CHAPTER I

Introduction

Hospital is health care setting where sick or injured are given medical care that includes observational, diagnostic, therapeutic and rehabilitative services. People in different health condition visit hospital and thus hospital holds the high opportunity to harbor pathogens that are responsible for causing variety of diseases. These pathogens whenever gets the suitable opportunities never miss to captivate its new host. Infection thus acquired in hospital is referred as Nosocomial infection (NI). A NI has been defined as "Infections occurring in patients in hospital in whom the infection was not present or incubating at the time of admission" (Beneson, 1995). The term "healthcare associated infection" is now widely used instead of the traditional NIs and is defined by the CDC as "A localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s). There must be no evidence that the infection was present or incubating at the time of admission to the acute care setting". The threat to NI is being a matter of concern due to development of multidrug resistant (MDR) strains among common isolates in hospital. Applying antibiotics caused breakthrough in treatment stepping out in ill's group infections treated in hospital however enlarging resistance, the formation of new mechanisms of resistance, and/or the spreading of gene of resistance has become the shortcomings of antibiotic therapy (Jane *et al*., 2006).

Multidrug resistant organisms (MDRO) are the microorganism predominantly bacteria, that are resistant to one or more classes of antimicrobial agents (Harrison *et al*., 1998). Although the names of certain MDRO describe resistance to only one agent (e.g., MRSA, VRE), these pathogens are frequently resistant to most available antimicrobial agents. These highly resistant organisms deserve special attention in healthcare facilities (Shales *et al*., 1997). In addition to MRSA and VRE, certain gram negative bacteria,

including those producing Extended Spectrum Beta-lactamases (ESBLs) and others that are resistant to multiple classes of antimicrobial agents, are of particular concern (Jane *et al*.,2006).

Resistant emerges from over utilization of antibiotics trying to sterilize the environment and also the inappropriate use of the antibiotics for treatment. Free availability and self medication of antibiotics, lack of access to health facilities, in adequate public awareness, uncontrolled antibiotics use in agriculture, lack of adequate antimicrobial resistance surveillance and lack of updated national antibiotic polices and guidelines are added worries. Antibiotics are commonly used in animals for prophylaxis or as performance enhances and such practices are likely to increase the development of resistance. Human factors such as international travel, movement of patients from hospitals to community, improper disposal of infectious wastages and unhygienic behaviors contribute to fast spread of resistant organisms as well as diseases (Jane *et al*., 2006).

Infection acquired in hospital has been the major cause of mortality and morbidity, leading directly or indirectly to enormous increases in the cost of hospital care to the emergence of new health hazards for the community. NIs occur worldwide and affect both developed and resource poor countries. It has been reported that in US NI appears in one in ten patients admitted and approx 2 million people annually (CDC, 1992). The estimated annual cost of those infections is in excess of $1 billion. Additionally 3% of NI may probably result directly in death of patient (Bennett *et al*., 1979). Similarly the increasing emergence of serious MDR infections has led to a new health-care crisis (Livermore, 2009). Overuse of antibiotics is continuing problem, 25 to 30 % of hospitalized patients receive systemic antibiotic therapy on any single day (Bennett *et al*., 1979).Such treatment are not only expensive but subjects patient to unnecessary risk. The pressure of excessive use of antibiotic may be shown by appearance of bacterial strains resistant to that antibiotic, which may result in specific therapeutic problems (Banjara, 2002).

In context of Nepal, it has been found that, about 10.5 % of patients admitted to acute care hospitals develop NIs (Tuladhar *et al*., 1990). In tertiary care hospital in Nepal, the overall point prevalence of NI is reported to be 2.4% (Lamichhane and Shrestha, 2001). In another study conducted among the isolates from environment and staff of Nepal Medical College it was found that of major gram negative isolates were resistant to amoxicillin followed by ciprofloxacin and nitrofurantoin. Similarly the occurrence of MDRO including ESBLs producers has been reported from the researches done in various hospitals in Nepal.

Nepal Police Hospital is a hospital providing health care facility to Police and their family since 2040 B.S. Hospital provides both long term and short term treatment to almost all types of cases. Despite of its long history of establishment and services no microbial investigation for surveillance of NIs and prevalence of MDRO has been conducted. So this study may be an evidence for the need of management of NI and control development of MDR strain.

CHAPTER II

Objectives

General objective:-

- To study the bacterial flora in Hospital's environment and clinical samples and correlate them to understand NI

Specific objectives:-

- To describe gram negative bacteria from various possible sources of NI
- To describe gram negative bacteria from clinical samples
- To correlate the above both finding to understand the source of infection and NI

Note: - Study site: Nepal Police Hospital

Chapter III

Literature review

3.1 Hospital and Hospital acquired infection:

The National Nosocomial Infection Surveillance System (NNIS) defines a NI as a localized or systemic condition that 1) results from adverse reaction to the presence of an infectious agent(s) or its toxin(s) and 2) was not present or incubating at the time of admission to the hospital (NNIS Manual, 1994). For most bacterial NIs, this means that the infection usually becomes evident in 48 hours (i.e., the typical incubation period) or more after admission. However, because the incubation period varies with the type of pathogen and to some extent with the patient's underlying condition, each infection must be assessed individually for evidence that links it to the hospitalization (Garner *et al*., 1988).

Hospital is an institution for health care, providing patient treatment by specialized staffs and equipments and often but not always providing for longer-term patient care. Hospitalized patients are at unusually high risk of infections. The hospital environment favors the acquisition of resistance to antimicrobial agents, complicating the treatment of NIs due to drug-resistant pathogens (WHO, 2002). Moreover NI not only encloses the disease of the patients but also the infection among the staff of the facility or anyone else who has contact with hospital, including volunteers, visitors, workmen, and delivery personnel (Benenson, 1995).

All the infections in hospitals are not considered to be NI. Only the information that follows the following criteria comprises the defination of NIs as according to NNIS Manual (1994). There are two special situations in which an infection is considered nosocomial: (a) infection that is acquired in the hospital but does not become evidence until after hospital

discharge and (b) infection in neonate that results from passage through the birth canal. Similarly there are two special situations in which an infection is not considered nosocomial: (a) infection that is associated with a complication or extension of infection already present on admission, unless a change in pathogen or symptoms strongly suggests the acquisition of a new infection, and (b)in an infant ,an infection that is known or proved to have been acquired transplacentally and becomes evident at or before 48 hours after birth.

3.1.1 Contributing agents for NI

There are several factors that contribute to the occurrence of NI; these factors may act as both source as well as route of transmission of infection.

Animate agent:-

Health givers: -

Health service providers who are directly or indirectly involve in patient care are at risk for spread as well as acquiring of NI. Improper hand washing contaminated fabrics are medium of spread. They involve in direct and indirect route of transmission of infections among patient group.

Patients:-

Patient in hospital are either immunocompromised or undergoing antibiotic therapy so are vulnerable to pathogenic microbial bugs hovering the hospital setting. Furthermore endogenous normal flora of patient are also equally capable of causing opportunistic infection favored by the state of patient and unhygienic status of patient care.

Others:-

This includes all those visitors whom are also the most potent source of bringing deadly pathogens to the hospital environment from outer environment. In hospital environment these organisms get suitable host to proliferate.

Inanimate agent:-

Hospital objects:-

During stay in hospital patient are in constant contact with various equipments , fabrics and other settings kept for patient's facility but due to poor maintenance these may act as a habitat of microbes which when acquired by suitable host acquires NI.

Hospital air:

Air is omnipresent and it supports the spread of pathogens by transferring droplets trapped pathogens bring the deadly consequences like nosocomial pneumonia, spread of tuberculosis.

Hospital water and food:

Food and water in hospital that are made for fast recovery of patients may be the source of infection if proper sanitation and purification process are not followed. Both food and water are essential factor not for only humans but also for pathogens so these act as best transport medium of pathogens to host.

Treatment procedure: -

Use of indwelling apparatus and excess antibiotic therapy more than needed along with above factors are responsible for supporting the NI.

3.1.2 Diseases possible of nosocomial origin (WHO 2002)**.**

a) Urinary tract infection
b) Surgical site infection
c) Respiratory tract infection
d) Septicemia
e) Skin and soft tissue infection
f) Gastroenteritis
g) Sinusitis and other enteric infection
h) Infections of eye and conjunctiva
i) Endometritis and other infection of reproductive organs following child birth

3.1.3 Some common etiological agents of NI (WHO, 2002)

Bacteria:-	**Parasites :-**	**Fungi :-**
Cogulase negative Staphylococcus	*Giardia lamblia*	*Candida albicans*
Staphlyococcus aureus	**Viruses**	*Aspergillus*
Escherichia coli	Respiratory syncytial virus	*Cryptococococcus*
Klebsiella spp.	Rota virus	*neofromans*
*Pseudomonas*spp.	Entero virus	
Proteus spp.	Influenza virus	
Legionella spp.		
Enterobacter spp.		
Citrobacter spp.		
Serratia marcesens		

3.1.4 Historical and Epidemiological aspects

NI has been established as threat since ancient times. Consequent mortality due to NI reached its peak in the 19th century (Smith and Easman, 1990). Urban overpopulation and hospital overcrowding made hospitals places of dread for the poor, with uncontrolled puerperal sepsis, surgical sepsis causing death in most cases of compound fracture admitted to hospital and cross-infection in children's hospitals increasing the mortality rate.

The work of Semmelweis (1861) on puerperal sepsis, drastically deduced the spread of the disease by the use of hand disinfection (by means of hand washing with chlorinated lime solution) in obstetrical clinics. Florence Nightingale established important principles of nursing and hospital design and hygiene, after experiencing sepsis at army medical centre. About same time the introduction of aseptic surgery by Lister (1867) and its replacement by Bergman's 'asepsis' with the introduction of gloves made contribution in infection control.

The victory over NI is however yet to be achieved. A prevalence survey conducted under the auspices of WHO in 55 hospitals of 14 countries representing 4 WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) showed an average of 8.7% of hospital patients had NIs. At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital (Tikhomirov, 1987).

The highest frequencies of NIs were reported from hospitals in the Eastern Mediterranean and South-East Asia Regions (11.8 and 10.0% respectively), with a prevalence of 7.7 and 9.0% respectively in the European and Western Pacific Regions (White *et al*., 1988).

Review of data done from 1992-1995 at the rural government hospital in West Indies showed over 7,158 NIs from 75,532 patients (10.0/100) admissions). High NI rates

were found on the ICU (67/100 admissions), urolology (30/100 admissions). The cost to the government was estimated to US$ 697,000 annually (Orrett *et al*., 1998).

The most frequent NIs is infections of surgical wounds, urinary tract infections and lower respiratory tract infections. The WHO study and other, has also shown that the highest prevalence of NIs occurs in ICUs and in acute surgical and orthopedic wards. Infection rates are higher among patients with increased susceptibility because of old age, underlying disease, or chemotherapy (WHO, 2000).

National Audit Office, England (2000) reported that at any time 9 % of hospital inpatients have NI. The efforts to lower infection risk has been continually challenges by growing number of immunocompromised patients, antibiotic resistant bacteria, super infection, invasive devices and procedures (Braunwald *et al*., 2001). Even after the adjustment of various factors patient with hospital acquired infections were 7.1 times more likely to die in hospital than other infected patients (Plowman *et al*., 2000).

A retrospective study on 12,418 inpatients was undertaken from January to December in Beijing Hospital (2000). 378 cases of NI were identified with rate of 3.04 % (Wang *et al*., 2001).

The point prevalence rate of NI in 42 hospitals involving 18,456 patients across Thailand (2001) was 6.4%. The prevalence was higher in male than female patients (7.8% vs 5.0%). The prevalence rates of over 10.0% were found in 4 hospitals. The infection rate was highest in surgical followed in rank by medical, pediatric and orthopedic departments (9.1%, 7.6% and 5.8%) respectively. In 2006 again study was done in 20 hospitals: three universities, five regional, five provincial, and seven other hospitals 9,865 patients were included Male and female patients were almost equal in number with an average age of 42.7 years. The NI proportion was 7.0% in male and 5.9% in female patients. The

prevalence rate of NI was highest in university and other hospitals (7.6%) followed by provincial (6.0%), and regional hospital (4.9%) (Danchaivijitrmd *et al*., 2006).

During the 6-year study by International Infection Control Consortium (INICC) infection from January 2003 through December 2008 in 173 intensive care units (ICUs) in Latin America, Asia, Africa, and Europe , using Centers for Disease Control and Prevention (CDC) US National Healthcare Safety Network (NHSN; formerly the NNIS system) definitions for device-associated health care-associated infection, prospective data was collected from 155,358 patients hospitalized in the consortium's hospital ICUs for an aggregate of 923,624 days, it was found that though the device utilization in the developing countries' ICUs was remarkably similar to that reported from US ICUs in the CDC's NHSN, rates of device-associated NI were markedly higher in the ICUs of the developing hospitals (Rosenthal *et al*.,2008).

In Nepal not much data to determine the prevalence of NI has been available however few studies have been conducted to analyze microbial load in environment has been done. In study done at Tribhuvan University Teaching Hospital it was found that the rate of occurrence of NIs was 10.5% (Tuladhar *et al*., 1990). In another study performed at the same hospital, the overall point prevalence of NI has been reported to be 2.4% (Lamichhane and Shrestha, 2001). Again in another random bacteriological study conducted in same hospital, to assess the level of medically important organisms prevailing in hospital it was found that the predominant organisms in the order of frequency were *S. aureus*, *Micrococcoccus* spp., CoNs other gram negative bacteria. About 23.5% of the *S .aureus* isolates were reported to be methicillin resistant strains (Pokharel *et al*., 1993). In study done in TUTH various bacterial isolates from air were *S. aureus*, CoNs, *Bacillus* spp., *Micrococcus* spp., *P. aeruginosa* and *K. pneumonia* (Banjara, 2002). Another research carried to analyze the microbial flora of hospital environment and visitors in TUTH it was found that the predominant organisms in ICU environment were *S. aureus* , *Micrococcus*

spp., *Bacillus* spp., *Serratia* spp. (Sharma, 2005). Similarly in the study carried out in Nepal Medical College the organisms isolated include: *S. aureus*, CoNs, *Streptococcus* spp., *E. coli*, *Klebsiella* spp., *Citrobacter* spp., *P. aeruginosa* (Pant *et al*., 2006). Analysis of the association in hospital environment and occurrence of infection among ICU patients done in Neuro Hospital showed that, occurrence of *P. aeruginosa , K. pneumonia, E. coli ,Acinetobacter* spp. and *S. aureus* in the ICU environment (Karki, 2010).

3.1.5 Prevention of NI:-

According to the practical guide by WHO (2002) the prevention of NI requires an integrated, monitored program which includes following key components:-

a. limiting transmission of organisms between patients in direct patient care through adequate hand washing and glove use, appropriate aseptic practice , isolation strategies, sterilization and disinfection practices
b. controlling environmental risks for infection
c. protecting patients with appropriate use of prophylactic antimicrobials, nutrition, and vaccinations
d. limiting the risk of endogenous infections by minimizing invasive procedures , and promoting optimal antimicrobial use
e. surveillance of infections, identifying and controlling outbreak
f. prevention of infection in staff members
g. enhancing staff patient care practices, and continuing staff education

Strategies for prevention of NI includes:-

1. Risk stratification
2. Reducing person-person contact
3. Preventing transmission from the environment

1. Risk stratification: -

Acquisition of NI is determined by both patient factors, such as degree of immunocompromise, and interventions performed which increase risk. The level of patient care practice may differ for patient groups at different risk of acquisition of infection. A risk assessment will be helpful to categorize patients and plan infection control interventions.

2. Reducing person-person contact: -

Person to person contact is an important route of transmission of infection. Contact may be direct or indirect. Direct contact involves a direct body, surface contact, surface-to-body surface contact and physical transfer of microorganisms between a susceptible host and an infected or colonized person. Indirect contact involves contact of susceptible host with the contaminated hands and fabrics of the health givers. The approach to prevent the spread of NI from person-person contact involves:-

i. Hand decontamination
ii. Personnel hygiene of health givers
iii. Preventing spread from health care worker's spread
iv. Use of gloves and masks

3. Preventing transmission from the environment: -

Inanimate surfaces, medical equipments, fabrics even hospital air and water have been often described as source of infection. In hospitals, surfaces with hand contact are often contaminated with nosocomial pathogens (Bures*et al*., 2000, Boyce *et al*., 1997 and Catalano *et al*., 1996) and may serve as vectors for cross transmission. The most common nosocomial pathogen may well survive or persist on inanimate surfaces and equipments for

months. Thus too minimize the transmission of microorganisms from equipment and the environment, adequate methods for cleaning, disinfecting and sterilizing must be in place. Written policies and procedures which are updated on a regular basis must be developed for each facility.

3.2 Antibiotics and development of resistance

Antibiotics are the chemotherapeutic agents that are either produced by microorganism or similar substances produced wholly or partly by chemical synthesis, which in low concentration inhibits the growth of other microbes. These agents show the characteristic property of the selective toxicity towards microbial cells with varying range of effectiveness, effect and accordingly the varying mechanism of its actions (Stephen *et al*., 2005)

3.2.1 Sources of antibiotics: (Stephen *et al*., 2005)

1. Microorganisms:-

a. Bacteria: e.g, - *Bacillus polymyxia* produces polymyxin B. *B. subtilis* and *B. liecheniformis* produce bacitracin.

b. Fungi: e.g.:- *Penicillium* produces penicillin and *Cephalosporin* produces cephalosporin.

c. Actinomycetes e.g.:- streptomycin and tetracycline are obtained from *Streptomyces* species and gentamicin is obtained from *Micromomosporapurpurea.*

2. Synthetic: Produced by synthetic process.e.g.:- sulphonamides, co-trimoxazole

3. Semi synthetic: - Molecules are partly formed by fermentation process and product is further modified by a chemical process. e.g.:- benzyl penicillin.

3.2.2 Classification of antibiotics:

Antibiotics can be classified on several basis:-

a. Based on spectrum of activity :

i. Broad spectrum antibiotics: - these antibiotics have wide range of activity against gram positive and gram negative organisms. e.g.:- tetracycline, aminoglycosides, sulnonamaides ,chloramphenicol etc.
ii. Narrow spectrum antibiotics: - these antibiotics have narrow spectrum activity. e.g.:-cloxacillin affects only gram positive cocci.

b. Based on the antimicrobial action :

i. Bacteriostatic:-these antibiotics prevent the active multiplication of bacteria. e.g.:- chloramphenicol, tetracycline and erythromycin.
ii. Bactericidal:-these are those antibiotics which at usual dosages kill bacteria. e.g.:- penicillin, cephalosporin, polymixin and aminoglycosides. Some bacteriostatic antibiotics become bactericidal when used at higher concentrations. e.g.:- erythromycin and tetracycline.

c. Based on chemical nature :

i. ß- lactam antibiotic :- possesses ß lactam ring as an active microbial component. e.g. penicillin and cephalosporin.
ii. Rifampicin:-semisynthetic derivative of rifampicin B.
iii. Aminoglycosides:-possesses two amino sugars. e.g.:- streptomycin, gentamycin and kanamycin.
iv. Polypeptide: - protein in nature. e.g.:- bacitracin, polymixins

v. Tetracycline: - possesses nucleus of 4 cyclic rings.

vi. Macrolides: - possesses macrocyclic lactone ring with attached sugars. e.g.:- erythromycin , azithromycin

3.2.3 Mechanism of action of antibiotics (Forbes *et al*., 2007)

Depending upon the chemical constituents, different antibiotics act on the different site. The major mode of action of antibiotics involves:

I. Cell wall synthesis inhibition

Peptidoglycan is a vital component of cell wall of virtually all bacteria, though it is composed in different proportions in gram positive (50%), gram negative (10-20%) and mycobacterial (30%). In all cases the peptidoglycan plays a vital role, for maintaining the shape and mechanical strength of the bacterial cell. If it is damaged or synthesis is inhibited, cells become distorted and lysis occurs. Mammalian cells lack peptidoglycan, so antibiotics which interferes synthesis and assembly of peptidoglycan show excellent selective toxicity. Antimicrobials showing inhibition of peptidoglycan structure of cell wall are glycopeptides (vancomycin/ teicoplanin) and ß lactam antibiotics. These interferes the peptidoglycan synthesis by blocking the action of transpeptidases and transglycosidase enzymes.

In case of *Mycobaterium* the inhibition of cell wall synthesis is usually due to inhibition in mycolic acid and arabinogalactan biosynthesis. Arabinogalactan forms the mycolic acid layer of the *Mycobaterium* providing acid fastness. Isoniazide and ethambutanol have been long known as specific antimycobaterial agents (Stephen *et al*., 2005).

II. Protein synthesis inhibition

The process of protein synthesis involves ribosome, mRNA, a series of aminoacyl transfer RNA (tRNA) molecules and accessory protein factors involved in initiation elongation and termination. Binding of antibiotics to any of these components of protein synthesis disrupts the mechanism of synthesis hence blocking the microbial cell proliferation. Though the process is essentially same in both prokaryotic and eukaryotic cells, antibiotics provides selective toxicity due to difference in the sedimentation coefficient of the ribosomal subunit (prokaryotic:30s and 50s / eukaryotic: 40s and 60s) and selective action in some antibiotics is derived through active uptake by microbial cells but only limited penetration of mammalian cells(Stephen *et al*., 2005).

Some antibiotics that inhibit protein synthesis are:-

i. Aminoglycoisides:- (streptomycin, gentamycin, kanamycin, amikacin) freezing initiation complex and distortion of 30s subunit
ii. Tetracyclines:- binds to 16srRNA of 30s subunit
iii. Chloramphenicol:- binds to 50s subunit in the region of acceptor site of ribosome involving 23s rRNA
iv. Macrolides:- (erythromycin, azithromycin)binds to the 50s subunit and blocks translocation step
v. Fusidic acid:- binds to elongation factor after first round of translocation blocking further synthesis

III. Inhibition of chromosome function and replication

The mechanism of chromosome replication and function are essentially same in prokaryotes and eukaryotes. There is however important differences in detailed functioning and properties in the enzymes involved and these differences are exploited by a number of

agents as the basis of selective inhibition. The chromosome replication is inhibited at several stages by different antibiotics.

i. Fluroquinolones: - interferes unwinding of chromosome by selectively binding the topoisomeraseII, rendering enzyme incapable to reseal DNA thus chromosome becomes fragmented.
ii. Rifampicin: - binding to the RNA polymerase hence inhibiting transcription process.

IV. Disruption of cytoplasmic membrane

The integrity of cytoplasmic membrane is vital for the normal functioning of all the cells. Bacterial cell membranes do not contain sterols and in this respect differs from membranes of mammalian cells, thus providing selective site for action of antibiotics. The most common antibiotics that act upon cytoplasmic membrane are polypeptide antibiotics (tyrocidin, polymixin), these cause pore formation causing leakage of intracellular ions and precipitation of biomolecules.

V. Inhibition of specific enzyme system

This involves the inhibition of folate metabolism. Folic acid is an important co-factor in all living cells. In reduced form used as tetra hydrofolate (THF) to carry of single carbon fragments which is used in synthesis of adenine, guanine, thymine and methionine. Antibiotics like sulphonamides and trimethoprim and pyrimethamine inhibits folate metabolism by inhibiting the conversion of dihydrofolate (reduced form of THF) back to THF by dihydrofolatereductase enzyme (DHFR).

3.2.4 .Antibiotic resistance:-

Resistant to antibiotic resistant the attack and counter attack of complex microbial flora to establish ecological niche and survive. Successive bacterial resistance to antimicrobial resistance to antimicrobial action requires interruption or disturbance of one or more of the steps essential for effective antimicrobial action.

Development of resistant in antibiotics usually attributed to following (Stephen *et al*., 2005):

1) Inactivation of antibiotics

    a. Hydrolysis
    b. Group transfer
    c. Oxidation- reduction

2) Modification of target site
3) Efflux pump
4) Target bypass
5) Alternation of membrane permeability

    1) Inactivation of Antibiotics:-

Bacterial population shows significant counter attack to the activity of antimicrobial drugs, rendering antibiotics inactive. Inactivation of antibiotics usually occurs by:

a. Hydrolysis:-

Hydrolysis includes the cleavage of bonds by introduction of water molecules. Different bacterial enzymes are known to destroy antibiotics by targeting and cleaving the specific

bond in the antibiotics. These enzymes are extracellular enzymes and mostly production of such enzymes is due to substrate induction. e.g.:- ß- lactamses . These are the hydrolytic amylases which cleave the ß- lactam ring of ß-lactam antibiotics.

Especially gram negative bacteria and also some gram positive bacteria produce number of ß-lactamse enzymes. More than 20 different ß-lactamases are known. Different ß-lactamases group include:

- Extended Spectrum ß-lactamse (ESBLs)
- Metallo ß-lactamse (MBLs)
- Ampicillin ß-lactamse
- Other hydrolases includes: - esterase (cleaves ester bond of macrolides) epoxidases (cleaves epoxide bond of polymixins)

b. Group transfer:-

This phenomenon includes inactivation of antibiotics takes place by chemical substitution of different radical groups to the periphery of antibiotic molecules. Most commonly added groups are adenyl, phosphoryl, acetyl group. e.g.:- chloramphenicol acetyl transferase, transfers acetyl group to chloramphenicol rendering them inactive.

c. Oxidation reduction reactions:-

Antibiotics are oxidized or reduced by various enzymes produced by the bacteria disrupting their structure and making them incapable to inhibit growth. e.g. :- tetracycline is oxidized by tet-x enzyme produced by tet-x gene in some gram positive and negative bacteria such that it could not bind to ribosome and thus protein synthesis is not blocked i.e. no inhibition occurs.

2. Target modification:-

Each antibiotic have their specific site of action modifications in these sites blocks the action of antibiotics. E.g.:-amino acid sequence alternation of penicillin binding protein on cell surface prevents the binding of penicillin and preventing its action. Post translational modification of 23s RNA and 30s RNA confers resistant to macrolides and aminoglycosides.

3. Efflux pump:-

This includes the group of protein that exports out the antibiotics out of cell and keep low intracellular concentration of antibiotics. Bacterial multidrug efflux pumps are classified into five families based on their sequence similarity: major facilitator, resistance–nodulation–cell division (RND), small multidrug resistance, multidrug and toxic compound extrusion, and ATP-binding cassette (Paulsen *et al.,* 2001, Putman *et al*., 2000, Brown *et al.,* 1999). Of these, RND family efflux pumps play major roles in conferring both intrinsic and elevated resistance of gram negative bacteria to a wide range of compounds, including ß-lactams (Nishino *et al*., 2001 and Nikaido *et al*., 1996). E.g. nor mediated efflux system against fluroquinones and quinones, resistance to chloramphenicol resistance in gram negative is also efflux mediated.

4. Target by pass:-

Resistance acquired by bypassing the specific target enzyme, e.g. trimethoprim and sulphonamide resistance in bacteria is acquired by bypassing inhibition of DHFR and dihydropteroatesynthetase enzyme.

5. Alternation of membrane permeability

For each antibiotic to work it must come in contact with their respective sites. Especially drug that confers its action within cytoplasm is inhibited by the organism by

altering their membrane permeability. E.g.:- polymixin and other cationic antimicrobial peptides have a self promoted uptake across the envelope. Addition of 4-amino-deoxy-arabinose moiety to phosphate group on the lipid A component of gram negative lipopolysachharide has been implicated in resistance to polymyxin.

Besides above biochemical alternation in antibiotics or structural composition several genetic factors are also found to be responsible for development of resistance. Mutation, spontaneous or adaptive, horizontal gene transfer involving cassettes of mobile genes are some genetical aspects of development of drug resistance (Brooks *et al*., 2007).

3.2.5 Resistance to commonly used antibiotics:

Resistant to ß-lactam antibiotics:-

These are the antibiotics possessing ß-lactam ring. The two major groups are penicillins and cephalosporins. Monobactams and carbapenems are the newer addition (Tripathi, 2003). ß-lactam antibiotics interfere with synthesis of bacterial cell wall particularly acting on enzyme transpeptidases so that crosslinking is inhibited. The penicillin-binding-protein (PBPs) aids the binding of ß-lactam ring to cell membrane and inhibits its synthesis. Resistance against ß-lactam antibiotics is due to production of enzyme ß-lactamase enzyme and alternation of PBPs. This breaks up the ß-lactam ring or prevents the binding of antibiotics and thus inactivates antibiotics.

In the present scenario the production extended spectrum ß-lactamase has become the rising threat in the treatment using ß-lactam antibiotics.

As defined by NCCLS, ESBLs are the enzymes that mediate resistance to extended spectrum (third generation) cephalosporins (e.g. ceftazidime, cefotaxime and ceftriozone) and monobactams (e.g. cefotixitin and cefotetan) or carbapenems (e.g. meropenem or

imipenem). The production of ESBLs is the result of mutation of TEM-1, TEM-2 and SHV-1 plasmid genes.

The first ESBLs isolates were discovered in Western Europe in mid 1980s and subsequently in US in late 1980s(Natnisuwans*et al.,* 2001).

Resistance to macrolide antibiotics:-

The macrolide-lincosamide-streptogramin B class (MLS) of antibiotics contains structurally different but functionally similar drugs, that all bind to the 50S ribosomal subunit. It has been suggested that these compounds block the path by which nascent peptides exit the ribosome drug (Tenson *et al*., 2003). An example of mechanism that confers resistance to macrolide antibiotic includes the gene that specifies a ribosomal RNA methylase (ermC) confers resistance to the macrolide-lincosamide-streptogramin B group of antibiotics. Synthesis of the ermC gene product is induced by erythromycin, a macrolide antibiotic (Dabnau, 1984).

Resistant to Aminoglycosides

These are group of natural and semi synthetic polybasic amino groups linked glycosidically to two or more amino sugar residues. Common examples are: - gentamycin, tobramycin, amikacin, and kanamycin. They act by interfering protein synthesis. The action of aminoglycosides includes diffusion of aminoglycoside through the bacterial cell wall and cytoplasmic membrane and binding to ribosome resulting inhibition in protein synthesis (Stephen *et al*., 2005).The bacterial uptake of aminoglycoside antibiotics requires respiration, which generates an electrical potential across the cytoplasmic membrane. A low level of transmembrane potential or even its absence is responsible for the intrinsic resistance of anaerobic bacteria (Bryan *et al*., 1979) and decreased susceptibility of facultative anaerobes such as enterococci to amino glycosides. Chromosomal mutations in

S. aureus resulting transmembrane electrical potential have also been shown to produce amino glycoside resistance (Miller *et al*., 1980). Such mutations lower growth rate and allows bacteria to survive during amino glycoside therapy. Recently, various multidrug active efflux systems have been identified as mechanisms of natural resistance to amino glycoside antibiotics in gram-negative bacteria such as: - P. *aeruginosa*, A. *baumannii* and *E. coli*. Efflux pumps extrude the incoming antibiotic and produce low-level broad-spectrum resistance that often includes antibiotics of different classes (Sergei *et al.*, 2003).

Resistance to Tetracycline:

The tetracyclines, which were discovered in the 1940s, are a family of antibiotics that inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site. Tetracycline is broad-spectrum agent, exhibiting activity against a wide range of gram-positive and gram-negative bacteria, atypical organisms such as Chlamydiae, Mycoplasmas, and Rickettsiae, and protozoan parasites. The favorable antimicrobial properties of these agents and the absence of major adverse side effects have led to their extensive use in the therapy of human and animal infections (Chopra *et al*., 2001).

The genetic heterogeneity of tetracycline resistance determinants in plasmids from members of the Enterobacteriaceae and Pseudomonadaceae and categorization of the tetracycline-resistant (Tc^r) plasmids was done by Mendez *et al*. (1980). The efflux proteins are best studied of the tet- proteins. The genes encoding them belong to the major facilitator super family (MFS), whose products include over 300 individual proteins. All the tet efflux genes code for membrane-associated proteins which export tetracycline from the cell. Export of tetracycline reduces the intracellular drug concentration and thus protects the ribosomes within. The efflux genes are found in both gram-positive and gram-negative species and confer resistance to tetracycline (Tukman *et al*., 2001).

3.3 Multi Drug Resistant Organisms (MDRO)

Organisms which confer resistance to two or more antibiotics are referred as MDRO. The development of MDRO is usually attributed to the multidrug efflux pump, conjugative R-plasmids, integrons (Stephen *et al*., 2005).

3.3.1 Gram negative MDRO:-

Gram negative organisms are the prokaryotes whose cell wall contains relatively little peptidoglycan but has as outer membrane composed of lipopolysaccharide, lipoprotein and other complex macromolecules (Madigan *et al*., 2000).

The most common gram negative MDRO in hospital environment include the members of family Enterobacteriaceae and non fermenters like *Acinetobacter* spp., *Pseudomonas* spp. Besides these some fastidious bacteria like *Legionella* spp., *Listerichia* spp. is also responsible for causing NIs (WHO, 2002 and Livermore, 2009).

Enterobacteriaceae

Enterobacteriaceae is family comprising large heterogeneous groups of medically important aerobic or facultative anaerobic gram negative enteric bacilli (Forbes *et al*., 2007).These are normal commensal flora of intestinal tract of man and animals, though few of the species in small proportion may occur as normal flora in other body parts such as upper respiratory and genital tracts. The family contains many genera, however the most common pathogenic genus of nosocomial importance includes: *E. coli. K. pneumoniae, K. oxtoca, S. marcesens, S. enteric* serovar Typhi, *S. enteric* serovar Paratyphi, *Proteus* spp., *E. cloacae, C. fruendii* etc.

E. coli is most frequently encountered Enterobacteriaceae in the urinary tract infection, intra abdominal and gut associated infection, wound infection and bacterimea. But the infection is always endogenous and occurs infrequently, and even the drug resistant strains occasionally spread among the hospitalized patients (Hart 1982 and Jarvis *et al*., 1992). Development of MDRO is often associated with multidrug efflux pumps that decrease drug accumulation in the *E. coli* (Nishino *et al*., 2010).

Klebsiella spp. is another important member of Enterobacteriaceae family that causes severe NIs. Most prevalent species include *K. pneumonia* and *K. oxytoca* which are responsible for causing UTI, bacterimia and sometime pneumonia in extremely immunocompromised patients. *K. pneumonia* is also emerging as blood stream pathogen. In US and Canada, *K. pneumonia* is among the top ten pathogens causing blood stream infection (Pfaller *et al*., 1998). In US, drug resistant *K. pneumonia* was found responsible for outbreaks of NIs (Woodford *et al*., 2004).

Salmonella spp. is also an important Enterobacteriaceae that has shown the multidrug resistance development. Development of MDR in *S. enterica* strains is attributed to conjugative DNA. The exchanges between plasmid(s) and the bacterial chromosome and the integration of resistance genes into specialized genetic elements, called integrons, play a major role in acquisition and dissemination of resistance genes (Alessandra, 2003). Similarly species of *Serratia* especially *S. marcescens* is also well known pathogenic organism of hospital setting that causes extra intestinal infections such as LRT and UTI among critically ill patients (Smith and Easmon, 1990).

Other genus like *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp. have been continuously reported causing health care associated infections.

Pseudomonas aeruginosa

P. aeruginosa is an aerobic gram negative bacterium ubiquitous in its distribution. It is the commonest cause of most NIs. This non fermentative MDR bacterium can survive for long periods on equipments around patients therefore is responsible for causing many device associated infections in the hospitalized patients. Expression of adhesions, production of biofilms, secretion of hydrolytic enzymes and production of toxins are the major virulence factor of *P. aeruginosa* that aid in its pathogenecity. Cross infection does not occur but endogenous infection is probably more common. Due to application of broad spectrum antibiotics has rendered selection of resistant strain. Isolation of *P. aeruginosa* from healthy carrier or environment is significantly only when there is risk of transfer to immunocompromised patients. The results showed that antimicrobial resistance among *P. aeruginosa* is gradually increasing for most anti-pseudomonal agents, particularly aztreonam, ceftazidime, piperacillin/tazobactam and imipenem. There were 19 (3%) and 12 (2%) MDR *P. aeruginosa* patients in 2004 and 2005 respectively, and was more commonly found in non-intensive care unit (ICU) patients. Most MDR isolates were from surgical and diabetic patients. The mortality rate was higher among ICU patients (Babay, 2005). Anaisssaie *et al*.,(2002) estimated that 1400deaths per year occurs in US hospitals due to water borne health care associated pneumonias caused by pseudomonas alone.

***Acinetobacter* spp**.

Acinetobacters are ubiquitous, free living saprophytes found in soil, water, foods and the clinical environment. They can be found on a range of dry or moist inanimate surfaces and as commensals on the skin of man and animal, it has been suggested that at least 25% of normal individuals carry *Acinetobacter* spp. on their skin. It have also been found occasionally in the oral cavity and respiratory tract of healthy adults but the carriage rate of *Acinetobacter* in nonhospitalized patients apart from on the skin is normally low. In recent

years, antibiotic resistant strains have also been recognized as an important pathogens involved in outbreaks of hospital acquired infection, particularly in high- dependency cases or ICUs. However the true frequency of NI caused by *Acinetobacter* spp. is not easy to assess, partly because the isolation of these organisms from clinical specimens may not necessarily reflect infection but, rather, may result from colonization (Bergogne and Towner, 1996). Members of genus *Acinetobacter* have been isolated from wide range of clinical specimens, including tracheal aspirates, blood cultures, CSF and pus. A relative lack of virulence factors limits their pathogenicity to patients that are immunocompromised especially those undergoing intensive care therapy. Because of the escalating trend in antibiotic resistance, the organism is posing challenge for the treating physician. *Acinetobacter* appears to have a propensity to develop antibiotic resistance extremely rapidly, perhaps as a consequence of its long-term evolutionary exposure to antibiotic-producing organisms in a soil environment. This is in contrast to more ‘‘traditional’’ clinical bacteria, which seem to require more time to acquire highly effective resistance mechanisms in response to the introduction of modern radical therapeutic strategies; indeed, it may be their ability to respond rapidly to challenge with antibiotics, coupled with widespread use of antibiotics in the hospital environment, that is responsible for the recent success of *Acinetobacter* spp. as nosocomial pathogens. Several mechanisms are responsible for ß-lactam resistance in *A. baumannii*, including: (i) the production of ß-lactamases; (ii) alterations of porin-like proteins, resulting in decreased permeability to antibiotics; and (iii) the activity of efflux pumps, decreasing the intracellular concentration of antibiotics (Silvia *et al*., 2009).

3.3.2 Epidemiological aspects of gram negative MDRO

During the last several decades, the prevalence of MDRO in hospitals and medical centers has increased steadily. Gram negative bacteria resistant to third generation

cephalosporin fluoroquinolones, carbapenems, and aminoglycosides also have increased in prevalence (Siegel *et al*., 2006).

In 1997, The Senetery Antimicrobial Surveillance Program found that among *K. pneumoniae* strains isolated in the US, resistance rates to ceftazidime and other third-generation cephalosporin were 6.6%, 9.7%, 5.4%, and 3.6% for bloodstream, pneumonia, wound and urinary tract infections respectively.

A 3-month survey of 15 Brooklyn hospitals in 1999 showed that 53% *of A. baumannii* strains were resistance to carbapenems and 24% of *P. aeruginosa* strains were resistant to imipenem (Landman *et al*., 2002).

In 2003, 20.6% of all *K. pneumoniae* isolates from NNIS ICUs were resistant to these drugs (NNIS, 2003). Similarly, between 1999 and 2003, *P. aeruginosa* resistance to fluoroquinolone antibiotics increased from 23% to 29.5% in NNIS ICUs (Fridkin, 2001).

During 1994-2000, a national review in 43 US found that the overall susceptibility to ciprofloxacin decreased from 86% to 76% and was temporally associated with increased use of fluoroquinolones in the US (Neuhauser *et al*., 2003).

The resistant type of *E. coli* is ESBLs producing strains has been shown in the study conducted in Taiwan in, 2004. Extended spectrum β-lactamases (ESBLs) were detected in 33 (94.3%) isolates among the 35 isolates from the clinical sample from National Cheng Kung University Hospital (NCKUH) (Yan *et al*., 2004). Similarly the occurrence of ESBLs producing *E. coli* has been reported from New Dehli in GB Pant hospital where of the of 2,870 blood samples of suspected cases of septicemia between January and December 2009 Forty-one (70.7%) *K. pneumoniae* isolates and ten (41.7%) *E. coli* isolates were ESBLs (Taneja *et al.*, 2009). In the study conducted in US 95% of the *K. pneumoniae* isolates showed resistance or decreased susceptibility to at least one of the three third generation

cephalosporin [3GC (ceftazidime, cefotaxime, ceftriaxone)] used for the study (Woodford *et al*., 2004). 87% of the *K. pneumoniae* isolates showed resistance to all the three third generation cephalosporin antibiotics and this resistance to all the three 3GC was found to coexist with resistance to other antibiotics (Subha and Ananthan, 2002).

Asia is regarded as one of the epicentres of antimicrobial drug resistance, there is an alarming number of antibiotic-resistant species, including MDR *A. baumannii*, extended-spectrum β-lactamase (ESBLs)-producing *K. pneumoniae* (particularly mediated by CTX-M-9, CTX-M-14 and CTX-M-15), New Delhi metallo-β-lactamase 1 (NDM-1) producing Enterobacteriaceae, MDR *S. enterica* serotypes Choleraesuis and Typhi, carbapenem-resistant *A. baumannii* (OXA-58 and OXA-23 carbapenemases (Yong *et al*., 2009). A few clones of MDR *A. baumannii* have been widely disseminated in hospital settings in Asia, and *K. pneumonia* carbapenemase (KPC)-producing *K. pneumoniae* strains have been widely distributed in China. Continuous surveillance of resistance data from clinical isolates as well as implementation of strict infection control policies in healthcare settings are required to mitigate the progression. Marked variations in the resistance profiles of bacterial pathogens as well as the quality of public hygiene have had a considerable impact on the effectiveness of antimicrobial agents in Asian countries(Ma *et al*., 1994, Nikaido *et al*., 1996, Zgurskaya *et al*., 2000)

In the current scenario development of organisms producing metallo-β-lactamase is the challenge for the microbiologist. Designated as NDM-1, it is the carbapenemases found in Enterobacteriaceae isolated from patients in the United Kingdom and elsewhere who have had healthcare contact in India or Pakistan (Yong *et al*., 2008 and Health protection report, 2009). NDM-1 was first detected in a *K. pneumoniae* isolate from a Swedish patient of Indian origin in 2008. The gene coding for this unique enzyme *blaNDM-1* was found in one of the three resistance-carrying regions of an integron. NDM-1 shares very little identity with other MBLs. As well as possessing unique residues near the active site,

NDM-1 also has an additional insert between positions 162 and 166, which is not present in other MBLs. NDM-1 have been isolated from *K. pneumoniae*, *E. coli*, *C. freundii*, *E. cloacae,* and *Morganella morganii*. Other classes of carbapenemases have already been found in *K. pneumoniae, E. cloacae, Pseudomonas* spp. (Krishna, 2010).

In Asian countries like India, Pakistan the emergence carbapenem resistant Enterobacteriaceae NDM-1 is potentially a major global health problem. The prevalence of NDM-1, in multidrug-resistant Enterobacteriaceae was studied in India, Pakistan, and the UK. 44 isolates with NDM-1 was identified in Chennai, 26 in Haryana. The occurrence of same strain has also been found in 37 in the UK and 73 in other sites in India and Pakistan. NDM-1 was mostly found among *E. coli* and *K. pneumoniae*, which were highly resistant to all antibiotics except to tigecycline and colistin. *K. pneumoniae* isolates from Haryana were found to be clonal but NDM-1 producers from the UK and Chennai were clonally diverse. Most isolates carried the NDM-1 gene on plasmids. Those plasmids from isolates obtained from UK and Chennai were readily transferable whereas those from Haryana were not conjugative. Many of the UK NDM-1 positive patients had travelled to India or Pakistan within the past year, or had links with these countries (Kumarasamy *et al*., 2010).

In Nepal the occurrence of MDRO among gram negative has been studied in different hospitals. Study done in B.P.Koirala Institute of Health and Sciences among *Acinetobacter* spp. isolated from various clinical samples 99.2% were resistant to cefotaxime, 98.4% were resistant to ceftazidime, 95.9% were resistant to tobramycin, 96.7% were resistant to ciprofloxacin among 123 resistant strains (Ghimire *et al.,* 2002).

In the another study done among the respiratory pathogens showed that more that 50% of gram negative isolates were resistant to ciprofloxacin, gentamicin, ampicillin and cephalexin (Shrestha *et al.*, 2005).Similarly on analyzing MSU samples 27.5% isolates of *E.*

coli and *K. oxytoca* among 371 samples were confirmed for production of ESBLs (Manandhar *et al*., 2005).

In a study done at Kathmandu Model Hospital, it was found that the predominant bacteria causing UTI were the gram negative isolates constituting 88.2% among them 67.9% were MDR strains whereas gram positive bacteria constituted only 11.8% out of which 38.9% were MDR strains (Shrestha *et al.*, 2005).

In the research done in TUTH total of 541 isolates of *S. enterica* serotypes Typhi (47%) and Paratyphi A (53%) were grown. Twenty-eight isolates (5%) of *S. enterica* were resistant to two or more antibiotics (MDR isolates), with a greater prevalence among serotype Paratyphi A (7%). All ESBLs producers (three isolates) were serotype Paratyphi A. Most of the MDR *S. enterica* showed reduced susceptibility to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, ofloxacin, and ciprofloxacin, and had good susceptibility to extended-spectrum cephalosporins and carbapenems. Among the fluoroquinolones, gatifloxacin demonstrated better in vitro activity compared to levofloxacin, ciprofloxacin, and ofloxacin (Dahal *et al*., 2005)

Regarding *P. aeruginosa* one of the study on MDR/ESBLs producing strains among respiratory pathogens more than 50% of total isolates of *P. aeruginosa* were resistant to ciprofloxacin, gentamycin, ceftazidime and ceftriaxone (Pokharel *et al.*,2006).

3.3.3 Prevention of MDRO:-

The spread of alarmingly increasing MDRO such as MRSA, VRE, ESBLs producing organism, carbepenem resistant *E. coli, Klebsiella* spp., *P. aeruginosa* etc., need to counter act. The only strategy that seems feasible is the implementation of an effective and integrated program that involves antimicrobial resistance surveillance, a rational antimicrobial use program and infection control approaches. According to Goldmann *et al*.

(1996), the strategies to prevent and control the emergence and spread of antimicrobial resistant microorganisms may be grouped into those aimed at optimizing antimicrobial use and those preventing the transmission of resistant organisms.

Preventing infections will reduce the burden of MDRO in healthcare settings. Prevention of antimicrobial resistance depends on appropriate clinical practices that should be incorporated into all routine patient care. These include optimal management of vascular and urinary catheters, prevention of lower respiratory tract infection in intubated patients, accurate diagnosis of infectious etiologies, and judicious antimicrobial selection and utilization (Seigel *et al*., 2006).

Control Interventions:

According to the Healthcare Infection Control Practices Advisory Committee (CDC, 2006) the various types of interventions used to control or eradicate MDRO. These interventions provide the basis for the recommendations for control of MDRO in healthcare settings. In the studies reviewed, these interventions were applied in various combinations and degrees of intensity, with differences in outcome.

1. Administrative support:

In several reports, administrative support and involvement were important for the successful control of the target MDRO and authorities in infection control have strongly recommended such support. There are several examples of MDRO control interventions that require administrative commitment of fiscal and human resources, that include: implementing system changes to ensure prompt and effective communications e.g. computer alerts to identify patients previously known to be colonized/infected with MDRO, providing the necessary number and appropriate placement of hand washing sinks and alcohol-containing hand rub dispensers in the facility maintaining staffing levels

appropriate to the intensity of care required enforcing adherence to recommended infection control practices (e.g. hand hygiene and Contact Precautions) for MDRO control.

2. Education:

Facility-wise, unit-targeted, and informal, educational interventions were included in several successful studies. The focus of the interventions was to encourage a behavior change through improved understanding of the problem MDRO that the facility was trying to control. Educational campaigns such as to enhance adherence to hand hygiene practices in conjunction with other control measures have been associated temporally with decreases in MDRO transmission in various healthcare settings.

3. Judicious use of antimicrobial agents:

This includes the effort that encourage healthcare settings to focus on effective antimicrobial treatment of infections, use of narrow spectrum agents, avoiding excessive duration of therapy and restricting use of broad-spectrum or more potent antimicrobials to treatment of serious infections when the pathogen is not known or when other effective agents are unavailable. Achieving these objectives would likely diminish the selective pressure that favors proliferation of MDRO.

4. MDRO surveillance:

Surveillance is a critically important component of any MDRO control program for detection of newly emerging pathogens, monitoring epidemiologic trends, and measuring the effectiveness of interventions. MDRO surveillance may be simple routine antibiogram that provide the information about MDRO incidence in routine clinical culture or based on detection of asymptomatic colonization; an approach is based upon the observation that, for some MDRO, detection of colonization may be delayed or missed completely.

5. Environmental measures:

The potential role of environmental reservoirs, such as surfaces and medical equipment, in the transmission and other MDRO has been the subject of several reports. While environmental cultures are not routinely recommended, environmental cultures were used in several studies to document contamination and led to interventions that included the use of dedicated noncritical medical equipment, assignment of dedicated cleaning personnel to the affected patient care unit, and increased cleaning and disinfection of frequently-touched surfaces (e.g. bedrails, charts, bedside commodes, doorknobs). Therefore, monitoring for adherence to recommended environmental cleaning practices is an important determinant for success in controlling transmission of MDRO and other pathogens in the environment.

CHAPTER IV

Materials and Methods

4.1 Materials

List of materials used during study are as in appendix I

4.2 Methodology

Study design: - Cross sectional descriptive type of study was done to determine the antibiotic sensitivity pattern of organisms from environment and clinical samples of Nepal Police Hospital.

Collaborating institutions:-

i. Nepal Police Hospital
ii. Shi-Gan Health Foundation
iii. Central Department of Microbiology

Sampling frame: -

i. Environmental sample: - inanimate objects, air and staff in direct contact with patients in different wards of Nepal Police Hospital.
ii. Clinical sample: -All clinical samples came for microbiological analysis in Microbiology Laboratory of Nepal Police Hospital.

Sample size:-

i. Environmental sample: -215 swab samples and 54 plate exposure were taken
ii. Clinical sample: - total of 1100 clinical sample were considered

4.3 Laboratory methodology

4.3.1 Collection of sample

The present work includes the samples such as air, inanimate objects present in immediate contact of patients (bed sheet, table, utensils, oxygen flow meter, thermometer) and hand swabs of health professionals, as well as other staff direct influencing patient's health. Further for determining the susceptibility among clinical sample, isolates from clinical sample was taken.

4.3.1.1 Air samples

54 air samples were collected using gravity settlement method whereby the culture plates, including nutrient agar were exposed to air of the wards for fixed period of time. The plates were left open with lid by its side at 4-6 feet height from the floor for a time period of 30 minutes in sterilized ward and 5-10 minutes in unsterilized wards. The plates were transported to the working laboratory in sealed condition (sealed with parafilm) in an ice box. The plates were then incubated at $37^{O}C$ for 24 hours. Utmost care was taken to prevent any flies or insects from entering and contaminating plate.

4.3.1.2 Surface samples

134 surface samples were collected from different wards. The surface samples include samples from table, bed sheet and bed bar, patient's utensils, mouth of water container, thermometer and equipments in nursing station of each ward.

Surface samples were taken using sterile cotton swabs dipped in BHI. For the purpose of quality control each batch of swab was tested for sterility by streaking on culture medium and incubated at $37^{O}C$ for observing colonies if growth occurs. For proper collection of

samples it was made sure that the swab was rotated during procedure. Collected swab sample was sealed in tube and transported to laboratory in sealed condition in an ice box.

4.3.1.3 Hand swab samples:-

The entire area of palm and between fingers was rubbed with sterile swab, dipped in BHI broth and samples were transported to laboratory as soon as possible in ice box.

4.3.1.4 Clinical isolates:-

Isolates from all clinical samples that had been processed in microbiology laboratory of Nepal Police Hospital were subcultured in Nutrient agar plates, sealed with parafilm and transported to laboratory in an ice box.

4.3.2 Processing of Samples:

All the samples were transported in sealed condition in an ice box to Shi-Gan health foundation where further processing were done.

4.3.2.1 Processing of air samples:-

i. Air samples were collected and sealed with parafilm in the site
ii. Samples were transported to laboratory, seal was removed
iii. Plates were incubated at $37^{O}C$ for 24 hours
iv. Enumeration and observation was done
v. Isolates were differentiated based on their morphology incase of large number of colonies
vi. Isolated colony was picked and gram staining was performed
vii. Gram negative isolate was subcultured on nutrient agar plates

viii. Identification of isolates done based on the morphological and biochemical characters

ix. Antibiotic susceptibility test was performed

4.3.2.2 Processing of surface swab samples :-

i. Swab sample from the inanimate was taken in BHI soaked swab

ii. Swab in sterilized tube and transported in icebox

iii. Swabs were then inoculated into BA/MA plates

iv. Plates were then incubated at 37^{O}C for 24hours

v. After 24 hours observation was done for isolated colony

vi. Isolated colony was picked and gram staining was performed

vii. Gram negative isolate was subcultured on nutrient agar plates

viii. Identification of isolates done based on the morphological and biochemical characters

ix. Antibiotic susceptibility test was performed

4.3.2.3 Processing of hand swab samples:-

Similar as surface swab sample

4.3.2.4 Processing of clinical isolates:-

i. Isolates from clinical samples were subcultured in nutrient agar plate and transported to laboratory

ii. Plates were incubated at 37^{O}C for 24 hours

iii. Colonies were screened for gram negative isolates

iv. Identification of gram negative isolates were done based on the morphological and biochemical characters

v. Antibiotic susceptibility test was performed

4.3.3 Identification of isolates:-

4.3.3.1 Identification with staining reactions:-

As the study includes only gram negative isolates, Gram staining was performed as according to standard technique using acid alcohol as decolorlizer and gram positive bacteria were excluded; only gram negatives were further processed. Staining procedure and reagents are given in appendix II

4.3.3.2 Identification with biochemical test:-

(Cheesebrough 2000; Collee*et al*., 1996, Forbes *et al*., 1998)

Appropriate biochemical test were performed for identification of bacterial isolates:-

a. Catalase test
b. Oxidase test
c. Indole test
d. Methly Red test
e. Voges Progeskaur test
f. Citrate Utilization test
g. Triple Sugar Iron Agar test
h. Urease test

Result interpretation of different results was done based on identifying characters of different organisms on appendix III.

4.3.4 Antibiotic susceptibility test (AST):-

Antibiotic susceptibility test of all isolates was performed by Kirby disc diffusion method. In this technique a disc impregnated with antimicrobial agents are laid over the carpet culture of test organism, antimicrobial agent diffuses radially from the disc into the medium. Following overnight incubation, the culture was examined for areas of no growth around the disc. Bacterial strains sensitive to the antimicrobial were inhibited at a distance from the disc where as resistant strain grew up to the edge of the disc.

Procedure for AST:-

1. Preparation of medium
2. Preparation of inoculum
3. Performing sensitivity test
4. Observation and result interpretation

1. Preparation of medium:

   Medium used for running AST was Muller Hinton agar. Medium was prepared as per directions provided by manufacturer, care was taken to maintain height of medium while pouring (4mm in 90mm diameter plates i.e. 25ml per plate).

2. Preparation of inoculum:

   Inoculum was prepared by adding pure colonies of organisms to 5ml Nutrient broth and incubated at $37^{O}C$ for 4 hours. The prepared inoculum was compared with McFarland tube number 0.5 (Preparation of McFarland tube number 0.5 is given in appendix IV).

3. Performing sensitivity test:-

After proper turbidity was achieved, a new sterile cotton swab was submerged in the suspension, lifted out of the broth, and the excess fluid was removed by pressing and rotating the swab against the wall of the tube. The swab was then used to inoculate the entire surface of the Mueller Hinton agar plate three times, rotating the plate 60 degrees between each inoculation. The inoculum was allowed to dry (usually taking only a few minutes but no longer than 15 minutes) before the discs are placed on the plates.

The discs was then placed on the agar with sterile forceps and tapped gently to ensure the adherence to the agar. The plates containing the discs were incubated at 37^{o}C for 24 hours.

4. Observation and result interpretation

After overnight incubation, the diameter of each zone of inhibition was measured with a ruler. The ruler was positioned across the center of the disc to make these measurements. The results were recorded in millimeters (mm) and interpretation of susceptibility was obtained by comparing the results to the standard zone interpretative chart provided by the company (appendix V)

4.3.5 Quality control (Forbes *et al.*, 2002)

Quality control is considered as one of the important factors for the correct result interpretation. During this study, quality control was applied in various areas.

i. During sample collection aseptic technique was followed for collecting samples in order to avoid contamination.
ii. During sample processing, all tests were carried out appropriately in aseptic conditions.

iii. While using readymade dehydrated media, the manufacturer's instruction for preparation, sterilization and storage were followed to prevent the alternation of the nutritional, selective, inhibitory, and biochemical properties of the media.

iv. The performance of newly prepared media was tested using control species of bacteria (i.e., known organisms giving positive and negative reactions).

v. The prepared media in each batch were incubated for sterility testing.

vi. The sterility of the swab in each batch was checked by swabbing the sterilized swab in media plates.

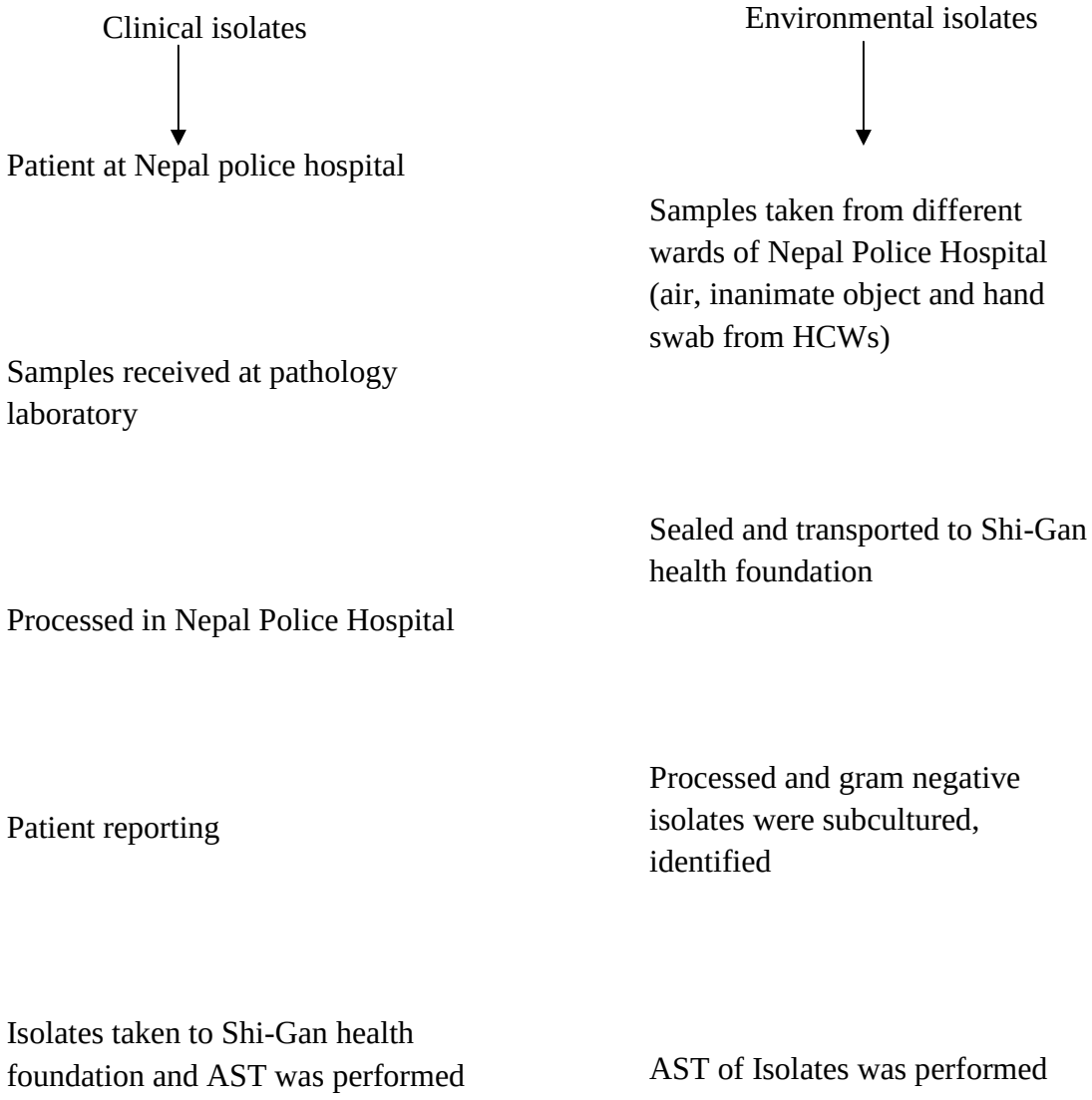
vii. For stains and reagents, whenever new batch of them was prepared, a control smear was stained to ensure correct staining reaction.

viii. Control strain of *E. coli* (ATCC 25922) was used for standardization of Kirby-Bauer test and also for correct interpretation of zone of diameter.

4.3.6 Data interpretation:-

On the basis of antibiotic susceptibility testing, organisms resistant to more than 3 antibiotics were considered as MDRO and interpretation was made as accordingly. Different isolates and antibiotics sensitivity pattern were expressed in percentage. Grouping of the isolates into different antibiotypes were done and comparison of environmental and clinical isolates was done.

Flow Chart of Methodology



Chapter VI

DISCUSSION

The NIs are caused by pathogens found in hospital, several of which are often resistant to many antimicrobials, probably because of the selective pressure by the extensive use of broad-spectrum antibiotics in hospital (Hassanzadeh *et al.,* 2008; Fridkin, 2001 and Kollef *et al.,* 2001). Most of these pathogens may remain viable in the environmental aspects of hospitals like air, dust, clothes and in inanimate surfaces and equipments, which can serve as important source of pathogens particularly to the immunocompromised patients (Neely *et al.,* 2000 and 2001; Hota, 2004; Kramer *et al.,* 2006).

The prevention of NI demands a thorough knowledge of the infection rates and of the source, type and nature of invading microorganisms along with the risk factors associated with infection (Weinstein, 1991). In developed countries many surveys and control programs are implemented so as to prevent transmission of pathogens from hospital environment to the patients (Wilks *et al*., 2006; Zolldan *et al*., 2005; Orsi *et al*., 2005 and Haley *et al*., 1985). On the other hand, in the context of resource poor countries like Nepal, there are even hardly any study carried out to identify the nosocomial pathogens that has hospital (exogenous) origin and are responsible for disease causation in immunocompromised patients. All hospitals follow various standard international guidelines to control the growth of microorganisms and so does Nepal Police Hospital. But despite following all the norms, persistence of organisms is inevitable hence, it is essential to check the efficacy of such guidelines followed. This cross sectional study therefore focuses on the MDR gram-negative organisms that may cause the health care crisis and their association with the organisms isolated from clinical sample during same period. But it was beyond the scope of this thesis to determine whether the isolates from environmental

samples played role in causing the NI or not. However current study examined the association between the clinical and environmental isolates on the basis of similarity in characters determined by conventional microbiological techniques (cultural, biochemical tests and antibiotic sensitivity pattern). Though the study was carried out in a single hospital, nonetheless this study will arrest the attention of all hospitals' management committee in making appropriate surveys and investigations to control infections caused by hospital strains.

From the total positive sample 87.4% gram positive cocci, 37.4% gram positive rods and 80.4% gram negative rods were isolated. This shows that among environmental sample gram positive isolates were found to be the most predominating. Similar result was obtained in the study done by Pokharel *et al*., (1993), Banjara (2002), Pant *et al*., (2006) and Karki (2010).

The AST of gram negative isolates included in this study showed higher sensitivity towards ciprofloxacin, gentamycin and co-trimoxazole and higher resistance against amoxicillin, amikacin, nitrofurantoin followed by cefotaxime and ceftriaxone. Similar type of results was reported by Shehabi *et al*., (2005) they reported excellent activity of ciprofloxacin against all gram-negative isolates. In the study by Kukukates (2005), high rate of resistance was observed among the gram negative isolates against all antibiotics studied and so was with this study. In the study conducted by Karki among the ICU patients, the AST of gram negative isolates showed lesser sensitivity to ciprofloxaxin and amikacin whereas higher resistance was observed towards ampicillin followed by cefotaxime. Such variations in the antimicrobial sensitivity pattern among different studies may be due to the variation in duration and dose of antibiotics used, spectrum of antibiotics used, and differing antibiotic policies among different hospitals.

In the different study it has been revealed that the unique nature of the intensive care unit (ICU) environment makes this part of the hospital a focus for the emergence and

spread of many nosocomial pathogens. In a surveillance study, Bdareen (2009) reported that the percentage of ICU contamination by nosocomial pathogens such as *S. aureus*, *E. coli* and CoNS was found to be 23.8% out of total contamination of whole hospital. Another study carried out by Sexton *et al.,* (2006) demonstrated the recovery of identical and closely related isolates from patients and their environment in 14 (70.0%) patients, indicating possible environmental contamination of the isolation room that may have contributed to the endemicity. However as far Nepal Police Hospital is concerned no ICU unit has been established but still from the result obtained, where about 80.0% of the environmental samples was found to be positive and 74.0% of the isolates found to be MDR, the possibility of NI among immunocommpromised patients cannot be left unseen.

Indoor air of a hospital can serve as a potential reservoir of nosocomial pathogens. Air movement aids in the transport and dispersal of particles and microorganism. The hospital indoor air environment can potentially place patients at greater risk than the outside environment because enclosed spaces can confine aerosols and allow them to build up to infectious levels. The population of microbial flora in the indoor air of any hospital relies on several factors including the number and hygienic standard of people present, the quality of the hospital system and mechanical movement within the enclosed space. These pathogens present in the air can be a potential source of infectious diseases (Sattar *et al.,* 1987), hazardous health effects such as respiratory problems (Jacobs, 1989), allergic and irritating reactions (*et al.,*1986), hypersensitivity reactions (Woodward *et al., 1988;* Tambekar *et al.,* 2003). This suggests that air especially of the hospital vicinity can harbor several varieties of microorganisms that can in some cases pose serious problem to patients with weakened immunity. In this study, the air flowing through different ward was not filtered so 100% growth positive samples were observed and the predominant gram negative isolate was *Acinetobacter* spp. However no significant resemblance was found among the samples from environmental and clinical samples, similar result was obtained in

an epidemiological study by Bauer *et al.* (1990) in which the spectrum of bacteria recovered from patients and air was different.

Though considered as nonessential or insignificant for microbiological investigation, fabrics or clothing materials that are being continuously used in the healthcare environment may play very significant role as an important source of nosocomial pathogens. Nosocomial pathogens can thrive for a long duration on medical fabrics or clothing materials such as towels, aprons, gowns, privacy curtains, bed sheet, etc and can serve as an active source or reservoir of those pathogens. In multiple studies carried out by Neely and her colleagues during the year 2000-2001 showed experimentally, the survival of several gram negative bacteria such as *P. aeruginosa, K. pneumoniae, E. coli, S. marcescens, P. mirabilis* and *Enterobacter* spp**.** for hours to days on different types of commonly used medical fabrics such as clothing, towels, scrub suits and lab coats, privacy drapes, splash aprons etc. Signifying these fabrics thus can serve as reservoir of various types of nosocomial pathogens**.** Therefore in our study bed fabrics, bed rails also included and it was found that these harbor the potential MDR and it cannot be avoided that such contaminated fomites may lead to NIs either through direct contact of patients with fomites or indirectly through the hands of HCWs.

Inanimate hospital environment (e.g., surfaces and medical equipment) often becomes contaminated with nosocomial pathogens either via contaminated hand contact or via contact with other environmental sources such as contaminated air, water, etc. Once the organisms contaminate the inanimate surfaces, it can then persist and remain viable for a long time. In one study it has been reported that gram negative bacteria survive longer on inanimate surfaces than gram positive bacteria (Hirai Y, 1991). However study by Pokhrel *et al*., (1993) in TU teaching hospital, samples collected from various areas of hospital showed higher prevalence of *S. aureus* (60.0%). In our study, swab samples of several inanimate surfaces of objects such as bed bars, bedside tables, equipment holders and wash

basin showed the overall prevalence of *Acinetobacter* spp., *E. coli, Klebsiella* spp., gram positive cocci, *Bacillus* and some fungal isolates. Microbiological reports of inanimate surface samples of this study approximately concurred to the previous report made by Pant (2006) which was however representative of all wards of a Teaching Hospital in Nepal. The predominant pathogens reported by Pant include: CoNS (30.3%) followed by *S. aureus* (26.1%), yeast (13.9%), Micrococci (13.7%), Streptococci (7.2%), gram positive bacilli (6.8%) and gram negative bacilli (2.4%). Similar type of reports was also made in the study by Sharma (2006) which however included the surface samples of inanimate objects from ICU and SICU. Similarly in another research by Karki (2010) analysis from ICU environment with swab samples of several inanimate surfaces of objects such as bed rails, bedside tables, report writing tables, bedside monitor, ventilator (air filter) and stethoscope showed the overall prevalence of CoNS, *S. aureus, P. aeruginosa, Acinetobacter* spp., *K. pneumoniae, E. coli,* Enterococci, *Bacillus* spp. and some fungal isolates.

In the mid-1800s, studies by Ignaz Semmelweis (WHO, 2009) in Vienna, Austria, and Oliver Wendell Holmes in Boston, USA, established that hospital-acquired diseases were transmitted via the hands of HCWs. Cross-transmission of microorganisms by the hands of HCWs is considered the main route of spread of nosocomial infections. *A. baumanii* strains isolated from the hands of HCWs and from the clinical isolates patient were reported to be similar by El Shafie and colleagues (2004) during an outbreak investigation of multidrug-resistant *A. baumannii* indicating HCWs hands as a source of outbreak of infection among the patients. Therefore hands should be properly washed before touching any patients. Many studies have shown a sustained decrease of the incidence of multidrug-resistant bacterial isolates and patient colonization following the implementation of hand hygiene improvement strategies (Brown *et al*., 2003; Gordin *et al*., 2005 and Girou *et al*., 2006). To access the hand hygiene in this study total of 81 hand swab samples were collected among which 63 hand swab were found to be positive and

total of 47 gram negative isolates were obtained. The predominant organism was *Acinetobacter* spp. which is also present as predominant organisms in other environmental samples followed by *E. coli* and *Klebsiella* spp. The latter two organisms were mostly isolated from staffs of dietric department. In the previous study carried out by Pant (2006), several isolates from the hands of HCWs have been reported including *S. aureus,* CoNS, Micrococci, *Streptococcus* spp., *Bacillus* spp. and *E. coli.* These reported isolates were similar with the isolates of this study.

Besides medical care units our study also included the dietric department from where the food for the patients is supplied. Analysis of the environment in the dietric department showed the need of attention towards sanitation and hygiene. All the swab sample from dietric unit was positive, further various pathogenic gram negative isolates like *E. coli, Acinetobacter* spp. and *Klebsiella* spp. were isolated from food carriage, food mixer, meat cutting log, rice cooker, vegetable cutting slab, washed utensils and wash basin. These isolates were isolated also from air and hand swab sample. The occurrence of these isolates suggests possibility of contamination of food supplied to the patient. In an investigation, including environmental sample, was undertaken after four leukaemic patients on the same hospital ward developed serious infections with *Klebsiella aerogenes*, capsular type K14, the source of this organism, common to all four patients, was found to be a food blender used for preparing milk-based drinks on the ward. (Kiddy *et al*., 1986). Therefore isolation of MDR isolates from the dietric department should also be taken as a matter of concern.

During this dissertation work on comparing cultural and biochemical characters, three species were found to be common in both the sample types (environmental and clinical) which include :- *Acinetobacter* spp., *Klebsiella* spp., *E. coli*. The relation between these organisms however could not be established since only single antibiogram type of environmental isolates of *Acinetobacter* spp. and *Klebsiella* spp. matched the antibiogram types from clinical sample and no similarity in antibiogram type of *E. coli* was found.

Acinetobacter spp. is normal environmental colonizers. The emergence of MDR *Acinetobacter* spp. has become an increasing problem in health-care-associated infections over the past few years (Maragaskis and Pearl, 2008, Fournier and Richert 2006, Bernards *et al*., 2004 and Seifert and Dowzicky, 2009). The NNIS reported a 6.9% increase in *Acinetobacter* spp. causing hospital-acquired pneumonias in ICUs in 2003 (up from 1.4% in 1975) (Gaynes and Edwards, 2005). Risk factors that have been linked to increased colonization and infection prolonged hospitalization, mechanical ventilation, recent surgery, invasive procedures and underlying severe illness. These types of infections present clinically as bacteremia, pneumonia, meningitis, urinary tract infections and wound infections (Ramazanzadeh *et al*., 2009). In addition, this organism has the ability to persist and colonize environmental surfaces for prolonged periods of time, thereby contributing to its pathogenicity, increased transmission rates and association with nosocomial outbreaks (Seifert and Dowzicky, 2009). However the true frequency of NI caused by *Acinetobacter* spp. is not easy to assess, partly because the isolation of these organisms from clinical specimens may not necessarily state infection but, rather, may result from colonization (Bergogne and Towner, 1996). In our study of the total (269), 154 samples *Acinetobacter* spp. was 71.9%. Among the gram negative isolates from the air and surface sample *Acinetobacter* spp. was the predominant almost 95.0 % and 82.7% of the isolates. This shows the dominance of the species and as discussed early pose a threat of NI.

E. coli obtained was second dominant organism isolated from hospital environment while most frequently organisms among clinical isolates. The environmental isolates were identified as of six different antibiogram types. While among the clinical samples *E. coli* were differentiated into nine different types; but none of them matched the antibiogram types from environmental isolates. Thus the relation between environmental isolates and the clinical isolates cannot be stated. In this study most resistant drug found to be nitrofurantoin followed by amoxicillin for both the sample type. While the finding of this study is contrast of the findings from the study of UTI from different hospitals of rural

Nepal by Jha and Bapat (2005) in which in study conducted in five hospitals showed that in three hospitals, amoxicillin was found to be most effective, and nitrofurantoin in next hospital amikacin and gentamycin was most effective in 3rd hospital. In Antimicrobial Resistance Surveillance for selected bacterial pathogens in done in Nepal by NPHL along with 11 labs from central, western and eastern region, increase in resistance was found ESBL isolates increased from 13-86 from 2009-2010 and similarly study in 2010 showed isolates were found resistant to most of the common antibiotics and 3rd generation cephalosporin as; amoxicillin 100.0%, furadantin 100.0%, ciprofloxacin 97.0% - 100.0%, ofloxacin 66.0% - 100.0%, ceftriaxone 92.0%, cefopodoxime 99.0%, ceftazidine 99.0%, cefotaxime 100.0% (Shakya, 2011). This supports our finding that the amoxicillin is least effective drug compared others.

Klebsiella spp. was third common isolate of environmental samples and different clinical samples. On grouping antibiogram types of the isolated species total of 4 different antibiogram types from the environmental sample and 1 antibiogram type from clinical sample was identified. However the single *Klebsiella* spp. from the inpatient which was resistant to all the antibiotics tested was identical, to the one of the antibiogram type of environmental isolate.

The rise in resistance of organisms to amkacin and amoxicillin in this study may be due to increased consumption aminoglycosides and aminopenicillins in the hospital. In present study Enterobacteriaceae (*E. coli and Klebsiella* spp.) isolates with decreased susceptibility to cefotaxime and ceftriazone were defined as extended spectrum β-lactamase (ESBL) phenotypes. Similarly, *Acinetobacter* spp. in this study showed the high MDR nature showing resistance to more than 3 groups of antibiotics among aminopenicillins aminoglycosides, sulphonamides, quinolones, cephalosporins and other miscellaneous drug. Thus this study highlights the trends of multidrug resistance among gram negative isolates, therefore indicating an alarm of threat of emergence of drug resistant pathogens.

The results of this study obtained by the application of conventional microbiological methods of isolation and identification for the microbes, aided us to know that the relation between the isolates from hospital environment and clinical sample could not be established as the antibiogram types of both the sample type shows the significant difference. Use of techniques such as immunoassays followed by analysis of gene through pulse field gel electrophoresis (PFGE), Polymerase Chain Reactions (PCR), etc. would have helped to develop concrete association between the isolates from clinical and environmental samples.

Conclusion

From this study of microbiological analysis of gram negative isolates of both the clinical samples it is found that the *Acinetobacter* spp. is the major microbial pollutant in hospital environment of Nepal Police Hospital. Antibiotyping based on resistivity of isolates from both sample types (environmental and clinical) showed 40.0% of the clinical isolate of *Acinetobacter* spp. is identical in its antibiotic resistivity pattern as that of the environmental isolates similarly 100.0% of the *Klebsiella* spp. from clinical sample showed similar antibiotic resistivity pattern as that of environmental isolates, while no E. coli isolates from two sample types showed similar character as environmental isolates. Hence it may be concluded that there is possibility of NI due to *Acinetobacter* spp. and *Klebsiella* spp.

Chapter VII

SUMMARY AND RECOMMENDATION

7.1 Summary

1. The prevention of nosocomial infection demands a thorough knowledge of the source, type and nature of invading microorganisms along with the risk factors associated with infection therefore this study aims to study the types of organisms existing in different wards of Nepal Police Hospital.

2. Overall all the ward of Nepal Police Hospital except OPD was studied, along with the swab from the available health care workers in respective wards. The dietric unit and CSSD unit were also included in the study.

3. Total of 169 environmental samples were taken, including air samples, surface swab from inanimate objects and the hand swab of health care workers. Along with this clinical samples obtained in microbiology laboratory of hospital was also considered.

4. 212 of the environmental samples were found to be positive and 181 clinical samples were positive. Three isolates *Acinetobacter* spp., *E. coli* and *Klebsiella* spp. were common in both the samples types. *Acinetobacter* spp. was dominant organisms from environmental sample and *E. coli* was dominant isolates from the clinical samples.

5. Among the wards Chest and TB, Emergency and MW, showed highly positive result with 96.9%, 86.2%, 79.4% growth positive result and in overall dietric department showed high microbial load (100.0% sample positive).

6. Antibiotics like ciprofloxacin and co-trimoxazole showed higher sensitivity towards the isolates of both type while amoxicillin, nitrofurantoin, amikacin , ampicillin were highly resisted by the isolates.

7. Analysis of MDR property revealed 409/154 isolates Acinetobacters from environmental samples to be MDR i.e. 70.8% isolates were MDR while 86.7 % (13/15) E. *coli* and 100% (14/14) of isolated *Klebsiella* spp. isolates was MDR.

8. Antibiogram analysis showed that environmental isolates of *Acinetobacter* spp. could be classified to into 12 antibiogram type while the clinical isolates could be classified into 4 antibiogram types. Among these only single antibiogram type of the both is identical i.e. group VI (resistant to aminopenicillins and cephalosporins)

9. Antibiogram analysis for *E. coli* revealed 6 antibitoype from environmental sample and 4 antibiogram types from clinical samples. But none of this antibiogram type was identical to each other.

10. Antibiogram of *Klebsiella* spp. revealed 4 antibiogram types from environmental samples and 1 antibiogram types from clinical samples. And single isolates from both the sample types were identical. This antibiogram type was resistant to all tested antibiotics.

11. The occurrence of the nosocomial infection from the environmental isolates though could not be established but the high microbial load of nosocomial pathogens like *Acinetobacter* spp. shows the need of attention of concerned authority towards hospital environment maintenance.

7.2 Recommendations:-

Based on the study following recommendations have been made:-

1. Sterilization and disinfection of inanimate surfaces, fumigation of air and monitoring of maintenance.
2. Development and implementation of guideline for hospital environmental control and antimicrobial use is recommended.
3. Take necessary prevention to control super bug like *Acinetobacter* spp. which was found to be dominant isolate from all the wards.
4. Conduction of workshops to make staffs aware about NI and their role in control should be given.
5. Analysis of fungal isolates and study of other samples like water will be helpful to develop effective strategies to prevent NI.

CHAPTER VIII

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Chapter IX

Appendices

Appendix-I

List of materials used in study:-

A. Equipments and materials used during the study

Autoclave	ALP Co. LTD.
Hot air oven	Memmert
Incubator	Memmert
Refrigerator	LG (Korea)
Microscope	Olympus (Japan)

B. Composition and Preparation of Different Culture Media

The culture media used were from HiMEDIA company.

1. Nutrient Agar (NA)

Composition	gram/litre
Peptic digest of animal tissue	5.00
Beef Extract	1.50
Yeast Extracts	1.50
Sodium Chloride	5.00
Agar	15.00
Final pH (at 25°C)	7.4 ± 0.2

28 gm of the medium was suspended in 1000ml of the distilled water and boiled to dissolve completely. Then medium was autoclaved at 121°C (15lbs pressure) for 15min. The

sterilized medium was then poured into the sterilized petridishes and then was allowed to cool.

2. Blood Agar Base (Infusion Agar)

Composition	gm/litre
Beef heart, infusion from	500
Tryptose	10.0
Sodium Chloride	5.00
Agar	15.0
Final pH (at 25°C)	7.3 ⸗ 0.2

40gms of the medium was suspended in 1000ml of the distilled water, dissolved by boiling and sterilized by autoclaved at 121°C (15lbs pressure) for 15minutes. After cooling to 50^{o}C, 5%v/v sterile defibrinated blood was added aseptically, then mixed with gentle rotation and poured into the sterilized petridishes and was allowed to cool.

3. MacConkey Agar (MA)

Composition	gm/litre
Peptic digest of animal tissue	17.00
Proteose Peptone	3.00
Lactose	10.00
Bile Salt	1.50
Sodium Chloride	5.00
Neutral Red	0.03
Agar	15.00
Final pH (at 25°)	7.1 ⸗ 0.2

51.53gm of the medium was dissolved in 1000ml of the distilled water and then boiled to dissolve completely. The media was autoclaved at 121°C (151bs pressure) for 15min. The sterilized medium was then poured in sterilized petridishes and was allowed to cool.

4. Nutrient Broth (NB)

Composition	gm/litre
Peptic digest of animal tissue	5.00
Sodium Chloride	5.00
Beef Extract	1.50
Yeast Extracts	1.50
Final pH (at 25°)	7.4 ⸗ 0.2

13gm of the medium was dissolved in 1000ml of the distilled water and then boiled to dissolve completely. The medium was then dispensed in test tube in amount of 3ml in each and autoclaved at 121°C (151bs pressure) for 15 minutes. The sterilized medium was then cooled to room temperature.

5. Muller Hinton Agar (MHA)

Composition	gm/litre
Beef infusion form	300.0
Casein Acid Hydrolysate	17.50
Starch	1.50
Agar	17.00
Final pH (at 25°)	7.3 ⸗ 0.2

38gm of the medium was dissolved in 1000ml of the distilled water and then boiled to dissolve completely. The medium was autoclaved at 121°C (15lbs pressure) for 15minutes. The sterilized medium was then poured in sterilized petridishes and was allowed to cool.

C. Composition and preparation of different biochemical media

1. Simmon Citrate Agar

Composition	gm/litre
Magnesium Sulfate	0.20
Monoammonium Dihydrogen Phosphate	1.00
Dipotassium Phosphate	1.00
Sodium Citrate	2.00
SodiumChloride	5.00
Agar	15.0
Bromothymol Blue	0.08
Final pH (at 25°C)	6.8 ⸗ 0.5

24.2 grams of the medium was dissolved in 1000ml of the distilled water and boiled to dissolve completely. 3 ml of the medium was dispensed in each test tube and autoclaved at121°C (15lbs pressure) for 15minutes. The sterilized medium in the test tube was then allowed to set in slopes or slant.

2. Urea Agar base (Christensen urea agar)

Composition	gm/litre
Peptic digest of animal tissues	1.000
Dextrose	1.000
Monopotassium Phosphate	0.800
Dipotassium Phosphate	1.200
Sodium Chloride	5.000
Agar	15.00
Phenol Red	0.012
Final pH (at 25⸢C)	6.8 ± 0.2

24 grams of the medium was suspended in 950 ml of distilled water, dissolved by boiling and autoclaved at 121^0C (15 lbs pressure) for 15 minutes. After cooling to 50^0C, 50 ml of sterile 40% urea solution was added aseptically, mixed with gentle rotation. Then 5ml of the medium was dispensed in test tube and set at slant position.

3. Sulphide Indole Motility (SIM) Agar

Composition	gm/litre
Peptic digest of animal	30.00
Beef Extract	3.00
Peptonized Iron	0.20
Sodium Thiosulfate	0.025
Agar	3.00
Final pH (at 25°C)	7.3 ± 0.2

36.23grams of the medium was dissolved in 1000ml of the distilled water, boiled to dissolve completely and then dispensed in test tubes to a depth of about 3 inches. Then the medium in tubes was autoclaved at121°C (15lbs pressure) for 15 minutes.

3. MR-VP Medium

Composition	gm/litre
Buffered peptone	7.00
Dextrose	5.00
Di-potassium phosphate	5.00
Final pH (at 25°C)	6.9 ± 0.2

17 grams of the medium was dissolved in 1000ml of the distilled water, boiled to dissolve completely and then 3ml of the medium was dispensed in each test tubes and then autoclaved at121°C (15lbs pressure) for 15 minutes.

4. Triple Sugar Iron (TSI) Agar

Composition	gm/litre
Peptic digest of animal tissue	10.00
Casein Enzymatic Hydrolysate	10.00
Yeast Extracts	3.00
Beef Extract	3.00
Lactose	10.00
Sucrose	10.00
Dextrose	1.00
Sodium Chloride	5.00
Ferrous Sulphate	0.20
Sodium Thiosulphate	0.30
Agar	12.00
Phenol red	0.024
Final pH (at 25°C)	7.4 ⦚ 0.2

65grams of the medium was dissolved in 1000ml of the distilled water. The medium was then dispensed in test tubes and autoclaved at 121°C (15lbs pressure) for 15minutes. The sterilized medium in the test tube was then allowed to set in slant with a butt of about 1 inch of thickness.

D. Composition and preparation of different staining reagent

1. Gram Stain

(a) Crystal Violet Solution

Crystal Violet	20.0 g
Ammonium Oxalate	9.0 g

Ethanol or Methanol	95.0 ml
Distilled Water (D/W) to make	1 litre

Preparation: 20 grams of Crystal Violet was weighed in a clean piece of paper and transferred to a clean brown bottle. Then 95 ml of ethanol was added and mixed until the dye is completely dissolved. To the mixture, 9 grams of Ammonium Oxalate dissolved in 200ml of D/W was added. Finally the volume was made 1 litre by adding D/W.

(b) Lugol's Iodine

Potassium Iodide	20.00g
Iodine	10.00g
Distilled Water	1000.0 ml

Preparation: To 250 ml D/W, 20 grams of Potassium Iodide was dissolved. Then 10 grams 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 Decolorizer

Acetone	500ml
Ethanol (absolute)	475ml
Distilled Water	25.0 ml

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

(d) Safranin (Counter Stain)

Safranin (2.5% solution in 95% ethyl alcohol)	10.00ml
Distilled Water	100.0 ml

Preparation: 2.5 % of Safranin solution was prepared in 95% ethanol. 10 ml of this solution was then suspended in 100 ml of D/W.

2. Normal saline

Sodium Chloride	0.85g
Distilled Water	100ml

Preparation: 0.85 grams of Sodium Chloride was weighed and added to a bottle containing 100ml of D/W and mixed well to dissolve the salt completely. The bottle was well labeled and stored at room temperature.

3. Biochemical Test Reagents

a. For catalase test

Catalase reagent (3%H2O2)

Hydrogen Peroxide	1ml
Distilled Water	9ml

Preparation: To 9ml of D/W, 1ml of Hydrogen Peroxide was added and mixed well so as to make a 3% solution of Hydrogen Peroxide.

b. For oxidase test

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

Tetramethyl *p*-phenylene diamine dihydrochloride(TPD)	1.00g
Distilled Water	100ml

Preparation: 1 gram of TPD was dissolved in 100 ml of D/W. To this 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

p-dimethyl aminobenzyldehyde	2.00gm
Isoamyl alcohol	30.0ml
Concentrated Hydrochloric Acid	10.0ml

Preparation: In 30 ml of isoamyl alcohol, 2 grams of *p*-dimethyl aminobenzaldehyde was dissolved and transferred to a clean brown bottle. Then to this solution, 10ml of concentrated Hydrochloric Acid was added and mixed well.

d. For methyl red test

Methyl red solution

Methyl red	0.05gm
Ethylalcohol(absolute)	28.0ml
Distilled Water	22.0ml

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

e. For Voges Proskauer test

Barrit's reagent

Solution A

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

Preparation: 5 gram of α napthol was dissolved in 25 ml ethanol and transferred into a clean brown bottle.Then the finel volume was made 100ml by adding ethanol.

Solution B

Potassium hydroxide (KOH)	40.0gm
Distilled Water	100ml

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

Appendix - II

A. Procedure for Gram Staining (Forbes *et al.*, 2007)

Gram staining is a differential staining that differentiates all bacterial species into two large groups: *gram positive* and *gram negative*. The following steps were involved in gram staining:

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
4. drying for 10-30 seconds.
5. The slide was rinsed with tap water, shaking off excess.
6. The slide was flooded with iodine solution and allowed to remain on the
7. surface without drying for twice as long as the crystal violet was in contact
8. with the slide surface.
9. The slide was rinsed with tap water, shaking off excess.
10. The slide was flooded with alcohol acetone decolorizer for 10 seconds and
11. rinsed immediately with tap water until no further colour flows from the slide
12. with the decolorizer. Thicker smear requires more aggressive decolorization.
13. The slide was flooded with counter stain (safranin) for 30 seconds and washed
14. off with tap water.
15. The slide was blotted between two clean sheets of bibulous paper and
16. examined microscopically under oil immersion at 100X.

B. Methods of biochemical test used for the identification of pathogens

a. Catalase test:

The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria; the main exception is Streptococcus species (catalase negative). Usually organisms which lack the cytochrome system also lack the Catalase enzyme and therefore are unable to break down hydrogen peroxide. Catalase is a heme protein. The prosthetic group is made up of four atoms of trivalent iron (ferric) per molecule, which retains its oxidized state during enzyme activity. Hydrogen peroxide is formed as an oxidative end

product of the aerobic breakdown of sugars. Reduced flavoprotein reacts directly with gaseous oxygen by way of electron reduction to form hydrogen peroxide and not by direct action between hydrogen and molecular oxygen.

With the help of a sterile glass rod, a small amount of culture from the Nutrient Agar was transferred to a clean glass slide and a drop of 3% Hydrogen peroxide solution was dropped on the surface of the slide. The positive test is indicated by the formation of active bubbling of the oxygen gas. The lack of catalase was evident by lack of or weak bubble production.

b. Oxidase test :

The oxidase test is based on the bacterial production of an oxidase enzyme. The oxides reaction is due to the presence of a cytochrome oxidase system which activates the oxidation of reduced cytochrome by molecular oxygen, which in turn acts as an electron acceptor in the terminal stage of the electron transport system. The cytochrome system is usually present only in aerobic organisms which make them capable of utilizing oxygen as a final hydrogen acceptor to reduce molecular oxygen to hydrogen peroxide, the last link in the chain of aerobic respiration.

A piece of filter paper soaked in oxidase reagent and dried was moistened with distilled water and a colony from the fresh culture was picked up with a sterile glass rod and smeared on the paper. The positive test is indicated by the appearance of blue-purple color within 10 seconds. The oxidase reagent (Tetra methyl *p*- phenylene diamine dihydrochloride) is a dye that is primary aromatic amines and diamine derivatives of benzene. Cytochrome oxidase in the presence of atmospheric oxygen oxidizes the Oxidase reagent to form a colored compound called indophenol. The Oxidase test is based on the bacterial production of an oxidase enzyme.

c. Indole production test :

Tryptophan is an amino acid that can be oxidized by certain bacteria to form three major indolic metabolites: indole, skatol (methyl indole), and indole acetic acid. Intracellular enzymes involved in this oxidation process are collectively called as tryptophanase. The enzyme tryptophanase catalyses the deamination reaction attacking the tryptophan molecule in its side chains and leaving the aromatic ring intact in the form of indole.

For this test, organism was stabbed in SIM (Sulfide Indole Motility) medium from the nutrient broth and incubated at 37^{o}C for24 hours . After incubation, 2-3 drops of Kovac's reagent (*p*- dimethyl aminobenzaldehyde in acid ethanol) was added and resulting color was noted .Indole, if present combines with the aldehyde present in Kovac's reagent to give a red color in the alcohol layer. The color reaction is based on the presence of pyrrole structure present in the indole.

d. Methyl red test

This test is used to determine the ability of an organisms to produce and maintain the stable acid end product from glucose fermentation, and to overcome the buffering capacity of the system.The methyl red test uses a pH indicator in the form of methyl red, to determine the hydrogen ion concentration (pH) arising out of fermentation of glucose by an organism. The hydrogen ion concentration depends on gas ratio (CO2 and H2), which in turn is an index to the different pathways of glucose metabolism exhibited by various organism. The different fermentation patterns are due to variation in enzymes concerned with pyruvic acid metabolism present in the organism. Methyl red positive organisms produce stable acids, maintaining a high concentration of hydrogen ions until a sudden concentration is reached. The validity of methyl red test depends upon a sufficient incubation period in order to permit the differences in glucose metabolism to occur. The organisms to be tested should be incubated at least 35^{o}C -37^{o}C. Methyl red is an indicator which is already acidic and well 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^{o}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 and negative with yellow color.

e. Voges Proskauer test

The principle of this test is to determine the ability of some organisms to produce a neutral end product, acetyl methyl carbinol (acetoin), from glucose fermentation.Glucose is metabolized to pyruvic acid which is a key intermediate in glycolysis. From pyruvic acid, there are many pathways that a bacterium may follow. The production of acetoin is one pathway for glucose degradation occurring in bacteria. The VP test for acetoin is used primarily to separate *Escherichia coli* from *Klebsiella* and *Enterobacter* spp.A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37^{o}C for 24 hours. After incubation, about 5 drops of Barrit's reagent was added and mixed well and kept for 15 minutes, positive test shows development of pink red color.

f. Citrate utilisation test:

This test is done to determine if an organism is capable of utilizing citrate as a sole source of carbon for metabolism with resulting alkalinity .The organism whose ability was to be tested were inoculated in the slant of Simmon's Citrate agar media and incubated at 37^{o}C for 24 hours. Result was interpreted as positive if there was a growth or change in color of slant from green to intense blue and negative if there is no growth and no change in color.

g. Triple sugar Iron (TSI) Agar:

Triple sugar Iron (TSI) agar is a medium used in the identification of Gram negative enteric rods. The medium measures the ability of a bacteria to utilize sugars: glucose, sucrose and lactose, the concentration of which are in 0.1%, 1.0% and 1.0% respectively. A pH indicator (Phenol Red) included in the medium can detect acid production from

fermentation of test carbohydrates. The test organism was streaked and stabbed on the surface of TSI and incubated at 37^{o}C. for 24 hours. The results are interpreted as:

a. Yellow (Acid) / Yellow (Acid), Gas, H_2S ⟶ Glucose, Lactose/ Sucrose fermenter, H_2S producer.
b. Red (Alkali) / Yellow (Acid), No Gas, No H_2S ⟶ Glucose fermenter, Lactose/Sucrose nonfermenter, Anaerogenic, H_2S nonproducer.
c. Red (Alkali) /No Change ⟶ Glucose, Lactose and Sucrose nonfermenter,
d. Yellow (Acid)/ No Change ⟶ Glucose oxidizer.
e. No Change / No Change ⟶ Nonfermenter.

h. Motility test:

Bacteria are motile by means of flagella. Flagella occur primarily among the bacilli; however a few cocci forms are motile. Motile bacteria may contain single flagella. The motility media used for motility are semi solid, making motility interpretation macroscopic. Motile organisms migrate from the stab- line and diffuse into the medium causing turbidity. They may exhibit fussy streaks of growth. Whereas nonmotile bacteria show the growth along the stab-line, and the surrounding media remains colorless and clear.

i. Urea hydrolysis test

This test demonstrates the urease activity present in certain bacteria which decomposes urea, releasing ammonia. The test organism was inoculated in a medium containing urea and the indicator Phenol red. The inoculated medium was incubated at 37^{o}C overnight. Positive organism shows pink red due to the break down of urea to ammonia. With the release of ammonia the medium becomes alkaline as shown by a change in color of the indicator to red pink.

Appendix- III

A. Features used to assist in the identification of gram negative isolates:-

Key: Lac=lactose, Man=Mannitol, Glu=glucose, Suc=sucrose, Ox= oxidase, Cit=citrate test, Mot=Motility, Ind= Indole test, H_2S=hydrogen sulphide (blackening), R= red- pink (alkaline reaction), Y= yellow (acid reaction), d= different strains give different results, S= slow reaction, L= late reaction, w= weak result.

Species	**Test/substrate**												
	Lac	Man	Glu	Suc	Ox	Cit	Mot	Ind	Urea	TSI			
										Slope	Butt	H_2S	Gas
Escherichia coli	+	+	+	d	-	-	+[5]	+[2]	-	Y[6]	Y	-	+[2]
Shigella spp.	-	d	+	-	-	-	-	d	-	R	Y	-	-[3]
Salmonella Typhi	-	+	+	-	-	-		+	-	R	Y	+ w	-
S. Paratyphi A	-	+	+	-	-	-	+	-	-	R	Y	-	+
Citrobacter fruendii	+L	+	+	d	-	+	+	-[3]	d	R /Y	Y	d	+
Klebsiella pneumoniae	+	+	+	+	+	-	-	-[3]	+S	Y	Y	-	+
Enterobacter spp.	+	+	+	d	-	+[2]	+	-	-	Y	Y	-	d
Serratia marcesens	d	+	+	+	-	+	+	-	d	R/Y	Y	-	d
Proteus vulgaris	-	-	+	+	-	d	+	+	+	R	Y	+	d
P. mirabilis	-	-	+	d	-	+[2]	+	-	+	R	Y	-	+
Morgenella morganii	-	-	+	-	-	-	+[5]	+	+	R	Y	-	d
Vibrio cholerae	-	+	+	-	+	d	+	+	-[3]	R	Y	-	-
Pseudomonas aeruginosa	-	-	d	-	+	+	+	-	d	R	R	-	-
Alcaligenes spp.	-	-	-	-	+	d	+	-	-	R	R	-	-

Notes:
1. *S. sonnei* ferments sucrose slowly
2. A minority of strains give positive result
3. A minority of strains give positive result
4. Test should be incubated at 20-28^{o}C
5. A few strains are non motile
6. A few strains produce a Red-pink slope (alkaline reaction)

B. Identification of *Acinetobacter* spp.

Species	Growth at 42^0 c	Gelatin liquifaction	Urease	Citrate	Growth at 37^0C	Acid production O/F medium
A. calcoaceticus	-	-	+/-	+/-	+	+
A. baumanii	+	-	-	+	+	+
A. jonsonii	-	+	-	+	-	-
A. junii	+	-	-	+	+	-
A. lowffi	-	-	-	-	+	-

+: positive
-: negative

Appendix- IV

Preparation of Turbidity standard equivalent to McFarland 0.5

- 1% v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99ml of water.
- 1% w/v solution of barium chloride prepared by dissolving 0.5 gram dihydrate barium chloride ($BaCl_2.2H_2O$) in 50 ml of D/W
- To 99.4 ml of prepared H_2SO_4 solution, 0.6 ml of, was added and mixed well.
- The standard was transferred into screw capped tubes of the same size and volume as those used for preparing the test and control inocula.
- The tubes were then sealed tightly to prevent loss by evaporation and stored protected from light at room temperature.
- The turbidity standard was then vigorously agitated before use.
- This standard when stored in well sealed container in the dark at room temperature (20-28°C), may be kept for up to 6 months.

Appendix-V

Zone size interpretation chart of antibiotics used in present study

Antibiotics used	Symbol	Disc potency (mcg)	Diameter of zone of inhibition in mm		
			Resistant	Intermediate	Sensitive
Ampicillin When testing Enterobacteriaceae	A	10	13	14-16	17
Amoxycillin	AM	10	13	14-17	20
Cotrimoxazole (Trimethoprim+ Sulfamethoxazole)	Co	1.25+ 23.75	10	11-15	16
Ciprofloxacin	CF	5	15	16-20	21
Gentamicin	G	10	12	13-14	15
Amikacin	Ak	10	14	15-16	17
Cefotaxime	CE	30	14	15-22	23
Ceftriaxone	CI	30	13	14-20	21
Nitrofurantoin	NF	100	14	15-16	17
Chloramphenicol	C	30	12	13-17	18
Cefixime	CFX	5	15	16-18	19

(Source: Product Information Guide, HiMedia Laboratories Pvt. Limited, Mumbai, India)

Appendix- VI

Collection sites in hospital

Location	Collection sites	Organism			Total
		E. coli	***Acinetobacter* spp.**	***Klebsiella* spp.**	
CSSD	wash basin	0	1	0	1
Chest and TB	bed bar	1	3	0	14
	bed fabric	0	2	0	
	equipment rack	0	2	0	
	thermometer	0	1	0	
	equipment box	0	1	0	
	table	1	1	0	
	wash basin	0	2	0	
	food carriage	0	1	0	
Dietric department	food mixer	0	0	1	13
	meat cutting log	0	1	0	
	rice cooker	0	1	0	
	slab	0	1	2	
	utensils	0	1	0	
	wash basin	2	3	0	
	bed fabric	0	1	0	
Dressing room	scissor for Gauze	0	1	0	5
	scissors for dressing	0	1	0	
	working table	0	1	0	
	leg support	0	1	0	
	bed	0	1	0	
Emergency ward	bed bar	0	4	0	12
	bed of Minor OT	0	0	1	
	equipment rack	0	1	0	
	table	0	4	0	
	equipment box	0	1	0	
	bed bar	1	1	0	
Female ward	bed fabric	1	1	0	5
	table	0	1	0	

	bed bar	0	2	0	
Infectious disease ward	bed fabric	0	2	0	8
	patient's Jug used	0	1	0	
	table	0	1	0	
	table of nursing station	0	1	0	
	thermometer	0	1	0	
	bed bar	0	1	0	
Male surgical ward	bed fabric	0	1	0	4
	patient's jug used	0	1	0	
	table	0	1	0	
	bed bar	0	2	2	
Medical ward	bed fabric	0	2	3	19
	patients utensils	0	2	0	
	table	0	1	1	
	table of nursing station	0	1	0	
	tables	0	1	0	
	thermometer	0	1	0	
	wash basin	0	3	0	
	bed of Minor OT	0	1	0	
Operation theater	table of minor OT	0	1	0	2
	bed bar	0	2	0	
Orthopedic ward	bed fabric	0	2	0	8
	patients utensils	0	1	0	
	table	0	1	0	
	table of nursing station	0	2	0	
	bed bar	0	1	0	
Post operative ward	bed fabric	0	1	0	2
Total		6	77	10	93
Sites of collection varies according to ward type and equipments used in respective ward.					