department, CoNS was present in 6 samples, *S. aureus* was present in 5 samples. Haemolytic *Bacillus* spp., non haemolytic *Bacillus* spp. and *Micrococcus* spp. in 3 samples each and *Pseudomonas* spp. was present in 2 samples.

Table 13. Pattern of bacterial colony count of 16 samples from general surgery department

S.N	**Sample**	**Total No. of colony**	**No of colony**				
			S. aureus	CoNS	*Bacillus* spp. (haemolytic)	*Bacillus* spp. (non haemolytic)	*Micrococcus* spp.
1.	Dressing drum	0	0	0	0	0	0

2.	Dressing drum	3	1	1	1	0	0
3.	Instrument tray	0	0	0	0	0	0
4.	Instrument tray	0	0	0	0	0	0
5.	Rack	17	2	4	3	6	2
6.	Dressing trolley	27	9	8	4	3	3
7.	Dressing trolley	31	11	9	6	3	2
8.	Dressing drum	0	0	0	0	0	0
9.	Dressing drum	0	0	0	0	0	0
10.	Dressing gloves drum	0	0	0	0	0	0
11.	Instrument tray	0	0	0	0	0	0
12.	Instrument tray	0	0	0	0	0	0
13.	Instrument tray	2	0	0	1	1	0
14.	Dressing trolley	11	3	2	1	3	2
15.	Dressing trolley	15	2	4	0	4	5
16.	Rack	13	2	7	1	2	3

Out of 16 samples, different types of organisms were obtained, which were *S. aureus*, CoNS, haemolytic and non haemolytic *Bacillus* spp. and *Micrococcus* spp. Their densities are summarized in the table.

Table 14**.** Pattern of bacterial isolates in 16 samples from general surgery department

S.N	**Bacterial isolates**	**Frequency**	**Percentage**
1.	*Staphylococcus aureus*	7	41.1%
2.	CoNS	7	41.1%
3.	*Bacillus* spp. *(*haemolytic*)*	7	41.1%
4.	*Bacillus* spp. *(*non haemolytic*)*	7	41.1%
5.	*Micrococcus* spp.	6	35.2%

Among the 17 samples collected from general surgery department, *S. aureus* and CoNS were present in 7 samples each. Haemolytic *Bacillus* spp., non haemolytic *Bacillus* spp. in 7 samples each and *Micrococcus* spp. was present in 6 samples.

Table 15**.** Pattern of bacterial colony count of 9 samples from gynaecology department

S.N	**Sample**	**No. of colony**	**No of colony**			
			S. aureus	CoNS	*Bacillus* spp.(haemolytic)	*Bacillus* spp.(non haemolytic)
1.	Speculum	5	2	-	-	3
2.	Speculum	29	7	13	3	6
3.	Speculum	2	-	1	1	-
4.	Speculum	1	-	1	-	-
5.	Speculum	1	-	-	1	-

6.	Speculum	13	-	9	4	-
7.	Speculum	3	-	2	-	1
8.	Speculum	2	-	2	-	-
9.	Speculum	6	-	4	-	2

Out of 9 sterilized speculum samples, different types of organisms were obtained, which were *S. aureus*, CoNS, haemolytic and non haemolytic *Bacillus* spp. Their densities are summarized in the table.

Table 16. Pattern of bacterial isolates in 9 instrument samples from gynaecology department

S.N	Bacterial isolates	Frequency	Percentage
1.	*Staphylococcus aureus*	2	22.2%
2.	CoNS	7	77.7%
3.	*Bacillus* spp. *(*haemolytic*)*	4	44.4%
4.	*Bacillus* spp. *(*non haemolytic*)*	4	44.4%

Among the 9 sterilized speculum samples collected from gynecology department, CoNS was present in 7 samples, *S. aureus* was present in 2 samples. Haemolytic and non-haemolytic *Bacillus* spp. in 4 samples each.

5.2.5 Antibiotic Susceptibility Profiling of Bacterial Isolates

Table 17. Antibiotic sensitivity pattern of *S. aureus* in various samples

Organisms (No. of isolates)	Antibiotics used	Sensitivity Pattern					
		Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
S. aureus (N=207)	Ampicillin	113	54.6	0	0.0	94	45.4
	Ciprofloxacin	179	86.4	0	0.0	28	13.6
	Cephalexin	188	90.9	0	0.0	19	9.1
	Cloxacillin	181	87.4	13	6.2	13	6.2
	Erythromycin	127	61.3	15	7.2	65	31.5
	Cotrimoxazole	126	60.8	19	9.1	62	29.9

Among the antibiotics used against *S aureus*, Cephalexin was found to be the most effective drug as out of 207 isolates, 188 (90.9%) isolates were sensitive, whereas181 (87.4%) isolates were sensitive to Cloxacillin. Similarly, 179 (86.4%) isolates were sensitive to Ciprofloxacin, 127 (61.3%) isolates were sensitive to Erythromycin and 126 (60.8%) isolates were sensitive to Cotrimoxazole. The least effective drug was Ampicillin as 113 (54.6%) samples were only sensitive to it. The results are shown in the table.

Table18**.** Antibiotic sensitivity pattern of the *Acinetobacter* spp. in various samples

Organisms (No. of isolates)	Antibiotics used	Sensitivity Pattern					
		Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Organisms (N=45)	Ampicillin	13	28.9	4	8.9	28	62.2
	Ciprofloxacin	9	20.0	7	15.6	29	64.4
	Cephalexin	37	82.2	4	8.9	4	8.9
	Gentamicin	45	100	0	0.0	0	0.0

Among the antibiotics used against *Acinetobacter* spp., Gentamicin was found to be the most effective drug as out of 45 isolates, 45 (100%) isolates were sensitive, followed by cephalexin as 37 (82.2%) isolates were sensitive. Similarly, 13 (28.9%) isolates were

sensitive to Ampicillin. The least effective drug was Ciprofloxacin as 9 (20.0%) samples were only sensitive to it. The results are shown in the table.