PREVALENCE OF MICROORGANISM IN THE INTENSIVE CARE UNIT (ICU) PATIENTS AND THEIR ASSOCIATION WITH INDOOR ENVIRONMENT

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PREVALENCE OF MICROORGANISM IN THE INTENSIVE CARE UNIT (ICU) PATIENTS AND THEIR ASSOCIATION WITH INDOOR ENVIRONMENT

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BY
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RECOMMENDATION

This is to certify that Ms. Menuka Karki has completed her dissertation work entitled "**Prevalence of microorganism in the Intensive Care Unit (ICU) patients and their association with indoor environment**" as a partial fulfillment of Master of Science Degree in Microbiology under our supervision. This dissertation work done by Ms. Karki, is an original research work and has not been submitted to any other Institutes/Universities to earn any other degree.

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CERTIFICATE OF APPROVAL

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ABSTRACT

A six month cross sectional study was undertaken with an objective to determine the prevalence of microorganisms in the clinical specimens of suspected patients admitted for more than 48 hours to the Intensive Care Unit (ICU) at the National Institute of Neurological and Allied Sciences (NINAS) Hospital, Kathmandu, Nepal and to trace out their possible sources of transmission. Microorganisms from 687 clinical specimens and 677 environmental samples (hands and anterior nares of Health Care Workers (HCWs), air, water, fabrics/clothes, equipments and other inanimate objects) were identified by conventional microbiological method and antibiogram was performed by Kirby- Bauer disc diffusion method and Clinical Laboratory Standard Institute (CLSI) guidelines. Microorganisms isolated from clinical and environmental samples were tested for their relatedness on the basis of their observational microbiological characters followed by statistical analysis. *Pseudomonas aeruginosa* (61.3%), *Klebsiella pneumoniae* (24.7%), *Acinetobacter* spp. (22.7%), *Escherichia coli* (7.6%) and *Staphylococcus aureus* (4.9%) were isolated from 404 (58.8%) growth positive clinical specimens. 83.0% (n=562) culture positive environmental samples also found to contain 38.9% of *P. aeruginosa*, 14.5% of *K. pneumoniae*, 15.3% of *Acinetobacter* spp., 10.3% of *E. coli* and 51.6% of *S. aureus*. Clinical and environmental isolates of *Acinetobacter* spp. demonstrated marked resistance to common antibiotic tested including Cefoperazone/Sulbactam, Piperacillin/Tazobactam and Imipenem except Polymyxin B (100% susceptibility). *P. aeruginosa*, *K. pneumoniae* and *E. coli* isolates from clinical specimens exhibited relatively higher sensitivity (84.6%, 94.0% and 83.8 % respectively) to Imipenem while from environment expressed 100% sensitivity. The most effective antibiotic for all the isolates of *S. aureus* was Vancomycin with 100% efficacy. Microbial analysis reflected similarity in occurrence pattern, microbiological characters and antibiotic sensitivity pattern among the clinical and environmental isolates. Simultaneous statistical analysis also demonstrated significant association between clinical and environmental isolates ($p<0.05$) indicating some degree of relatedness among them. This signified that environment of ICU may act as potent source of pathogens. The allocation of environmental sources harboring pathogens aided the hospital to implement the intervention strategies to control the particular sources of pathogens.

Key words: ICU, HCWs, fabrics/clothes, inanimate objects, relatedness.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AATF	-	Antimicrobial Availability Task Force
ACSQH	-	Australian Commission on Safety and Quality in Healthcare
AST	-	Antibiotic Sensitivity Test
BA	-	Blood Agar
BHI	-	Brain Heart Infusion
BSI	-	Blood Stream Infections
CA	-	Chocolate Agar
CDC	-	Centres for Disease Control
CLSI	-	Clinical Laboratory Standard Institute
CoNS	-	Coagulase Negative Staphylococci
CRI	-	Catheter Related Infections
CSF	-	Cerebro Spinal Fluid
CVC	-	Central Venous Catheterisation
CVP	-	Central Venous Pressure
EPIC	-	European Prevalence of Infection in Intensive Care
ESBL	-	Extended Spectrum β –Lactamase
EVD	-	External Ventricular Drain
HCW	-	Health Care Worker
HICPAC	-	Hospital Infection Control Practices Advisory Committee
ICU	-	Intensive Care Unit
IDSA	-	Infectious Diseases Society of America
MA	-	MacConkey Agar
MDR	-	Multi Drug Resistant
MHA	-	Muller Hinton Agar
MPN	-	Most Probable Number
MR	-	Methyl Red
MRSA	-	Methicillin Resistant *Staphylococcus aureus*
MSA	-	Mannitol Salt Agar
MSSA	-	Methiciilin Sensitive *Staphylococcus aureus*
NA	-	Nutrient Agar
NB	-	Nutrient Broth
NI	-	Nosocomial infection
NINAS	-	National Institute of Neurological and Allied Sciences

NNISS	-	National Nosocomial Infection Surveillance system
PCR	-	Polymerase Chain Reaction
PFGE	-	Pulse Field Gel Electrophoresis
SIM	-	Sulphide Indole Motility
SOAP	-	Sepsis Occurrence in Acutely ill Patients
SSI	-	Surgical Site Infection
TMTC	-	Too Many To Count
TPD	-	Tetramethyl p-Phenylene Diamine dihydrochloride
TSI	-	Triple Sugar Iron
UTI	-	Urinary Tract Infection
VAP	-	Ventilator Associated Pneumonia
VISA	-	Vancomycin Intermediate *Staphylococcus aureus*
VP	-	Voges Proskauer
VRE	-	Vancomycin Resistant Enterococci
VSE	-	Vancomycin Sensitive Enterococci
WHO	-	World Health Organisation

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOGRAPHS

LIST OF APPENDICES

CHAPTER I
INTRODUCTION

An ICU of a hospital is a specialized high technology units equipped with life-support systems especially for treating critically ill patients. The role of ICU in the control and treatment of most variable and severe illnesses of the human body is incredible and noteworthy (Hassanzadeh *et al.,* 2009). In spite of its great effort in caring patients, infection acquired in ICU brings about some degree of morbidity, mortality as well as economic burden to the patients (Vincent *et al.,* 1995; Girou *et al.,* 1998 and Tennant *et al.,* 2005). Dismayingly, the prevalence rate of nosocomial infections is found to be 5 to 10 times greater in ICU than in other ordinary wards (Vincent *et al.,* 1995). In a large multicentre studies carried out in Europe and United States of America, it has been documented that the rate of infections acquired in ICU is highest amongst all nosocomial infections which ranges from 12.0 to 45.0% (Tennant *et al.,* 2005; Vincent *et al.,* 1995; Jarvis *et al.,* 1991 and Craven *et al.,* 1988). This high prevalence is associated with three major factors: i) intrinsic or patients factor that contribute to the susceptibility of patients include extremes of age, immune compromised condition, malnutrition and severe underlying diseases, ii) use of invasive procedures or instrumentation such as central intravenous catheterization, bronchial endoscopy, urinary catheterization, endotracheal intubation, tracheostomy etc. and iii) crowding or animate reservoirs that promote the cross transmission of infections (Vincent *et al.,* 1995; Weber *et al.,* 1999 and Esen *et al.,* 2009).

Because of immunocompromised status of patients and invasive procedure performed, ICU patients are likely to be infected at multiple sites with different kind of microorganisms. The most common and frequently reported infections acquired in ICU are ventilator associated pneumonia (VAP), blood stream infection (BSI), urinary tract infection (UTI) and surgical site infection (SSI) (Richards *et al.,* 1999; Ylipalosaari *et al.,* 2006 and Fridkin *et al.,* 1997). Data from the National Nosocomial Infection Surveillance System (NNIS) conducted between 1992 and 1997 from medical ICUs in

the USA identified UTIs as the most frequent nosocomial infection (31.0%), followed by pneumonia (27.0%) and bloodstream infections (19.0%) (Pittet *et al.,* 1994). In the European Prevalence of Infection in Intensive Care (EPIC) study done in 1992, pneumonia was the most common nosocomial infection (46.9%) followed by urinary tract infection (17.6%) and bloodstream infections (12.0%) (Vincent *et al.,* 1995). A one-day point-prevalence multicentre study conducted in Mexico in 1995 showed that the most frequently reported ICU-acquired infections were pneumonia (39.7%), urinary tract infections (20.5%), and bacteraemia (7.3%) (de Leon-Rosales *et al.,* 2000). These infections are caused by diversity of microorganisms which may vary among different institutions. This means that the prevalence of a particular type of infections caused by particular type of microorganisms varies among ICUs of different hospitals and even within the ICUs of a hospital (Tennant *et al.,* 2005). A NNIS study through 1989 to 1998 identified the eight most common pathogens associated with nosocomial infections in ICU to be Coagulase-negative staphylococci, *S. aureus, P. aeruginosa, Enterococci* spp., *Enterobacter* spp., *E. coli, C. albicans, K. pneumoniae* and others (Fridkin *et al.,* 1999). These microorganisms which could be patient's own flora (endogenous) or the flora acquired through the contact via HCWs , visitors, other patients or via various inanimate objects like air, water, clothing or through inanimate surfaces that are in nearby vicinity of the patients (exogenous).

ICU on the other hand, also presents an ambient environment for the rapid evolution of antimicrobial resistant pathogens through the frequent and prolonged use of antimicrobial agents (Fridkin, 2001). Antibiotic usage, especially the broad spectrum agents, in ICU is relatively high due to the types of infections which patients have and also the predominant organisms found in such situations. Exposure to these antimicrobial agents may further complicate hospitalisation of ICU patients and create conditions conducive to resistance selection among host bacterial flora or nosocomially transmitted pathogens (Savaş *et al.,* 2005) ranging from *Pseudomonas* spp., *Acinetobacter* spp., *Klebsiella* spp. and other Gram-negative aerobic bacilli to vancomycin resistant enterococci (VRE) and methicillin resistant *S. aureus* (MRSA) ,

which are found to pose a clinically significant danger of infection among ICU patients (Trilla, 1994 and Elliott *et al.,* 1999). Studies have demonstrated that rates of antimicrobial resistance are greater in bacteria isolated from ICUs compared with other hospital wards and outpatient clinics (Archibald *et al.,* 1997). Colonization with such antimicrobial-resistant pathogens can lead to serious nosocomial illnesses due to breakdown of normal host defenses following the application of invasive devices (Jarvis *et al.,* 1991). Since, ICU represents a hot zone for acquisition of infections caused by drug resistant pathogens, it is, therefore, important to restrict the use of antibiotics and to be selective in the types of treatment schedules used (Elliott *et al.,* 1999).

Several factors are involved in cross transmission of nosocomial pathogen in ICU. Urgent nature of critical care is one factor where universal precautions of aseptic techniques are frequently missed. The other factor involved is cross contamination through contact. Evidence suggests that nosocomial pathogens are carried from patient to patient (exogenous flora) via the unwashed hands of health care workers (Fridkin *et al.,* 1996). Furthermore, the degree of asepsis used in maintaining invasive devices, inanimate objects around patients and the level of crowding in ICUs may impact on the crosstransmission of these pathogens as well (Doebbeling *et al.,* 1992; Fridkin *et al.,* 1996, Haley *et al.,* 1982 and Mayer *et al.,* 1986). Additionally, the fomites, including equipment and clothes and faulty air handling systems, can also enable organisms to spread around units (Daschner *et al.,* 1982). Similarly, the introduction of nosocomial bacteria into an ICU may occur upon 1 (Fridkin *et al.,* 1999). Invasive procedures, high antibiotic usage and transmission of microbial pathogens between patients due to inadequate infection control procedures may explain why ICUs are “high risk area” for the emergence and spread of life threatening nosocomial infection (Vincent *et al.,* 2000 and 2006).

In the context of hospitals of underdeveloped countries like Nepal, it is necessary to carry out extensive investigations or surveillance regarding the microorganisms and patients within the ICU, as there are only few studies and researches based on these

subjects. Few studies that were carried out so far were focused on either environment of ICU or the clinical isolates from ICU patients. A study conducted on ICU environment of Tribhuvan University Teaching Hospital of Nepal, reported several microorganisms from air, hands of visitors, clothing and several other inanimate objects like door handle, bed bar handle, ventilator monitor, and suggested these objects may be the possible sources of microorganisms that serve as etiological agent of infections in ICU patients (Sharma *et al.,* 2006). Another study carried out by Koirala (2009), on the other hand focused on clinical samples of the tracheostomized ICU patients of NINAS hospital. The study, reported several microbiota including *P. aeruginosa*, *Klebsiella* spp*., E. coli, S. aureus, S. pyogens* and other gram negative bacteria from tracheal aspirate and provided the indication of the possible route of transmission including equipments like suctioning machine, nebulizers, etc. (inadequately washed) and the HCWs. However, from these single sample (either environment or clinical but never both) based studies it is difficult to determine whether the nosocomial pathogens isolated from clinical specimens are associated with the isolates of environment or not and therefore difficult to find out the major transmission source. Hence, in view of the relevance and impact of such observations and also limited information about ICU related infections in Nepalese scenario, it is crucial to perform study on the occurrence of microorganisms in ICU patients and then investigate if there is any association with the environment isolates.

NINAS is established in (2006) to treat the traumatic and severely ill neurological patients. ICU of NINAS is 12 bedded with the provision of almost all the facility needed for a severe patient and it follows all the standard international norms for the effective control of microorganisms. The purpose of this study was to determine the prevalence of positive cultures and to identify the common organisms and their antimicrobial sensitivity patterns from different clinical specimens of patients in ICU of NINAS over a period from July to December 2009. Secondary goals included determining the possible sources of microorganisms in the ICU environment including HCWs, responsible for developing positive cultures. The study then aims to establish

association between the isolates of positive cultures and ICU environment by evaluating the similarity in occurrence pattern, microscopic observations, pigmentation, cultural characteristics, biochemical test reactions, coagulase test results and then by statistical analysis.

Throughout this study period, isolates from the suspected clinical specimens of every patient that was repeatedly sent for the culture, identification and sensitivity, on daily basis, were analyzed. Occurrence of the isolate after 48 hours or more from the time of admission of the patients in the ICU was carefully investigated and if found to be consistently present on the consecutive samples sent each day, then the possible sources such as hands and nasal carriage of HCWs, air and water circulated in ICU and several other inanimate objects that are in close proximity to the patients were sampled and processed microbiologically for the identification of isolates. Isolates were considered related if they showed similarity in their occurrence pattern, microscopic observation, biochemical test results and statistical analysis.

The result of this study will help clinicians to make the most rational choices of empiric antibiotic regimes based on common organisms, their antimicrobial sensitivity patterns and to track down the major sources of nosocomial pathogens and to take quicker effective actions to control the transmission. Additionally, a part of the present study will also help to bring awareness about cleaning and disinfection of the environment as well as improving the personal hygiene of HCWs and admitted ICU patients in NINAS as well as in other hospitals. The present study is the first study in NINAS, so far known first time in Nepal, assessing the association between the clinical isolates of patients and environment of ICU.

CHAPTER II
OBJECTIVES

2.1 General objective

To determine the prevalence of microorganisms in ICU and their association with indoor patients

2.2 Specific objectives

1) To isolate, identify and perform antibiotic sensitivity test of microorganisms from various clinical specimens of suspected ICU patients.
2) To determine the frequency of occurrence of microorganisms, their isolation and identification from air, water (reservoirs and taps), fabrics/clothing samples (patient's bed sheet, curtains around the patient and gowns worn by HCWs, patients and visitors) and from the inanimate surfaces (bed rails, door handle/knob, ventilator (air filter), bedside monitor, bedside table, report writing table and stethoscope) and determine their antibiotic sensitivity pattern.
3) To find out the prevalence of microbial isolates in the hands and anterior nares of HCWs (doctors, physiotherapist, nurses and housekeepers).
4) To investigate the association between the microbial isolates from clinical specimens of patients and from the environment (animate and inanimate) of ICU by evaluating their similarity in occurrence pattern, microscopic observation, cultural characters, pigmentation and biochemical reaction.

CHAPTER III
REVIEW OF LITERATURE

3.1 Hospital and hospital acquired infection

Hospital is a special environment serving health care to patients and as a work environment for medical and other staffs. According to World Health Organisation (WHO,1994), a hospital is a residential establishment that provides short term and long term medical care consisting of observational, diagnostics, therapeutics and rehabilitative services for person suffering from a disease or injury and for parturient. Sick people go to hospitals with the expectation that they will get better but unfortunately their expectation go into vain when instead of being healthier, they become much sicker at the hospital they visit. The increment in degree of sickness may occur due to acquisition of infection which was neither present nor incubating at the time of admission but acquired during the hospital stay or that may appear after discharge. Such infections are frequently referred to as hospital acquired infections or nosocomial infections (NIs) or health care associated infections. National Nosocomial Infection Surveillance (NNIS) system of Centeres for Disease Control and Prevention (CDC) in Atlanta, Georgia defines a nosocomial infection as a localized or systemic condition that results from adverse reaction to the presence of an infectious agent(s) or its toxin(s) that was not present or incubating at the time of admission to the hospital.

Nosocomial infections have been recognized for over a century as a critical problem affecting the quality of health care and a principal source of adverse healthcare outcomes. Nosocomial infections constitute an important worldwide health problem resulting in high morbidity and mortality as well as economic consequences. It is estimated that out of 100 admissions, approximately 5-6 admission develop nosocomial infections and that in 1995, nosocomial infections cost $4.5 billion and contributed to more than 88000 deaths –one death in every 6 minutes (Weinstein, 1998).WHO in 55

hospitals of 14 countries representing four WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) showed an average of 8.7% of hospital patients had nosocomial infections. At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital (Tikhomirov *et al*., 1987). The highest frequencies of nosocomial infections 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 (Mayon-white, 1988). A study in Brazil reported that in every year 45000 deaths occur due to nosocomial infection and imply an indirect cost of 4.8 billion (Zuliani-Maluf *et al*., 2002). Even in the developed countries like United States, nosocomial infection appears in one of every ten patient admitted and affects approximately two million people annually (CDC, 1992). Nosocomial infection and the consequent mortality reached its peak during the 19^{th} century (Smith *et al.,* 1990). However, recent national prevalence study in different European countries has revealed that during the past twenty years the rate of nosocomial infection has varied from 3.5% to 14.8% (Pittet *et al*., 2005). In Nepal there are very few reports of nosocomial infections and most of the studies are based in capital city only. Tuladhar *et al.,* in 1990 found the occurrence of nosocomial infection to be 10.5%, Similarly Lamichhane *et al*. (2001) showed the overall point prevalence rate of NI to be 2.4%. These findings from around the globe and Nepal indicate that the nosocomial infections are eventually becoming a great threat to the patients and visitors in the hospital where they go to treat for disease.

Nosocomial infections typically affect patients who are immunocompromised because of age, underlying diseases, or medical or surgical treatments. Aging of our population and increasingly aggressive medical and therapeutic interventions, including implanted foreign bodies, organ transplantations, and xenotransplantations, have created a cohort of particularly vulnerable persons. As a result, the highest infection rates are in ICU patients (Weinstein, 1991). NI's have become especially prominent in ICUs, where the incidence is two to five times greater than in general inpatients population (Ewans *et al*.,

1999). ICUs of a hospital are the most frequently identifiable source of nosocomial infections with the high infection rates ranging from 5-30% as well as high rates of antimicrobial resistance (Spencer *et al*., 1994; Archibald *et al.*, 1997 and Fridkin *et al.*, 1999). Although ICU comprise only about 5.0% of all hospital beds, it accounts for 20-25% of all nosocomial infections (Brown *et al.*, 1985; Fraise *et al*., 1997; Spencer *et al*., 1994 and Craven *et al.*, 1996). As NNIS is established recently, there are no documented studies done on this hospital about nosocomial infection, however bacteriological profile of tracheal aspirate of patients attending NINAS showed that out of 50 cases 45 demonstrated bacterial growth whereby most predominat were *P. aeruginosa* and gram negative enteric bacteria (40.30%) followed by *S. aureus* (10.45%), Viridans Streptococci (2.98%) and other gram negative bacteria (5.96%). This is the first study carried out to determine the pattern of microbial isolates in clinical and environmental samples of ICU from NINAS hospital and the association of etiological agent of infection or simply only colonisation present in the clinical specimens to that of environment.

3.2 Intensive Care Unit (ICU)

ICU is a specialized department of a hospital that is well equipped with advanced technological system and run by highly trained medical personnel to provide intensive care to the critically ill patients. ICUs have contributed greatly to the survival of patients with trauma, shock states, and other life-threatening conditions.

Our understanding of ICU pre-dates the infancy of microbiology as a discipline. During the 1854, Florence Nightingale, the lady with a lamp left for the Crimean war where she separated severely wounded soldiers from the less severely wounded thereby reducing the mortality from 40.0% to 2.0% on the battle field and thus creating the concept of intensive care unit. To date, many hospitals all around the world have at least one ICU equipped with required facilities. Therefore, the provision of ICU in a hospital has aided in saving the lives of those that were to reach the end of their lives.

ICU of NINAS hospital in Nepal is one among those that provides facility of health care to the critically ill neurological patients, who otherwise may have left to suffer, get paralyzed or seek expensive treatment outside the country. ICU of NINAS is 12 bedded with high range bedside monitors, good ventilation, excellent air conditioning system as well as an isolation room for barrier nursing of patients with infections. In addition, cleanliness, appropriate evidence based interventions and well trained health care workers is the strength of ICU in NINAS hospital. Thus, ICU of NINAS hospital is effortful in providing the intensive care therapies and medications thereby reducing the rate of mortality of the patients with neurological disorders.

3.3 Nosocomial infections in ICU

ICU patient may not only receive intense care but may acquire NIs that could severely affect their already restrained health status and may lead even to untimely demise. It was reported that the risk of death in nosocomially infected patients was 2.1 times greater than in the patients without such infection (Bueno *et al*., 1994). It has been reported that the rates of NIs in ICU ranges from 12-45% (Tennant *et al.*, 2005; Weber *et al.*, 1999; Esen *et al.*, 2009 and Richards *et al.*, 2003) and are associated with substantial morbidity, mortality and cost. Several studies have shown that survival rate of infected patients is lower than those of non- infected. NNIS data indicate that today's typical hospitalized patient may be sicker than in former years. ICU acquired infections, which are often caused by microorganisms especially by the drug resistant ones, pose a great threat to the patients admitted in ICU. Invasive procedures, high antibiotic usage, transmission of pathogens between patients, and between staffs and patients due to inadequate infection control procedures may explain why ICUs are "hot zones" for the emergence of Multi Drug Resistant (MDR) pathogens and spread of associated NIs.

3.4 Epidemiology of nosocomial infections in ICU

3.4.1 Risk factors

Since the 1980s, infectious disease specialists have recognized that ICU patients acquire nosocomial infections at a much higher rate than patients elsewhere in the hospital. For ICU patients, the risk of acquiring NIs is as much as 5 to 10 times greater than for those on general medical wards of a hospital (Donowitz *et al.*, 1982; Chandrasekar *et al*., 1986, Brown *et al*., 1985; Brawley *et al*., 1989 and Vincent *et al*., 1995). A likely explanation for such increased risk is that ICU patients are frequently exposed to multiple risk factors such as invasive diagnostic and therapeutic procedures, immunosuppressive and sedative medication etc. which ultimately ruins the patients' immune condition. Thus the risk factors that aid in acquisition of nosocomial infections can be broadly categorized into three major groups (Weber *et al*., 1999):

1. Intrinsic risk factors

Intrinsic risk factors related to the need for intensive care include extremes of age, gender, severe underlying diseases and comorbid illnesses, malnutrition, length of ICU stay etc. Aged or elderly people whose immunity has been highly lowered are more prone to infection in ICU. Several studies report that age greater than 60 years is a significant risk factors for ICU acquired NIs (Legras *et al.*, 1998 and Caglayan *et al*., 2005).On the other hand gender has not been considered as the important risk factor in many studies however some studies suggest that male are more prone to NIs than the females (Shaikh *et al*., 2008 and Wang *et al.*, 2001). Similarly underlying diseases and comorbid illness also influence the susceptibility of patients to specific ICU acquired infection. Neurological failure and certain diagnostic categories such as trauma (Appelgren *et al.*, 2001; Vincent *et al*., 1995 and de Leon Rosales *et al*., 2000) and shock (Craven *et al*., 1988) are regarded as major significant risk factors.

The length of ICU stay is another important risk factor for ICU patients in acquisition NIs. The longer the ICU stays, the higher the risk of contracting NIs (Caglayan *et al*., 2005). In a study carried out by Ylipalosaari *et al*. (2007), 23.9% of the patients whose ICU stay was longer than 48 hours acquired a total of 107 infections (1.3 per patient) during their ICU stay.

2. Invasive procedures

Invasive procedures such as central intravenous catheterization (CVC), surgical drainage, haemodialysis, bronchial endoscopy, urinary catheterization, nasogastric tube, tracheostomy, endotracheal intubation and blood transfusion are considered as significant risk factors for nosocomial infection and mortality (Appelgren *et al*., 2001, Fernandez- Crehuet *et al*., 1997 and Wenzel *et al*., 1981***).*** Most frequent nosocomial infection in ICU was generally associated with the use of invasive devices. For example in a study carried out by de Leon-Rosales (2000), 86.0% of pneumonia in ICU patients were ventilator associated, 87.0% of blood stream infections were central venous associated and 95.0% of urinary tract infections were catheter associated. Device associated infections and the significant risk factors related to it are discussed later in separate heading.

On the other hand, excessive use of high dose of therapeutic medications including several immunosuppressive and sedative drugs or antibiotics in ICU has also been reported to play vital role in causing NIs in susceptible patients (Erbay *et al.,* 2003; de Leon Rosales *et al.,* 2000 and Vincent *et al*., 1995).

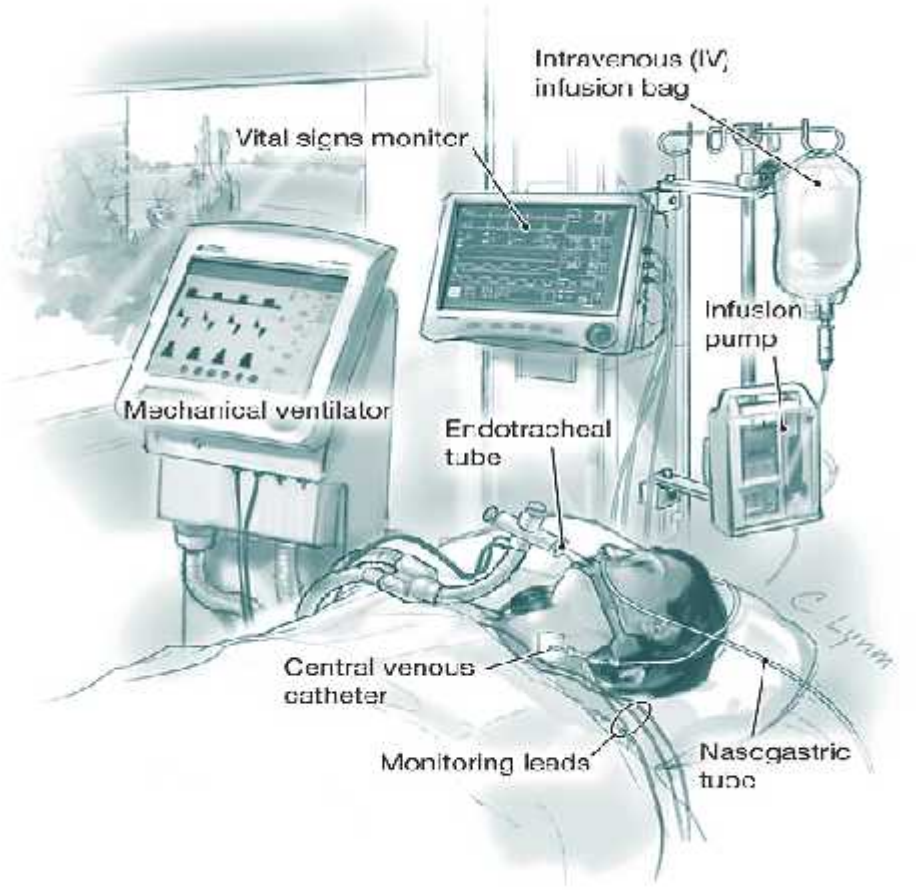


Figure 1: Different invasive devices used in patients in ICU (Vincent *et al.*, 1995)

3. Crowding and animate reservoirs

Crowding (e.g., neonatal ICU) and animate reservoirs (example colonized or infected patients), which increase the risk of cross-infection in the ICU (Chandrasekar *et al*., 1986 and Brawley *et al*., 1989) are also the major risk factors in development of infection. At the other point, breaks in aseptic procedures which are frequently associated with overcrowding, understaffed and low nurse to patient ratio has also assisted in establishment of NIs among ICU patients (Hugonnet *et al*., 2007).

3.4.2 Device associated infections in ICU

NIs from invasive medical devices in the ICU is major threat to patient safety. A device-associated infection is not present or incubating at the time of the patient's admission to the ICU, but becomes apparent during the ICU stay or within 48 hours after transfer from the ICU to another acute care unit within the hospital. Infection in ICU can be categorized as device associated infection such as ventilator associated pneumonia (VAP), catheter related urinary tract infection, catheter related blood stream infection, surgical site infection, etc. which are associated with application of the specific invasive medical procedures in the specific site of patient's body. The prevalence of a particular type of infection may differ in ICU of different hospitals. In some hospitals, VAP is the major ICU acquired infection while it is not in others. Urinary tract infection (UTI) accounted to be the most frequent (31.0%) NIs in a study conducted by NNIS system between 1992-1997 in medical ICUs in the USA, followed by pneumonia (27.0%) and primary bloodstream infection (19.0%) (Pittet *et al*., 1994). However in another study, UTI was the second most prevalent infection accounting 17.6% after pneumonia (46.9%) while blood stream infection (BSI) accounted only 12.0% (Vincent *et al.*, 1995).

3.4.2.1 Urinary tract infection (catheter related)

UTI is an infection of the urinary tract that involves the kidney, ureter, urinary bladder, urethra; tissue surrounded the retro-peritoneal and peri-nephric spaces (Garner *et al*., 1996). The genitourinary tract is the most common site of nosocomial UTI in ICU patients accounting for 20-40% of all NIs (Richard *et al*., 2000; Laupland *et al*., 2002 and Erbay *et al*., 2003). Infections are usually defined by microbiological criteria: positive quantitative urine culture (≥105 microorganisms/ml, with a maximum of 2 isolated microbial species) (WHO, 2002). Catheterization and instrumentation of the urinary tract are considered to be the major predisposing factors in approximately 60 - 80% of the cases of nosocomial UTI (Arunodoya, 2001; Manley *et al*., 2000 and Ozinel

et al., 2004). Study by Burke *et al*. (2004) showed that the vast majority of UTIs occur in patients with temporary indwelling bladder catheters and is frequently termed as catheter associated urinary tract infection (CA-UTI). The causative organisms of catheter-associated urinary tract infections are mainly *E. coli*, *Klebsiella* spp., *Proteus* spp., *Enterococcus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Serratia* spp., and *Candida* spp.. Many of these microorganisms are part of the patient's endogenous bowel flora, but they can also be acquired by cross-contamination from other patients or hospital personnel or by exposure to contaminated solutions or non-sterile equipment.

3.4.2.2 Pneumonia (Ventilator associated)

The definition of pneumonia may be based on clinical and radiological criteria which are readily available but non-specific: recent and progressive radiological opacities of the pulmonary parenchyma, purulent sputum, and recent onset of fever. Diagnosis is more specific when quantitative microbiological samples are obtained using specialized protected bronchoscopy methods (WHO, 2002). Microorganisms colonize the stomach, upper airway and bronchi, and cause infection in the lungs (pneumonia): they are often endogenous (digestive system or nose and throat), but may be exogenous, often from contaminated respiratory equipment such as ventilators. Pneumonia is the second most common NI affecting 27.0% of all critically ill patients in ICU, 86.0% of which are associated with mechanical ventilation (Richards *et al*., 1999) and is termed as Ventilator Associated Pneumonia (VAP). VAP is defined as pneumonia occurring in a patient within 48 hours or more after intubation with an endotracheal tube or tracheostomy tube and which was not present before (Wagh *et al.*, 2009).

VAP may appear within 48 hours and/or beyond 48 hours of tracheal intubation. VAP that occurs within 48 hours after tracheal intubation is usually termed as early onset often resulting from aspiration, which complicates intubation process and often due to antibiotic sensitive bacteria (e.g. Oxacillin-sensitive *S. aureus*, *Hemophilus influenzae* and *Streptococcus pneumoniae*) whereas VAP occurring after 48 hours of intubation is called *late onset* and is frequently caused by Oxacillin-resistant *S. aureus*, antibiotic

resistant *Pseudomonas aeruginosa, Acinetobacter* spp. and *Enterobacte*r spp. (Pingleton *et al*., 1990; Niederman *et al*., 1990; Kollef *et al*., 1995 and Rello *et al*., 1993). In the United States, VAP is the most common and fatal infection of ICU affecting 9-27% of intubated patients thereby resulting in doubling of the risk of mortality as compared with similar patients without VAP (Jimenez *et al*., 1989; Heyland *et al*., 1999; Fagon *et al*., 1996 and Safdar *et al*., 2005).

3.4.2.3 Blood stream infection (catheter related)

Blood stream infection is the infection of blood due to the presence of bacteria or fungi. Nosocomial bloodstream infections (BSIs) occur 2 to 7 times more often in intensive care unit (ICU) patients than in other ward patients with concomitant increases in attributable mortality and economic costs (Jarvis *et al*., 1991; Dashner *et al*., 1982; Donowitz *et al*., 1982 and Pittet *et al*., 1994). It is reported that blood stream infection is the eighth leading cause of death in the United States with attributable mortality rate of 35.0% (Pittet *et al*., 1994). The Australian Commission on Safety and Quality in Health Care (ACSQH) states that many studies in Australia document 17-29% patients with nosocomial blood stream infection die while still in hospital.

Nosocomial blood stream infection associated with the use of intravascular catheters and is often termed as catheter related blood stream infection (CR-BSI), stands on third position among all NIs in critically ill patients in ICU setting (Braunwald *et al*., 2001). Intravascular devices are indispensable in modern-day medical practice. They are used to administer intravenous fluids, medications, blood products, and parenteral nutrition fluids, and to monitor the hemodynamic status of critically ill patients. However, the use of intravascular devices frequently is complicated by a variety of local or systemic infectious complications, including septic thrombophlebitis, endocarditis, BSI and metastatic infection (e.g., osteomyelitis, endophthalmitis, arthritis) resulting from hematogenous seeding of another body site by a colonized catheter.

In the recently published SOAP study, the overall incidence of catheter related blood stream infection was 20.0% (Vincent *et al*., 2006). The most common pathogens responsible for catheter related blood stream infection include Coagulase Negative Staphylococci (CoNS), *S. aureus*, *Enterococcus* spp. and *Enterobacter* spp. (O'Grady *et al*., 2002).

3.4.2.4 Surgical site infection

Surgical site infection (SSI) is an infection that occurs at the incised site. These types of infections are also considered as important nosocomial infections frequently acquired in ICU setting. The NNIS system of CDC defines an infection as a Surgical site infection only if one out of four criteria is fulfilled: (1) purulent drainage (2) a positive culture result from wound swab (3) local symptoms and opened by a surgeon, unless culture result is negative (4) when the diagnosis is made by a surgeon or physician. The infection is considered as hospital-acquired SSI if it occurs within 30 days of the operative procedure if no implant is left in place and within a year if implant is in place and the infection appears to be related to the operative procedure (Garner *et al*., 1996). The infection is usually acquired during the operation itself; either exogenously (e.g. from the air, medical equipment, surgeons and other staff), endogenously from the flora on the skin or in the operative site or, rarely, from blood used in surgery (WHO, 2002).

Based on the type of operation and the underlying status of patient, WHO (2002) reported that the incidence of surgical site infection ranges from 0.5-15%. The proportion of SSIs of all NIs in prevalence studies across Europe has varied between 14.0% and 48.0%, whereas overall NI rates were 4–10% (Gelber *et al*., 2002). In a prospective multicenter study of Italian hospitals, Nicola *et al.* (2008) reported the occurrence of SSI in 241 (5.2%) out of 4,665 patients. In one study, wound infection was shown to be the most common NI (34.0%) in a series with 80.0% of patients admitted due to surgical causes (Appelgren *et al*., 2001). In another study carried in tertiary care hospital in Pakistan by Shaikh *et al*. (2008) in all the patients who were referred from different wards, 22.68% of them developed wound infection in ICU. The

major causative agent responsible for surgical site infections in the ICU may be associated with the unique micro flora around the individual ICU setting.

3.4.2.5 Other nosocomial infections

There are many other potential sites of infection. For example: Skin and soft tissue infections such as open sores (ulcers, burns and bedsores) that encourage bacterial colonization and may lead to systemic infection. Gastroenteritis is the most common nosocomial infection in children, where rotavirus is a chief pathogen while *Clostridium difficile* is the major cause of nosocomial gastroenteritis in adults in developed countries. Similarly, sinusitis and other enteric infections, infections of the eye and conjunctiva, endometritis and other infections of the reproductive organs following childbirth are also important NIs (WHO, 2002).

3.5 Etiological agents of nosocomial infections in ICU

A large number of microorganisms are responsible in etiology of nosocomial infection. In fact any microbe may have the ability to cause an infection in the hospitalized patients. The causative organisms may be broadly classified into three categories: **conventional** pathogens that could cause disease in healthy persons in the absence of any specific immunity to them, **conditional** pathogens that could cause disease (other than simple localized infections) only in persons with lowered resistance to infection or when implanted directly into tissue or normally sterile area and the **opportunistic** pathogens that could cause generalized disease, but only to those patients having a greatly diminished resistance to infection.

The etiological agents differ depending on the origin and source of infection. Many studies report the gram negative rods to be the predominant etiological agent of ICU acquired infections followed by gram positive cocci and fungi (Vincent *et al*., 2006; Alberti *et al.,* 2002; Legras *et al*., 1998 and Vosylius *et al*., 2003). Gram negative rods especially *P. aeruginosa, E. coli* and *Klebsiella* spp. have been shown to be the leading

etiological agent of respiratory and urinary tract infections whereas gram positive cocci predominantly the coagulase negative staphylococci (CoNS), *S. aureus* and Enterococci have been shown to be the cause of catheter related blood stream infection and surgical site infections in the ICU setting (Erbay *et al*., 2003 and Vosylius *et al.*, 2003). The most important organism of concern in ICU mainly constitutes of:

Gram positive cocci

i) Staphylococci

The genus contains many medically important gram positive, nonsporeforming, nonmotile spherical cocci that appears singly, in pairs or in grapes like bunches. The members of this genus are ubiquitously scattered in nature with about a dozen of species being indigenous to humans occurring as a part of human flora and on fomites (inanimate objects). The genus is of immense concern in the hospital settings as they are frequent cause of several nosocomial infections especially in critically ill patients. Staphylococcal infection ranges from the trivial to the rapidly fatal. They are frequently encountered causative agents of surgical site infections, blood stream infections and many other post operative ward infections prevailing in the hospital. Based on the ability to clot blood plasma, the genus Staphylococcus can be categorized into two groups: coagulase positive Staphylococci (CoPS) mainly *S. aureus* and CoNS.

Staphylococcus aureus

It is the most virulent amongst the species of genus Staphylococci and is among the hardiest nonsporing bacteria with greater tendency to survive well in the environment under both moist and dry condition (Forbes *et al*., 2000). Most of the healthy individuals (20-40%) are the carrier of *S. aureus*, often carrying the bacterium on their skin, mucous membranes of the anterior nares and on the mucous membranes of vagina. Such carriers serve as the source of infection to themselves as well as to others through the direct contact route or via contaminated fomites such as doorknobs. The organism is

mainly the cause of soft tissue and skin infections such as impetigo, folliculitis, furuncles, carbuncles , abscesses, pneumonia, sepsis, toxic shock syndrome, etc. and is the commonest cause of late onset VAP. The EPIC (1995) and SOAP (2006) study revealed that *S. aureus* stands on the top position as the most prevalent ICU pathogen and is the cause of 30.0% of nosocomial infections acquired in ICU (Vincent *et al*., 1995 and 2006). Therefore, this bacterium especially the multidrug resistant ones such as MRSA are of great concern as they are difficult to be treated by common antibiotics.

Coagulase negative staphylococci (CoNS)

CoNS are other important etiological agents of NIs frequently in the ICU. These have been recovered as normal commensals of human skin and anterior nares and as a colonizer of implanted invasive devices and catheters (Frebourg *et al*., 1999; Agvald – Ohman *et al*., 2003 and Ronveaux *et al*., 1998). Among the CoNS, *Staphylococcus epidermidis* is the most abundant medically important species and is reported to be the third most commonest etiological agent of nosocomial infection and the most frequent cause of blood stream infections (Spencer 1996 and Pittet *et al*., 1994). *S. epidermidis* can survive for months on medical equipments and devices in the ICU, and is therefore more prone to cause catheter related infections (CRI) due to their ability to adhere and produce biolfilms in such devices (Spencer 1996). CoNS are difficult to treat as they have developed resistant to most of the drugs being commonly used therefore these organisms are often reported to pose serious problems in critically ill patients.

ii) Enterococci

Enterococci are gram positive, round or ovoid, facultatively anaerobic bacteria. They are part of the normal faecal flora however they also colonize oral mucous membranes and skin especially in the hospital settings. These organisms are intrinsically tolerant to chemical agents and unfavorable environmental condition and this power renders them to remain viable on the dry surfaces and fomites for a long period. Enterococci seldom cause diseases in normal healthy individuals but can cause infections like urinary tract

infections, endocarditis, surgical wound infections, intra-abdominal and pelvic infections and abscesses etc. in those patients who have reduced immunity such as the ICU patients. Enterococci are transmitted from one patient to another primarily by the contaminated hands of health care workers, some of whom may carry the enterococci in their gastrointestinal tract or through the contaminated fomites. *Enterococcus faecalis* and *Enterococcus faecium* are of the greatest clinical significance in the ICU setting. These organisms have inherent resistance to many common antibiotics such as cephalosporins, aminoglycosides, β- lactams, penicillinase resistant penicillin, monobactams etc. Therefore these multidrug resistant organisms have extensively increased their number in causing nosocomial infections mainly in the severely ill patients as a result of higher use of broad spectrum antibiotics.

Gram negative rods

i) Enterobacteriaceae

Enterobacteriaceae is a family comprising of large heterogenous group of medically important aerobic or facultative anaerobic gram negative enteric bacilli. They 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 ones include: *E. coli, Klebsiella* spp., *Serratia* spp., *Proteus* spp.

Among the Enterobacteriaceae, *E. coli* is the frequently encountered bacterium in urinary tract infection, intra-abdominal and gut associated wound infection and bacteraemia but the infection is always endogenous and occurs infrequently, even the drug resistant strains occasionally or never spread among the hospitalized patients (Hart 1982 and Jarvis *et al*., 1992).

Klebsiella is another important member of enterobacteriaceae family that causes severe nosocomial infections. Most prevalent species include *K. pneumoniae* and *K. oxytoca*

which are responsible for causing urinary tract infections, bacteraemia and sometime pneumonia in extremely hospitalized patients. *K. pneumoniae* is also emerging as blood stream pathogen. In United States and Canada, *K. pneumoniae* is among the top ten pathogens that cause BSI (Pfaller *et al*., 1998). In United States, drug resistant *K. pneumoniae* was responsible for outbreaks of infections (Woodford *et al*., 2004).

Similarly species of *Serratia* especially *S. marcescens* is also a well known pathogenic organism of hospital setting that causes extra intestinal infections such as those of the lower respiratory and urinary tract among the critically hospitalized patients. The organism has also been found to cause epidemic outbreaks of NI in the ICU and NICU due to their ability to resist action of many drugs which assist them in infecting the immunocompromised patients (Smith *et al.,* 1990).

Proteus spp. is also a commensal of intestinal tract and can cause infection only when it leaves this niche. Proteus infection tends to occur in patients with obstructive lesions of UTI following diagnostic instrumentation or during prolonged catheterization. Of importance in hospital are the *P. mirabilis* and *P. vulgaris* which may cause wound infections, septicaemia and other post operative infections among the hospitalized patients.

ii) Pseudomonas aeruginosa

P. aeruginosa, an aerobic gram negative bacterium, is an opportunistic human pathogen widely distributed in nature including soil, water, air, plants and animals. It is the commonest cause of most of the NIs in the hospital. This nonfermentative multidrug resistant bacterium can survive for long periods on equipments around the patients, therefore is responsible for causation of many device associated infections in the hospitalized patients especially in the intensive care unit. It is the commonest pathogen causing VAP as well as bacteraemia, UTIs, etc. in the ICU patients (Pollack, 2000). Rello *et al.* (1996) reported that about 40-50% of the mortality of intubated patients in hospital is caused mainly due to infection by *P. aeruginosa*. 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 the application of broad spectrum antibiotics that render selection of resistant strain. Isolation of *P. aeruginosa* from healthy carrier or environment is significant only when there is risk of transfer to immunocompromised patients for example by the hands of health care workers or via respirators, etc.

iii) Acinetobacter

Members of the genus *Acinetobacter* are nonmotile, obligate aerobic, encapsulated gram negative coccobacilli widely distributed in nature and are commonly found in soil, water, foodstuffs, on inanimate objects (fomites) and as a part of normal flora of humans and animals. Although *Acinetobacter* are well adapted to survival in diverse environment, a relative lack of virulence factors limits their pathogenecity to patients that are immune debilitated especially to those who are undergoing intensive care therapy. In such patients, *Acinetobacter* can infect virtually any body sites, organ system or tissues and cause respective site specific diseases. This bacterium has been isolated from skin, blood, sputum, pleural fluid and urine, usually in device associated infections. Outbreaks of nosocomial infection due to *Acinetobacter* spp. are extremely problematic in the ICU setting due to their inherent resistivity to multiple drugs that have even forced the unit to close (Onarheim *et al*., 2000). Because of the escalating trend in antibiotic resistance, *Acinetobacter* spp. is posing a great challenge for the treating physician.

iii) Stenotrophomonas maltophilia

This aerobic, nonfermentative gram negative bacterium is a nosocomial pathogen of increasing importance in the intensive care units of many hospitals. Being distributed widely in the ICU environment, it has been found to colonize different equipments used in ICU such as ventilators, dialysis machines, nebulizers etc. It has also been found to

colonize different solution used in the hospital setting (Denton *et al*., 1998 and 2003) and through these solutions, penetrate and diffuse into wounds, mucosal- barriers and urine. The bacterium is responsible to cause lower respiratory tract infections, bacteraemia as well as complications of gastro-abdominal surgery as a result of previous antibiotic therapy.

c) Candida

Fungal infections are also arising as alarming problem in hospital patients most potently in ICU patients. Fungal infections represent nearly 15.0% of all nosocomial infection in ICUs in the United States, with Candida species being the most frequent fungal pathogen. It is regarded as the third most common pathogen causing ICU acquired Intravenous device related BSIs and fourth most common to isolate for causing nosocomial BSIs. The incidence of candidaemia has been shown to be highest in the ICU patients thereby making this pathogen a subject of concern. This fungal species is found as normal body flora of the skin, mouth, vagina and intestine however opportunistically serve as pathogen in immunosuppressed patients. Among the species, *C. albicans* is the most frequently isolated yeast pathogen in the hospitals. It can cause UTI, pneumonia, septicaemia, abdominal abscesses, etc. in patients with lowered immunity. Non albicans species such as *C. glabrata, C. tropicalis, C. guilliermondii and C. dublinien*sis are also emerging in the clinical setting (Tortorano *et al*., 2004 and 2006, Klingspor *et al*., 2004).

3.5.1 Drug resistant pathogens in ICU

ICU is the breeding place for drug resistant pathogens. The occurrence of such multiple drug resistance in hospital associated pathogens has resulted in the emergence and reemergence of those nosocomial infections that are difficult to treat. In 2006, The Antimicrobial Availability Task Force (AATF) of the Infectious Diseases Society of America (IDSA) identified six resistant pathogens with increasing incidence of infections for which there are only a few or no drugs available or will be available in

near future (Talbot *et al*.,2006). These resistant pathogens include: *A. baumannii*, *Aspergillus* spp., extended spectrum β-lactamase (ESBL)–producing Enterobacteriaceae, Vancomycin-resistant *E. faecium*, *P. aeruginosa*, and Methicillin-resistant *S. aureus* (MRSA). These pathogens mainly cause infections in those patients who are already sick enough to be admitted to hospital and more frequently to the ICU.

The emergence of resistance is a result of factors such as excessive and haphazard use of antimicrobial agents (notably the extended-spectrum cephalosporins), increased use of invasive devices and procedures, a greater number of susceptible hosts, and lack of infection control practices leading to increased transmission of resistant organisms. In the hospital, widespread use of broad spectrum antimicrobials in the ICU and for immunocompromised patients has resulted in the selection of multidrug-resistant organisms. Microorganisms have a remarkable array of mechanisms with which to overcome the effects of antimicrobial agents. These include the production of structure-altering or inactivating enzymes (*e.g.,* β-lactamase- or aminoglycoside-modifying enzymes), alteration of penicillin-binding proteins or other cell-wall target sites, altered DNA gyrase targets, permeability mutations, and ribosomal modification (Thornsberry, 1995; Barg, 1995; Gold *et al*., 1996 and Shlaes *et al*., 1995). Microbes acquire such resistance either extrinsically or intrinsically. Similarly, infection caused by multidrug-resistant bacteria constitutes a serious problem for intensive care patients throughout the world as reported by Trilla (1994). Vancomycin Resistant Enterococci (VRE) and Methicillin Resistant *S. aureus* are the major gram positive pathogens of concern in ICU whereas *Pseudomonas* spp. and a wide variety of Enterobacteriaceae are among the important gram negative pathogens. In addition to these organisms, multidrug resistant *Acinetobacter* spp., fluconazole resistant *Candida* spp. and multiply resistant *M. tuberculosis* are responsible to cause significant death by infection among ICU patients. The mortality rate associated with multidrug-resistant Gram-negative enteric bacteria in the patients is high in some intensive care units (ICUs) and in some ICUs the rate is low. In Iran, gram negative bacteria accounted to be in highest number to cause NI in ICU patient followed by Gram positive bacteria and less frequently the yeast

(Hassanzadeh *et al*., 2009). Hence, it is likely that patterns of microbial infection and antibiotic resistance in ICU patients differ widely from one hospital or country to another and are often facilitated by the increasing use of invasive techniques, immunosuppressive drugs and inappropriate antibiotic therapy. In addition, certain types of pathogens are becoming common in each local community that may represent an important risk factor for the morbidity and mortality of ICU patients (Shehabi *et al*., 1996)

Rise of MRSA isolates from 2.4% in 1975 to 29.0% in 1991 suggests that the MRSA is increasing as one of problematic drug resistant pathogen in the hospitals (Emori *et al.*, 1993). As a consequence, empiric use of glycopepetide antibiotics such as Vancomycin for the treatment of MRSA appears to be on the rise. But most recently, strains of *S. aureus* with reduced susceptibility to Vancomycin (VISA) have now also been isolated in Japan and the United States (Hiramatsu *et al*., 1997; CDC, 1997) signaling the possibility of emergence of complete drug resistant pathogen. Such rising use of Vancomycin have even aided in the emergence of Vancomycin resistant enterococci (VRE), especially in the ICU setting (Emori *et al*., 1993**)** as well as CoNS (LeClercq *et al*., 1988 and Schwalbe *et al*., 1987). Infections caused by such resistant pathogens thus pose an alarming threat to the physician leaving very few therapeutic options.

Many studies in recent years have reported and emphasized the development of antibiotic resistance among gram-negative bacilli, with especial focus on *K. pneumoniae*, *P. aeruginosa* and *Enterobacter* spp.These organisms are increasing in incidence among nosocomial pathogens largely because of their ability to express certain resistance phenotypes. Concern over resistance to β-lactam agents among nosocomial gram-negative pathogens has heightened recently because of the increased availability and use of these drugs, particularly cephalosporins.

Emerging resistance has caused a shifting practice in antimicrobial use. The Antimicrobial Availability Task Force (AATF) considers this situation of emergence of drug resistant pathogens in the hospital as a terrible unavoidable crisis. This challenging

crisis associated with antimicrobial resistance should serve as strong incentives for responsible and prudent use of antimicrobial agents. There is a need for both proper use of antibiotics and strict appropriate infection control policies to reduce the emergence of resistance.

3.6 Sources of pathogens in ICU

For an infection to occur in the hospital, there are three important prerequisites: i) a microbe capable of producing an infection ii) susceptible host and iii) an environment that is favorable for the multiplication of microbe. These three factors are referred to as epidemiological triad. Mere presence and the delicate interplay of these three factors finally culminate in the occurrence of infection. ICU patients may acquire the nosocomial pathogens by two major sources:

1) Endogenous sources (self infection)

2) Exogenous sources (cross infection/environmental infection)

3.6.1 Endogenous sources

Endogenous sources , the ones that are normal microbial flora living in complete harmony with the host, however become opportunistic and cause infections in those host whose body's immune system depletes and become vulnerable to opportunistic infections. These may be the transient or permanent flora of the patient. Bacteria present as the normal flora cause infection because of transmission to sites outside the natural habitat (urinary tract), damage to tissue (wound) or inappropriate antibiotic therapy that allows overgrowth (*Clostridium difficile*, yeast species). For example, Gram-negative bacteria in the digestive tract frequently cause surgical site infection after abdominal surgery or urinary tract infection in catheterized patients. Around 80% of theNIs are caused by a patient's own endogenous microbial flora present upon admission to the hospital (Ali *et al.,* 2007).

3.6.2 Exogenous sources

These include microbial sources other than the patients' own flora. In an ICU of a hospital, the exogenous sources may be the animate sources (or source of cross infection) such as HCWs, other patients within the same room and the visitors, while the inanimate sources (or sources of environmental infection) include equipments around patients, air, water, fabrics used by and around the patients, etc.

i) Sources of cross infection: These include flora from other patient or member of staff in ICU. Bacteria are transmitted between patients by following ways:

a) through direct contact between patients (hands, saliva droplets or other body fluids),
b) in the air (droplets or dust contaminated by a patient's bacteria),
c) via staff contaminated through patient care (hands, clothes, nose and throat) who become transient or permanent carriers, subsequently transmitting bacteria to other patients by direct contact during care
d) via objects contaminated by the patient (including equipment), the staff's hands, visitors or other environmental sources (e.g. water, other fluids, food).

ii) Sources of environmental infection: These include flora from the health care environment. Several types of microorganism survive well in the hospital environment:

a) in water, damp areas, and occasionally in sterile products or disinfectants (*P. aeruginosa, Acinetobacter* spp., *Mycobacterium* spp.)
b) in items such as linen, equipment and supplies used in care; appropriate housekeeping normally limits the risk of bacteria surviving as most microorganisms require humid or hot conditions and nutrients to survive.
c) in food

d) in fine dust and droplet nuclei generated by coughing or speaking (bacteria smaller than 10 µm in diameter remain in the air for several hours and can be inhaled in the same way as fine dust) (Ducel *et al.,* 2002).

Thus, animate sources like medical staffs , visitors and patients represent the primary reservoir who carry the organisms in their hands, noses, clothing, etc. whereas the inanimate sources or environment suitable for microbial growth such as air, water, ventilators, humidifiers, nebulizers, urinary catheters, intravenous catheters, endotracheal tubes and other prostheses, foods, fabrics and all those objects that are in close vicinity with patients represent secondary reservoirs of microorganisms (Prescott *et al.,* 2003 and Pelczar *et al.,* 1993). In either of the both exogenous and endogenous case, the infecting organisms may invade the patient's tissues spontaneously or be introduced into them by surgical operation, instrumental manipulation or nursing procedures resulting in deterioration of body's natural defense mechanism and prone to the devastating stage of survival.

Potential for developing NI, both endogenous and exogenous, is higher in critically ill patients admitted in ICU. Infection in ICU has been a problem since these units have existed, primarily in those undisciplined units where policies for antibiotic usage and infection control are conspicuous by their absence (Gaya H, 1976). In ICU, the availability and use of complex and sophisticated machinery for maintaining life in moribund patients increases the proportion of patients who are vulnerably susceptible to infections and who then serve as potent sources for its transmission. The extensive and indiscriminate application of broad spectrum antibiotics as well as prolonged use of immune suppressive drugs is a favorable environment for the endemicity of antibiotic resistant flora in the ICU.

3.7 Mode of transmission

The mode of spread of pathogens within ICU occurs basically by four major ways:

1. Airborne transmission: This type of transmission consists of particles of size five micrometer or less in the air, either as airborne droplets or as dust particles containing the infectious microorganisms. The airborne droplets or dust can be produced during coughing, sneezing or talking as well as from procedures such as suctioning, bronchoscopy etc. In such airborne droplets or dust, the infectious microbe remains viable for up to several hours and can spread around the room over longer distances serving itself as a potent source of airborne infection. In a study carried out by Brachman in 1970 on airborneNIs, reported that around 10-20% of endemic NIs are due to airborne route of transmission. The airborne route of transmission is important for number of pathogenic microorganisms in hospital buildings (Beggs, 2003).These pathogens then reside on several ecological niches inside the hospital setting and subsequently proliferate into air and act as source of airborne NIs especially in the ICU patients who are highly susceptible.

Several gram positive and gram negative bacteria have been isolated from hospital air. Gram positive cocci such as *S. aureus, S. saprophyticus, S. epidermidis, M. luteus* and gram negative rods such as *P. aeruginosa, P. mirabilis, E. coli, E. aerogenes, K. pneumoniae, C. freundii, P. vulgaris, P. fluorescence, M. morganii and S. marcescence* were isolated from the indoor and outdoor air of seventy-six hospitals of Amravati, India (Tambekar *et al.*, 2007). In study at T.U. Teaching Hospital of Nepal, the most prominent bacterial isolates were *Streptococcus* species, *Bacillus* species, Micrococci, *P. aeruginosa* and *K. pneumoniae* (Banjara, 2002). In air sample of ICU and SICU of the same hospital, the microbial flora present were CoNS, CoPS, Micrococci, *Bacillus* spp., Gram negative cocci, Gram negative rods and yeast (Sharma, 2006). Similar study on indoor air of ICU and Post operative ward of Nepal medical college showed presence of *S. aureus*, CoNS, Micrococci, *Streptococcus* spp., *Bacillus* spp., *P. aeruginosa*,

Aspergillus spp., *Rhizopus* spp., Yeast, *Scopulariopsis* spp. (Panta 2006)**.** Occurrence of such pathogens in the indoor air of ICU indicates a serious threat of airborne infections to the immune debilitated patients of that unit. Exposure to these airborne microorganisms to the susceptible host may lead to hazardous health effects such as respiratory problems (Jacobs, 1989), allergic and irritating reactions (Croft *et al*., 1986), infectious diseases (Sattar *et al*., 1987), and hypersensitivity reactions (Woodward *et al*., 1988; Tambekar *et al.*, 2003).

2. Droplet transmission: This involves transmission of pathogens by respiratory droplets produced during coughing, sneezing, talking and several respiratory therapeutic procedures such as bronchoscope, suctioning etc. Respiratory droplets larger than 5 microns do not remain suspended (airborne) in air for long periods of time and fairly close contact with patients (within 1-2 meters) is required for transmission to occur. Organisms such as *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, and the aetiological agents of pneumonic plague, streptococcal pharyngitis and viral infections caused by influenza viruses are among the many organisms transmitted via this route. Nasal carriage of pathogenic organisms either in patient or in health care workers is also an important factor associated with transmission of infection by this mode. Health care workers and patient may carry pathogenic hospital strains in their nose and may disseminate it to the other susceptible patients through droplets or by colonized hands. The droplets formed during coughing, sneezing, talking, etc. by the HCWs or by the patient in the health care setting may contain pathogen present in their nose which may be transmitted to susceptible ICU individuals through airborne route. It is estimated that almost 25.0% of HCWs are nasal carrier who may occasionally carry *S. aureus* in their nares which can cause outbreaks of surgical site infections (Cespedes *et al*., 2002; Luzar *et al*., 1990). MRSA colonization in health care staff from intensive care units of a hospital in Turkey showed that 14 of 98 health-care staffs carried MRSA on their nose (Zer *et al*., 2009) which is a signal of potential source of MRSA to the ICU patients.

3. Contact transmission: Contact transmission is the most important and frequent mode of transmission of NIs. Transmission occurs from one infected or colonized person to a susceptible patient in two methods:

a) Direct-contact transmission: It involves a direct body surface-to-body surface contact and physical transfer of microorganisms between a susceptible host and an infected or colonized person. The direct contact can occur between two patients or between patients and HCWs, with one serving as the source of the infectious microorganisms and the other as a susceptible host. Infections transmitted by direct contact include Streptococcal sepsis, Staphylococcal sepsis, Leprosy, STDs, etc.

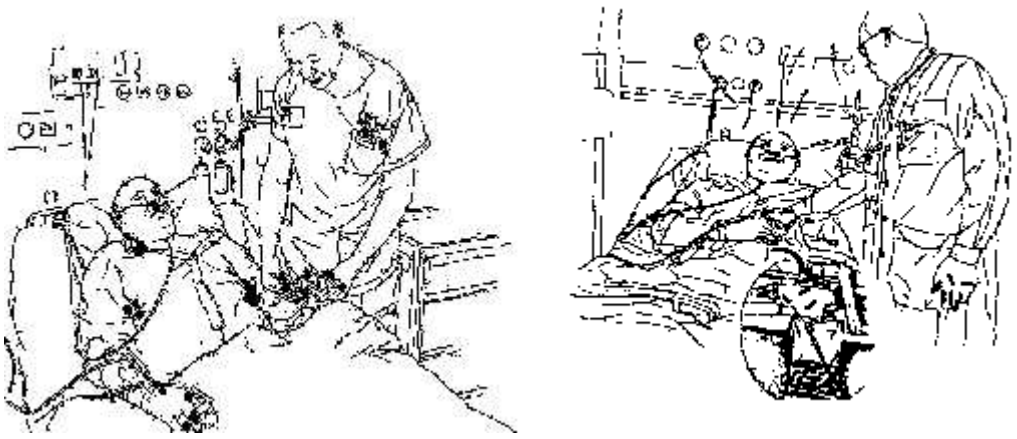
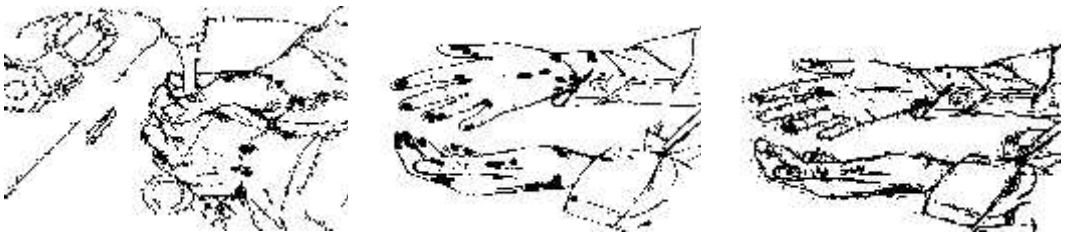


Figure 2: Organism transfer from patient to HCWs' hands (WHO, 2009)



a) **b)** **c)**

Figure 3*:* Organism survival on HCWs' hands (a- improper hand washing, b & c- survival of pathogen on the hands of HCWs) (WHO, 2009)

b) Indirect-contact transmission: It involves contact of a susceptible host with a contaminated intermediate object, usually inanimate, such as contaminated instruments, needles, or dressings, or contaminated hands that are not washed and gloves that are not changed between patients. Most of the NIs that occur among the severely ill patients in the health care facility are due to indirect transmission, mainly via the hands of HCWs. Hands are the most common medium by which pathogenic agents are transferred directly or indirectly from skin, nose and several inanimate surfaces from one HCW to patient or from patient to patient. The hands of nurses and other personnel have been shown to become contaminated with *P. aeruginosa* and to be responsible for its transmission (Kominos *et al.,* 1972).

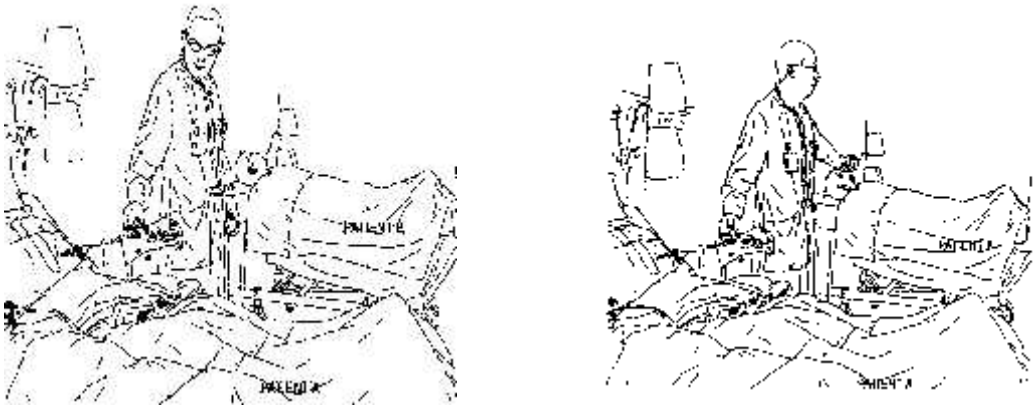


Figure 4*:* Failure to cleanse hands results in between-patient cross-transmission (WHO, 2009)

Hands of HCWs become contaminated either by their own unhygienic behavior or during their routine patient care activities such as respiratory tract care, handling of body fluid and secretion and rupture in the sequence of patient care (Pittet *et al*.,1999). Pittet *et al.* (1999) also demonstrated that the duration and type of patient care adversely affect the hand contamination. A study carried out in a hospital of Germany reported that gram positive bacteria were more frequent than the gram negative bacteria in the hands of medical personnel (Lemmen *et al.,* 2005). Similar study carried out in Nepal medical college (NMC) hospital in Nepal, CoNS was the most prevalent isolate (100%)

followed by *S. aureus*, Micrococci, *Bacillus* spp., *Streptococcus* spp. and *E. coli* in the hands of health care workers (Panta, 2006)**.** These studies suggested that hands of HCWs are in fact the transient habitat of various pathogenic organisms that when gets in entry into the body of immunocompromised patient would not leave without causing infection then and there.

Thus unclean hands and fingers are important risk factors for the transmission of nosocomial infections from patients to patients and from healthcare workers to patients. Overviews of epidemiological evidence conclude that hand mediated contact transmission is the major contributing factor in the current infection threat to the hospitalized patients (Gould, 1991 and Reybrouk, 1986).These include both MRSA and MSSA and multiple drug resistant gram negative aerobes and enterococci. However, regular hygienic hand practices would lower the carriage pattern of hands of health care workers thereby reducing the rates of NI.

4. Common vehicle transmission: It implies the transmission of infectious microorganisms through contaminated items such as food, water, medications, devices, and equipment, fabrics such as privacy curtains around patient, gowns, bed sheet and aprons being worn by patients as well as several inanimate surfaces such door handles, bed rails, bedside tables, working tables, etc.

Water: Water is used in vast quantities in health-care premises. The potable water distributed in health care facility for hydration and hygiene of both patient and health care worker is presumed to be safe. However, many aquatic microorganisms can survive and flourish in water with minimal nutrients and can be transferred to vulnerable hospital patients in direct (e.g., inhalation, ingestion, surface absorption) and indirect ways (e.g., by instruments and utensils). Contamination of the healthcare facility water supply with potentially pathogenic organisms is very common. Hospitals generally draw their water from the municipal water supply. As a consequence of the fact that municipal water, once disinfected at the treatment plant, travels through a system of biofilm-laden pipes before reaching the hospital, waterborne microorganisms have been

found in hospital water tanks, as well as the tap water that flows from faucets and showers. These pathogenic organisms in potable water particularly present in the slime layer or biofilms of the plumbing system under infallible environmental influence may proliferate into active growth forms or may remain for longer period as environmentally resistant forms. Common waterborne pathogens of primary clinical significance include *P. aeruginosa, L. pneumophila, Acinetobacter* spp., *Klebsiella* spp., *Nocardia* spp., *and Mycobacterium avium* complex, *A. fumigates, F. solani, etc.* In a study carried out in TU Teaching Hospital in Nepal, Banjara (2002) indicated that quality of hospital water was not good. The bacterial count (CFU/100ml) of water was observed similar in samples collected from all wards investigated and the isolated bacteria were *E. aerogenes, Serratia* spp., *Acinetobacter* spp., *K. oxytoca* and *P. vulgaris*. If by any means (e.g., direct contact with water during hydrotherapy, ingestion of contaminated water in the form of ice, indirect contact from an improperly processed medical device, inhalation of aerosols dispersed from contaminated water, aspiration of contaminated water) these organisms come in contact to seriously ill patients could serve as a sharp bullet to cause infection. Patient exposure to waterborne microorganisms in the hospital occurs while showering, bathing, drinking water, or ingesting ice. It can also occur through contact with contaminated medical equipment such as tube feed bags, flexible endoscopes, and respiratory equipment that have been rinsed with tap water. The hands of healthcare personnel washed using tap water can also lead to patient exposure (Marrie *et al*., 1992; Darelid *et al*., 1994 and Anaissie *et al*., 2002). Anaissie *et al.* (2002) estimated that 1,400 deaths per year occur in U.S. hospitals due to waterborne healthcare-associated pneumonias caused by *P. aeruginosa* alone. One study reported that healthcare-associated BSIs have been traced to water in the operating room environment, with water or healthcare workers' hands playing a critical role in the contaminating event (Jarvis *et al*., 1996).Healthcare-associated pneumonias account for 20 to 45% of all HAIs and 23,000 deaths per year in the U.S., with 20% of these pneumonias associated with *P. aeruginosa*. This suggests that waterborne *P. aeruginosa* may be a significant contributor to healthcare-associated pneumonia in U.S. hospitals (Anaissie *et al*., 2002).During a seven-month period, Trautmann *et al.* (2001) observed

that 29.0% (5/17) of patients in a surgical intensive care unit were infected with P. *aeruginosa* genotypes that were the same as those detected in the unit's tap water. Many outbreaks of infection or pseudo infection occur through lack of prevention measures and ignorance of the source and transmission of opportunistic pathogens.

From July 1995 to November 1996, multi-resistant *P. aeruginosa* O11 that was multidrug resistant was isolated from 36 patients admitted to a neurosurgery intensive care unit. Nine patients were colonized only; the remaining 27 patients had at least one infected site (17 urinary infections, 10 pneumonias and 4 with sinusitis). *P. aeruginosa* O11 with the same resistance pattern was isolated from tap water (Bert *et al*., 1998). Tap water used for drinking, showering, bathing, ice preparation, and rinsing medical devices presents a potential hazard, especially to at-risk patients. Schaberg *et al*. (1991) reported *P. aeruginosa,* other *Pseudomonas* spp., *B. cepacia, Acineotbacter* spp. and *Enterobacter* spp. as the clinically important opportunistic organisms in tap water that are responsible for colonization, bloodstream infections, Pneumonia and urinary tract infections among medically compromised patients in ICU and burn unit. Faucets and showers are also a source of aerosolized tap water. They produce vaporized water droplets that migrate on almost imperceptible air currents to distant locations within the healthcare environment. The water droplets, which carry infectious pathogens, then settle on surfaces and contact patients and staff through touch contamination or inhalation by susceptible individuals.

Fabrics: Fabrics or clothing being used by health care workers (e.g., uniforms or aprons) or those being used for patients (patient's apron, bed sheet, privacy curtains etc.) can be a source of microorganisms. Fabrics can be found everywhere in the hospital, and sometimes the fabric used most often is the least likely to be cleaned regularly which ultimately indicates the existence of microorganisms. Numerous studies 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 and gram-positive bacteria including Vancomycin-sensitive and -

resistant enterococci (VSE and VRE) and Methicillin-sensitive and -resistant staphylococci (MSSA and MRSA) for at least a day and or more than 90 days on common hospital medical fabrics (Neely, 2000; Neely and Maley, 2000). Similarly, fungi such as *Fusarium*, *Paecilomyces, Mucor, Aspergillus,* various *Candida* spp.were also determined to survive for days to months on such fabrics signifying that fabrics thus can serve as reservoir for transmission of fungal species (Neely and Orloff , 2001). All these studies suggest that fabrics such as healthcare uniforms, scrubs and lab coats can be ideal vehicles for the carriage and transmission of infectious microorganisms.

Gowns worn by **HCWs** have been implicated in the spread of microbes. Pilonetto (2004) analyzed the microorganisms from the uniforms (gowns) of 31 HCWs in a general ICU and reported that 48% of the gowns sampled revealed the presence of *S. aureus*, *A. baumannii*, *K. pneumoniae* and *S. rubidae* and noted that gowns can pick up bacteria from patients and disseminate it within the environment or even to other patients, with increased opportunities for transmission as the HCWs' shift progressed. Such bacterial contamination through hospital gowns, especially in the ICUs where the rate of hospital infections is very high, would help in contact transmission of such pathogens to the patients admitted in that particular unit. In similar studies, MRSA and VRE have been isolated from the gowns worn by patient as well as by health care workers (Boyce *et al*., 1997 and Gould *et al*., 1993). These studies revealed that the gowns used in health care setting which may have become contaminated from environment or any other sources could assist the pathogens to colonize the susceptible host and subsequently result in infections. So proper use and handling of gowns by physicians and other HCWs could minimize cross-contamination and improve patient safety by potentially reducing NIs

Other inanimate hospital environment (surfaces or medical equipments):

Inanimate surfaces and medical equipments have often been described as the source for outbreaks of NIs since a decade. In hospitals, surfaces with hand contact are often contaminated with nosocomial pathogens (Bures *et al*., 2000; Boyce *et al*., 1997 and

Catalano *et al*., 1999) and may serve as vectors for cross transmission. A single hand contact with a contaminated surface results in a variable degree of pathogen transfer. Transmission to hands was most successful with *E. coli*, *Salmonella* spp., *S. aureus* (all 100%) (Scott *et al*., 1990) *C. albicans* (90%) (Rangel-Frausto *et al*., 1994) and several viruses. The most common nosocomial pathogen may well survive or persist on inanimate surfaces and equipments for months and can thereby be a continuous source of transmission if no regular preventive surface disinfection method is processed forth. A recent systematic review by Kramer *et al.* (2006) revealed that most gram-positive bacteria, such as *Enterococcus* spp. (including VRE), *S. aureus* (including MRSA), or *S. pyogenes*, survive for months on dry surfaces. Many gram negative species, such as *Acinetobacter* spp., *E. coli*, *Klebsiella* spp., *P. aeruginosa*, *S. marcescens* and *Shigella* spp.can also survive for months. A few others, such as *B. pertussis*, *H. influenzae*, *P. vulgaris* and *V. cholerae*, however, persist only for days. Mycobacteria, including *M. tuberculosis*, and spore-forming bacteria, including *C. difficile*, can also survive for months on surfaces. *C. albicans* as the most important nosocomial fungal pathogen can survive up to 4 months on surfaces. Persistence of other yeasts, such as *T. glabrata*, was described to be for 5 months or shorter while *C. parapsilosis* survived for 14 days. Similar reports were documented by Hota (2004) despite his data also suggested that contaminated fomites lead to NIs do so indirectly.

Contamination of the inanimate environment-especially bed rails, bed sheets etc. has been most closely associated with Methicillin-resistant *S. aureus* (MRSA), *C. difficile* and antibiotic-resistant *Enterococcus* spp.. These microorganisms also have been found on blood pressure cuffs, dietary trays, intravenous pumps, stethoscopes, utility room sinks, bathroom doors and a sink drain in a patient room. In a study carried out by Gould *et al*. in 1993, VRE has been isolated from different medical equipments, microsphere beds and many environmental surfaces. Those environmental surfaces and sites that are in close proximity to the patients such as bed rails, bedside tables, pullover sheets etc have the highest probability of being contaminated with VRE (Blom *et al*., 2000)**.** Similarly, MDR *A. baumanii* has also been isolated throughout the inanimate

environment- on the beds of the colonized patients and on nearby surfaces (e.g., on mattresses and bedside equipments), in hospital rooms (e.g., on floors, sinks, countertops and door handles) (Das *et al.,* 2002 and Simor *et al.,* 2002). These studies indicate that patients in hospitals, basically in ICU are at greater risk of acquiring pathogens from such contaminated inanimate surfaces which are improperly disinfected. Therefore apt method of disinfection is a must in hospital settings so as to reduce the occurrence of outbreaks of any NI.

As described earlier several equipments or medical devices may also serve as reservoir of pathogenic microorganisms and therefore as source of NI to the patients undergoing invasive procedural treatments. Catheters, ventilators, tracheostomy tube, nebulizers, etc. may become contaminated with microorganisms either from animate sources like hands of HCWs or by inanimate sources like water, air, etc., which when inserted into a immune weak patients lead to entry of the pathogen as well thus leading to a simple infection to life threatening infection. One of the tool that has always been the part of the physician's basic paraphernalia when examining patients, which we call stethoscope, even has been shown to harbor various organisms with CoNS being the most predominant one (Marinella *et al*., 1997; Breathnach *et al.*, 1992). Many studies report other organisms as well including *S. aureus, Corynebacterium* spp., *Bacillus* spp., *Neisseria* spp., Streptococci, *M. luteus, Enterococcus* spp., *Candida* spp., Gram negative organisms and *Aspergillus* spp. (Smith *et al*., 1996; Wright *et al*., 1995 and Jones *et al*., 1992). A study conducted on an Indian Hospital showed high carriage of Methicillin resistant *Stahylococcus* (69.76%) and MDR gram negative bacilli (20.39%) on stethoscopes regularly used by medical professionals (Sengupta *et al*., 2000). In a study carried out by Zuliani Maluf and colleagues (2002) at a hospital in Brazil, random surface samples of diaphragm of 300 stethoscopes employed by medical personnel presented a high rate of contamination with *S. aureus* (58.67%) followed by *CoNS* (51%), yeasts (49.33%), *Sarcina* spp. (21.33%), *Bacillus* spp.(15%), *Streptococcus* spp. (2.33%), *Acinetobacter* spp. (0.67%), *P. putida* (0.33%) and *K. pnemoniae* (0.33%). Because of their universal use among health professionals, it is

advisable to disinfect the stethoscopes on regular basis, which would otherwise become potential vector in the dissemination of hospital pathogens and associated infections.

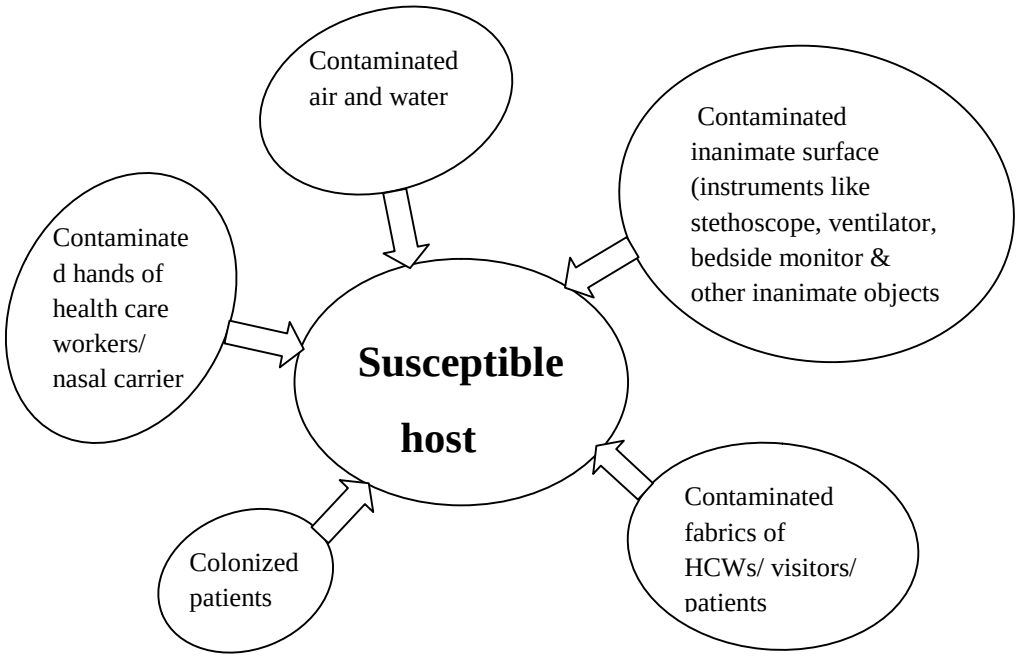


Figure 5: Modes of transmission of microbes from different sources to the susceptible host

3.8 Prevention and control of nosocomial infection at ICU

Because of its association with high morbidity and mortality among patients, it has become extremely necessary to take prompt actions in order to prevent and control NIs in ICU. Traditional infection control measures in ICUs have been directed at limiting person to person spread of infection and improving care of invasive devices but these measures often fail because they have only little effect on patients' endogenous flora, which is an important source of infection in ICUs. Weinstein RA (1991) has summarized the traditional infection control measures used in ICUs which include 4 major steps: **A. Identifying reservoir:** i) Colonized and infected patients and ii) Environmental contamination; common sources. **B. Halting transmission among**

patients: i) Improvement in hand washing and asepsis, ii) Barrier precautions (gloves, gown) for colonized and infected patients, iii) Elimination of any common source; disinfection of environment, iv) Separation of susceptible patients and v) Closing the unit to new admissions if necessary. **C. Halting progression from colonization to infection:** i) Discontinuation of compromising factors when possible (*e.g., m*extubate, remove nasogastric tube, discontinue bladder catheters, as clinically indicated; rotate IV catheter sites; proper ventilator and pulmonary care) and **D. Modifying host factors**: i) Treatment of underlying disease and complications and ii) Control of antibiotic use (rotate, restrict, or cease)

Key elements of an effective infection control measure include: *i) a surveillance system* which is defined as "the ongoing, systematic collection, analysis and interpretation of health data essential to the planning, implementation and evaluation of public health practice, closely integrated with the timely dissemination of these data to those who need to know" (CDC, 1988). It provides the data to identify the infected patients and determine the site of infection and the factors contributing to the infection. Broadly, we can say that surveillance system helps in maintenance of quality of care in hospital. *ii) proper hand washing* before and after contact with each patient or patient equipment (Garner, 1996). *iii) appropriate isolation of patients* with transmissible pathogens, prompt evaluation and intervention in cases of outbreaks (Wendt *et al.*,1997). *iv) adherence to standard guidelines* on disinfection and sterilization of medical equipment (Rutala, 1996) and *v) an effective program* of occupational health focusing on pre-exposure and post-exposure management of health care providers (Bolyard *et al.*,1998).

Infection control is the responsibility of all health care professionals - doctors, nurses, therapists, pharmacists, engineers and others. Proper hand washing, isolation, and disinfection are critical to prevent transmission of resistant pathogens between patients via contaminated equipments and/or contaminated hands of HCWs. Despite these activities, prospectively monitored use of antibiotics constitutes the essential component of infection control programs. It has been reported that infection control programs can

prevent up to 33.0% of nosocomial infection especially those originating from exogenous sources (Greenwood *et al.*, 1997). Thus, by monitoring various factors and implementing various preventive measures wherever required, the incidence of NI can be controlled to a great extent.

Selected patients may require specific precautions to limit transmission of potential infecting organisms to other patients. Isolation and other barrier precautions must be clearly written policies which are standardized, and adaptable to the infectious agent and the patients. These include:

1. standard or routine precautions to be followed for all patients
2. additional precautions for selected patients.

3.8.1 Standard (routine) precautions

To be applied to the care of all patients. This includes limiting health care worker contact with all secretions or biological fluids, skin lesions, mucous membranes, and blood or body fluids. Health care workers must wear gloves for each contact which may lead to contamination, and gowns, mask and eye protection where contamination of clothes or the face is anticipated. Considerations for protective clothing include: *gowns* that should be of washable material, buttoned or tied at the back and protected, if necessary, by a plastic apron; *gloves* that are inexpensive and plastic made are available and usually sufficient; *surgical masks* made of cloth or paper may be used to protect from splashes

3.8.2 Additional precautions for selected patients

These are also known as transmission based precautions that are used in conjunction with standard precautions for selected group of patients such as for those in intensive care unit or other critical care units. These precautions include:

i. Airborne precautions: These precautions are designed to reduce the nosocomial transmission of particles 5 μm or less in size that can remain in the air for several hours and be widely dispersed. Airborne precautions are recommended for patients with either known or suspected infections with particular infectious agents. It is used in addition to Standard Precautions for a patient known or suspected to be infected with microorganisms transmitted by the airborne route. The following is required:

a) individual room with adequate ventilation; this includes, where possible, negative pressure; door closed; at least six air exchanges per hour; exhaust to outside away from intake ducts
b) staff wearing high efficiency masks in room
c) patient to stay in room.

ii. Droplet precautions: These precautions reduce the risks for nosocomial transmission of pathogens spread wholly or partly by droplets larger than 5 μm in size. Used in addition to Standard Precautions for a patient known or suspected to be infected with microorganisms transmitted by large-particle droplets (larger than 5 μm). The following procedures are required:

a) individual room for the patient, if available
b) mask for health care workers
c) restricted circulation for the patient; patient wears a surgical mask if leaving the room

iii. Contact precautions: These are required for patients with enteric infections and diarrhoea which cannot be controlled or skin lesions which cannot be contained. These precautions are used in addition to Standard Precautions for a patient known or suspected to be infected or colonized with microorganisms transmitted by direct contact with the patient or indirect contact with environmental surfaces or patient care items.

a) individual room for the patient if available; cohorting of patients if possible
b) staff wear gloves on entering the room; a gown for patient contact or contact with contaminated surfaces or material
c) wash hands before and after contact with the patient, and on leaving the room
d) restrict patient movement outside the room
e) appropriate environmental and equipment cleaning, disinfection, and sterilization

iv. Absolute (strict) isolation: Such isolation is required where there is risk of infection by a highly virulent or other unique agent of concern where several routes of transmission are implicated.

a) individual room, in an isolation ward if possible
b) mask, gloves, gowns, cap, eye protection for all entering the room
c) hygienic hand washing at entry to and exit from the room
d) incineration of needles, syringes
e) disinfection of medical instruments
f) incineration of excreta, body fluids, nasopharyngeal secretions
g) disinfection of linen
h) restrict visitors and staff
i) daily disinfection and terminal disinfection at the end of the stay
j) use of disposable (single-use) equipment
k) appropriate transport and laboratory management of patient specimens.

(WHO, 2002; Garner JS and HICPAC, 1996)

All hospitals should be capable of providing such preventive and control measures in order to successfully treat and cure their patients.

3.9 Prevention and control of spread of MDR microorganisms

To counteract the spread of alarmingly increasing MDR pathogens such as MRSA, VRE, ESBL producing organisms, carbapenem resistant *P. aeruginosa*, *K. pneumoniae*, etc., 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. 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.

Interventions targeted for optimizing antimicrobial use include*:* optimizing antimicrobial prophylaxis for operative procedures; optimizing the choice and duration of empiric treatment; improving antimicrobial prescribing by educational and administrative means and monitoring and providing feedback regarding antimicrobial resistance and defining and implementing healthcare delivery system guidelines for important types of antimicrobial use.

Similarly, interventions aimed at preventing nosocomial transmission of resistant organisms include: developing systems to recognize and report trends in antimicrobial resistance within institutions; developing systems to rapidly detect and report resistant microorganisms in individual patients and ensuring rapid response by caregivers; increasing adherence to basic infection control policies and procedures; incorporating detection, prevention and control of antimicrobial resistance into institutional strategic goals and providing the required resources and developing a plan for identifying, transferring, discharging, and readmitting patients colonized patients colonized with specific antimicrobial resistant pathogens.

The surveillance and infection control carried out in ICU of a 2,000-bed, university-affiliated hospital in Italy by Orsi *et al.* in 2005 proved that routine surveillance for NIs, coupled with new measures to prevent infections and a revised policy for antimicrobial therapy, reduces the HAIs in ICU and the mortality rate.

CHAPTER IV
MATERIALS AND METHOD

The present study was conducted from July to December 2009 at the microbiology laboratory of NINAS hospital, Bansbari, Nepal. The study was carried out on patients, and animate and inanimate environment including hands and anterior nares of HCWs, air, water, fabrics/clothes and various inanimate surfaces of ICU. Altogether 687 clinical specimens and 677 animate and inanimate environmental samples of ICU were analyzed microbiologically. The materials used during the study period are presented in the Appendix II.

4.1 Clinical specimens

4.1.1 Collection of specimens

All the clinical specimens from ICU patients were collected by experienced physician on daily basis as per the guidelines of the department or when the attending physician suspected infection based on systemic signs (unexplained fever, chills, and hypotension), and/or local signs (purulent tracheal aspirates in mechanically ventilated patients or tracheostomised patients, purulent urinary drainage, or pus or pain at a vascular catheter insertion site). The specimens included tracheal aspirate, CSF, blood, urine, CVP tip, catheter tip, Foley's catheter tip, EVD tip, lumber drain tip, nasal swab, gastrostomy site collection, intracerebral collection, sputum, pus aspirate and wound swab. Immediately the collected specimens were sent to the routine microbiological laboratory for investigation (culture and sensitivity). The clinical isolates were first identified by conventional method. A positive culture was defined as identification of the organism on Gram stain followed by growth of the organism in the suitable culture medium (Hassanzadeh *et al.,* 2009; Esen *et al.,* 2009 and Eggimann *et al.,* 2001). The result obtained from the repeated specimens was included in this study however the

specimens that were improperly labeled and not collected in a sterile vile were excluded from the study.

4.1.2 Processing of the specimen

I. **Macroscopic examination:** All the specimens were visually observed for color, consistency, turbidity, presence or absence of blood or pus depending upon the type of specimen.

II. **Microscopic examination:** Gram's staining of some specimens like Tracheal aspirate, CSF were prepared and observed under microscope for presence of distinguishable bacterial cells as well as pus cells (Forbes *et al.*, 2007).

III. **Culture:** All the specimens (except blood which was preinoculated with BHI broth) were inoculated in the MacConkey agar (MA), Blood agar (BA) and Chocolate agar (CA). The CA plates were then incubated at 37°C for 24 hours in candle jar while BA and MA plates were incubated under aerobic condition at 37°C for 24 hours.

IV. **Isolation and identification:** After 24 hours of incubation, the visual growth of the organism was observed on the media plate. Then the organism were isolated and identified on the basis of colony morphology, Gram reaction and biochemical properties which included Catalase test, Oxidase test, Methyl Red (MR) test, Voges- Proakauer (VP) test, Citrate utilization test, Triple sugar iron (TSI) agar test, Sulphide Indole motility (SIM) test, Urea hydrolysis test and Coagulase test (Forbes *et al.*, 2007, Collee *et al*., 1996). The details of these biochemical processes are given in the appendix VI.

V. **Antibiotic sensitivity testing:** Antibiotic sensitivity tests of all clinical isolates were performed by modified Kirby- Bauer disc diffusion method recommended by Clinical Laboratory Standard Institute (CLSI) guidelines using the antibiotic discs of HiMedia Company and Becton-Dickinson and Company (Cefoperazone/Sulbactam).The detail procedure is given in the appendix IV.

4.2 Animate and inanimate environmental samples

4.2.1 Collection of the samples

Once it was identified that a particular organisms was being constantly and repeatedly isolated from the specimens of admitted ICU patients, sent repeatedly on each day, it was suspected that the patient might have become infected by a pathogen that may have remained as endemically in that unit of hospital or elsewhere around the hospital. Therefore, animate objects including HCW's hands and their anterior nares and the inanimate objects in the vicinity of patients as well as air and water circulated in the ICU were randomly sampled and microbiologically processed in the laboratory so as to trace out any possible source of the pathogen causing colonization or infection.

4.2.1.1 Samples from HCWs

Hand imprint samples: Hand imprint samples were collected from the HCWs of ICU in two different times: before they wash their hands (i.e., before the initiation of their regular duties) and after they wash their hands (i.e., after they complete their routine work) in order to determine the carrier pattern of their hand as well as their hand hygiene practices. The samples were collected by direct fingerprinting into MacConkey agar (MA), Mannitol Salt agar (MSA) and Nutrient agar (NA) media plates one half each for before wash and after wash. Then these plates were carefully transported to microbiology laboratory.

Nasal samples: Nasal samples were collected from the HCWs of ICU to determine their carrier pattern of the organisms. The samples were collected by swabbing the two anterior nares with the help of presterilized cotton swabs. The swabs were then immediately transferred to the microbiology laboratory.

4.2.1.2 Swab samples of fabrics/clothes

Clothes or fabrics that are regularly used or are in close contact with patients, ICU HCWs and the visitors were sampled to determine the prevalence of microorganisms present within the clothes. The clothes sampled included the Bed sheet of patients, Gowns worn by patients, Privacy curtains hung around patients, ICU entry Gown of HCWs and visitors. Sampling was done by swabbing the entire portion of the used clothes with the aid of sterile cotton swabs. Then these swabs were immediately transported to the microbiology laboratory for investigation.

4.2.1.3 Air sample

30 indoor air samples of ICU were collected on MA, MSA and NA media plate by plate exposure method for a predefined period of time (i.e. 30 minutes.). Air sampling was done from all around the corners and the isolation room of ICU. The exposed plates were immediately transported to the microbiology laboratory for further processing. The sample of air was collected using s Plate exposure technique whereby the plates containing solid culture media were exposed to the air of ICU for a pre defined period. The plates were left open with the lid by its side in normal position for a time period of 30 minutes. The plates were then covered with the lid, labeled properly and then carefully transferred to the microbiology laboratory in sealed condition and incubated at 37°C for overnight.

4.2.1.4 Water sample

500 milliliters of water samples each from two of the ICU taps (main hand washing tap which is used by HCWs, patients and visitors and the other restroom tap used only by HCWs) and its collecting tanks and reservoirs and from the drinking water tap were collected in a stoppered sterile glass bottles for bacteriological examination of water. The samples were then transported to laboratory for bacteriological examination and processed by standard MPN method for coliforms in treated water as described by

Cheesebrough (2000). Conventional culture and identification technique was performed for noncoliforms.

4.2.1.5 Swab samples of various inanimate surfaces

Surface samples were collected from different inanimate objects. These objects included Bed rails, Door handles, Tables and Equipments. The samples were collected by swabbing the surfaces with the help of sterile cotton swabs dipped in normal saline prior to sterilization. The swabs were then transported to microbiology laboratory for further processing to determine the pattern of microbial occurrence.

4.2.2 Processing of the samples

Culture: The samples including hand imprints of ICU HCWs and indoor air of ICU collected directly into the solid culture medium and transported to microbiology laboratory, were immediately incubated at 37°C for 24 hours. The samples that were collected in swabs were first streaked into culture medium (MA, BA & MSA), appropriately labeled and then incubated at 37°C for 24 hours.

The water samples collected in sterile bottles were processed by standard MPN method for coliforms in treated water as described by Cheesebrough (2000) and by conventional culture technique for noncoliforms. Presumptive coliform count was done by most probable number (MPN) method of coliform organism in 100 ml of water for diagnosis of bacteriological contamination of coliforms and by conventional culture techniques for presence of noncoliform in the treated water samples. The test was carried out by inoculation of measured quantities of water sample i.e. 50ml and 10ml respectively into a bottle of 50ml and into 5 bottles of 10ml of double strength Mac Conkey broth (purple) for 24 hours at 37°C.

Isolation and identification: After proper incubation the plates were examined for the appearance of colonies of microorganisms and the colony characters were studied.

Similarly, the MPN bottles were observed for color change and gas production to determine the presumptive coliform count in water. The bottles showing, color change and gas formation were regarded as 'Presumptive Coliform Positive'. The results of MPN were interpreted by probability tables from the no. of tubes showing acid and gas (fermentation by coliform organisms). WHO standard which recommend not more than 5 *E. coli* count per 100ml in chlorinated water supplies was used in the study to define the sample as satisfactory or unsatisfactory. On the other hand, the samples of all tubes including even the ones not showing color change and gas formation but were highly turbid, were also inoculated into MA and BA plate in order to isolate and identify the noncoliforms present in water samples and incubated at 37°C for overnight and the colony characters were studied the next day. Based on the colony morphology, grams reaction, biochemical properties (Catalase test, Oxidase test, Citrate utilization test, TSI agar test, MRVP test, SIM test and Urea hydrolysis test), the organisms from all the samples were identified accordingly. The details of biochemical tests and media are given in the appendix III and VI.

Antibiotic sensitivity testing: Antibiotic sensitivity tests of 43 environmental isolates were performed following the recommendation of CLSI.

4.3 Purity plate culture

Purity plate culture of each biochemical test of all clinical, animate and inanimate environmental isolates was performed to ensure that the inoculum used for biochemical test was a pure culture and also to observe whether the tests were preceded in an aseptic condition. For this , the 4 hours inoculated broth culture prepared for biochemical test was inoculated on one half of the Nutrient Agar (NA) plate prior to the processing of biochemical test whereas the other half of the same NA plate was inoculated with same inoculum immediately after completion of biochemical test. The plate was then incubated at 37°C for 24 hours. The pure growth of same organism in both pre and post

inoculated portion of the media plate was considered as the indication of aseptic condition throughout the processing.

4.4 Quality control

Quality control was applied in various areas during the study period for the accurate interpretation of results.

a. Aseptic technique was followed during the collection and transportation of samples from staffs, clothing and environment so as to avoid contamination.
b. During sample processing, all the tests were performed carefully in aseptic zone.
c. During the preparation, sterilization, storage and use of media, instructions provided by the manufacturer were strictly followed to avoid alteration of nutritional, selective, inhibitory and biochemical properties of the media.
d. The performance of newly prepared media were tested using the control species of bacteria (i.e., known organisms giving positive and negative reactions).
e. The QC of stains and reagents were maintained by preparing a control smear and staining it with the stains and reagents to be checked.
f. Control strains of *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *Enterococci* (ATCC 29212) were used for the standardization and correct interpretation of zone of inhibition of antibiotics during Antibiotic susceptibility testing.

4.5 Associating clinical and environmental isolates

On the basis of similarity in occurrence pattern, microscopy, colony morphology, pigmentation, biochemical characters, coagulase test results and antibiotic sensitivity pattern, the association between the clinical isolates from patients and environmental isolates from the HCWs, Clothes/Fabrics being used by HCWs, visitors and patients, air, water and other inanimate objects, was determined and the sources of

microorganism (that may be contaminating, colonizing or may be in fact infecting the patient) in the clinical isolates were identified.

Simulatneously, statistical analysis using Chi–square (χ^2) test was done to show the significant association of the clinical isolates with that of different environmental isolates from various animate and inanimate objects. If p-value calculated at 5% level of significance was less than 0.05, the results were considered significant.

CHAPTER V

RESULTS

During the six months study period, a total of 687 clinical specimens from ICU patients and a total of 677 possible environmental source samples (including both animate and inanimate objects) were collected and microbiologically processed in the Microbiology laboratory of the hospital. The clinical specimens included tracheal aspirate (498), CSF (98), urine (34), blood (10), sputum (6), pus aspirate (6), wound swab (5), gastrostomy site aspirate (1), intracerebral collection (1), peritoneal fluid (1), nasal swab (1) and tip cultures of clinical devices being inserted in patients (26). Environmental samples included air samples (30), water samples (24), fabrics/cloth samples (217) and swab samples from inanimate surfaces (251) in close vicinity of patients. Similarly, 100 hand imprint samples (50 each before and after hand wash) and 55 nasal samples of HCWs employed in ICU were also taken and microbiologically analyzed to determine the carriage pattern and to determine whether or not the carriage organism was associated with the clinical isolates.

5.1 Microbiological investigation of clinical specimens

During this study period, a total of 57 ICU patients admitted in ICU were investigated. Of these 57 patients, 46 (80.70%) were male and 11(19.30%) were female with the age range from 1 to 80 years (median age = 47.5). Among these 57 patients, 21 of them expired when the study was ongoing.

Table 1: Distribution of total clinical ICU cases according to Gender and Age

Age groups	Male n (%)	Female n (%)	Total N (%)	Median age
1-10	3 (6.52)	-	3 (5.26)	**47.5**
11-20	3 (6.52)	2 (18.18)	5 (8.77)	
21-30	9 (19.57)	1 (9.09)	10 (17.54)	
31-40	7 (15.22)	-	7 (12.28)	
41-50	5 (10.87)	-	5 (8.77)	
51-60	9 (19.57)	5 (45.46)	14 (24.56)	
61-70	8 (17.38)	2 (18.18)	10 (17.54)	
71-80	2 (4.35)	1 (9.09)	3 (5.26)	
Total	**46 (100)**	**11 (100)**	**57 (100)**	

Among the 57 patients in ICU, almost half of them (n=21, 36.85%) were admitted with Road traffic accident (RTA) or traumatic injury. Rest of the other patients admitted with cerebro-vascular diseases (22.81%), post operative complications (21.05%), brain associated tumor (14.04%) and Japanese-B Encephalitis (1.75%), Guillain- Barre syndrome (1.75%) and meningitis (1.75%).

Table 2: Distribution of total clinical ICU cases on the basis of diagnosis

Diagnosis	Male		Female		Total	
	N	**%**	**N**	**%**	**N**	**%**
RTA (Trauma)	19	41.31	2	18.18	**21**	**36.85**
Meningitis	1	2.17	-	-	**1**	**1.75**
Guillain-barre syndrome	1	2.17			**1**	**1.75**
Japanese-B encephalitis	1	2.17	-	-	**1**	**1.75**
Post-operative complications	9	19.57	3	27.27	**12**	**21.05**
Cerebro-vascular diseases	9	19.57	4	36.37	**13**	**22.81**
Tumor	6	13.04	2	18.18	**8**	**14.04**
Total	**46**	**100**	**11**	**100**	**57**	**100**

(-) = not present, (n) = number of male or female cases, (N) = Total number of particular cases

Among the 687 clinical specimens (including tip cultures of clinical devices that were being inserted into patients), tracheal aspirates were received from 64.91% (n=37) patients, CSF from 31.58% (n=18) patients, urine from 36.84% (n=21) patients, blood cultures from 15.79% (n=9) patients and CVP tips from 21.05% (n=12) patients. Similarly, sputum from 10.53% (n=6) patients, pus aspirate from 6 patients, Foley's catheter tips from 5.26% (n=3) patients and catheter tips from 3.51% (n=2) patients were received. Further, wound swabs from 7.02% (n=4) patients, EVD drain tips from 3.51% (n=2) patients, lumber drain tips from 3.51% (n= 2) patients and rest of other were received only once from single individual patient.

Table 3: Distribution of clinical specimens requested for microbiological investigation

Type of specimens	Total specimens received		ICU patients whose clinical specimen was sent for investigation	
	Frequency (n)	Percentage (%)	Frequency (n*)	Percentage (%)
Tracheal aspirate	498	72.49	37	64.91
CSF	98	14.26	18	31.58
Urine	34	4.95	21	36.84
Blood cultures	10	1.46	9	15.79
CVP Tip	13	1.89	12	21.05
Catheter Tip	4	0.58	2	3.51
Foley's catheter Tip	4	0.58	3	5.26
Sputum	6	0.87	6	10.53
Pus aspirates	6	0.87	6	10.53
Wound swab	5	0.73	4	7.02
Gastrostomy site aspirate	1	0.15	1	1.75
Intracerebral collection	1	0.15	1	1.75
Lumber drain Tip	2	0.30	2	3.50
Peritoneal fluid	1	0.15	1	1.75
Nasal swab	1	0.15	1	1.75
EVD drain Tip	2	0.30	2	3.50
Venous cut Tip	1	0.15	1	1.75

(n) = No. of total specimens received & (n*) = No. of patients from whom particular specimen was sent

Out of the total specimens investigated, significant growth was obtained in 58.81% (n=404) specimens from 43 (male=36 and female=7) patients, 20 (46.51%) of whom had polymicrobial growth. On the other hand, specimens such as EVD tip culture, lumber drain tip culture, intracerebral collection and peritoneal fluid showed no growth of microorganisms. Table 4 shows the data of significant growth positivity of microorganisms in different specimens.

Table 4: Distribution and analysis microbiological growth in various specimens

Types of specimen	Growth obtained/Total No. received	Percentage (%)
Tracheal aspirate	356/498	71.48
CSF	2/98	2.04
Urine	20/34	58.82
Blood cultures	3/10	30.00
CVP Tip	2/13	15.38
Catheter Tip	3/4	75.00
Foley's catheter Tip	4/4	100.0
Sputum	3/6	50.00
Pus aspirates	5/6	83.33
Wound swab	3/5	60.00
Gastrostomy site aspirate	1/1	100.0
Intracerebral collection	-/1	_
Lumber drain Tip	-/2	_
Peritoneal fluid	-/1	_
Nasal swab	1/1	100.0
EVD drain Tip	-/2	_
Venous cut Tip	1/1	100.0
Total	**404/687**	**58.81**

(-) = Growth not obtained

Of the total culture positive specimen**,** 61.39% (n=248) were positive for *P. aeruginosa* followed by 24.75% (n=100) for *K. pneumoniae*, 22.77% (n=92) for *Acinetobacter* spp.,

7.67% (n=31) for *E. coli*, 4.95% (n= 20) for *S. aureus*, 1.24% (n=5) each for *K. oxytoca* and *P. mirabilis* and 0.74% (n=3**)** for *M. catarrhalis.*

Table 5: Distribution of clinical isolates obtained from the culture positive specimen

Type of sample	**Types of clinical isolates**							
	***P. aeruginosa* n(%)**	***Acinetobacter* spp. n(%)**	***E. coli* n (%)**	***K. pneumoniae* n(%)**	***K. oxytoca* n(%)**	***M. catarrhali* n(%)**	***P. mirabilis* n(%)**	***S. aureus* n(%)**
Tracheal aspirate (N=356)	228(64.04)	92(25.84)	23(6.46)	86(24.16)	3(0.84)	3(0.84)	5(1.40)	13(3.65)
CSF (N=2)	2(100)	_	_	_	_	_	_	_
Urine (N=20)	6(30.0)	_	6(30.0)	6(30.0)	2(10.0)	_	_	_
Blood (N=3)	2(66.67)	_	_	_	_	_	_	1(33.33)
Catheter tip (N=3)	1(33.33)	_	_	2(66.67)	_	_	_	_
CVP tip (N=2)	2(100)	_	_	_	_	_	_	_
Foley's catheter tip (N=4)	1(25.0)	_	1(25.0)	2(50.0)	_	_	_	
Gastrost-omy site aspirate (N=1)	_	_	1(100)	_	_	_	_	_
Nasal swab (N=1)	1(100)	_	_	_	_	_	_	_
Pus aspirate (N=5)	2(40.0)	_	_	1(20.0)	_	_	_	4(80.0)
Wound swab (N=3)	1(33.33)	_	_	_	_	_	_	2(66.67)
Sputum (N=3)	1(33.33)	_	_	2(66.67)	_	_	_	_
Venous cut tip (N=1)	1(100)	_	_	_	_	_	_	_
TOTAL (N=404)	**248(61.39)**	**92(22.77)**	**31(7.67)**	**100(24.75)**	**5(1.24)**	**3(0.74)**	**5(1.24)**	**20(4.95)**

(N) = Total No. of culture positive specimen, (n) = Total No. of positive specimen for particular microorganism and (-) = Absence of particular organism

5.1.1 Antibiotic sensitivity pattern of organisms isolated from different growth positive clinical specimen

P. aeruginosa isolated from 248 growth positive specimen was found to be highly sensitive towards Imipenem (84.68%, n=210) and Cefoperazone/Sulbactam (64.92%, n= 161) while was resistant to Cefepime/Tazobactam (99.19%, n= 22) followed by Cefotaxime (97.58%, n= 223) and Piperacillin/Tazobactam (87.90%, n=218). Some isolates of *P. aeruginosa*, consistently present and susceptible to usual drugs was in later stages found to show resistance to most antibiotics used, except Polymyxin-B in some of the tracheal aspirate samples as presented in the table (16).

Table 6: Antibiotic sensitivity pattern exhibited by *P. aeruginosa*

Antibiotics	Total *P. aeruginosa* (N)	Sensitive		Intermediate		Resistance	
		n	%	n	%	n	%
Amikacin (10mcg)	248	117	47.18	6	2.42	125	50.40
Ciprofloxacin (5mcg)	248	116	46.78	3	1.20	129	52.02
Cefotaxime (30mcg)	248	6	2.42	-		242	97.58
Cefoperazone/Sulbactam (75/30µg)	248	161	64.92	-		87	35.08
Cefepime/Tazobactam (30/10mcg)	248	2	0.81	-		246	99.19
Gentamicin (10 mcg)	248	113	45.56	6	2.42	129	52.02
Imipenem(10 mcg)	248	210	84.68	-		38	15.32
Ofloxacin (5 mcg)	248	114	45.97	3	1.21	131	52.82
Piperacillin/Tazobactam (100/10mcg)	248	30	12.10	-		218	87.90

(n)= no. of sensisitve or resistance organisms

K. pneumoniae isolated from 24.75% (n=100) of the growth positive specimens was found to be highly sensitive towards Imipenem (94.0%, n=94) followed by

Cefoperazone/Sulbactam (85.0%, n=85), Chloramphenicol (79.0%, n=79) and Amikacin (75.0%, n=79) but was found resistance towards Ampicillin (99.0%, n=99), Cefotaxime (99.0%, n=99), Piperacillin/Tazobactam (95.0%, n=95), Gentamicin (80.0%, n=80) and Cotrimoxazole (79.0%, n=79). But *K. pneumoniae* isolated from 2.0% (n=2) of the total specimens that showed resistance to almost all antibiotics used in the test were however sensitive towards Polymyxin B (table 16)

Table 7: Antibiotic sensitivity pattern exhibited by *K. pneumoniae*

Antibiotics	**Total *K. pneumoniae* (N)**	**Sensitive**		**Resistance**	
		n	**%**	**n**	**%**
Ampicillin (10 mcg)	100	1	1.0	99	99.0
Amikacin(10 mcg)	100	75	75.0	25	25.0
Ciprofloxacin (5mcg)	100	41	41.0	59	59.0
Cefotaxime (30mcg)	100	1	1.0	99	99.0
Cefoperazone/Sulbactam (75/30µg)	100	85	85.0	15	15.0
Cotrimoxazole (25 mcg)	100	21	21.0	79	79.0
Chloramphenicol (30mcg)	100	79	79.0	21	21.0
Gentamicin (10mcg)	100	20	20.0	80	80.0
Imipenem (10 mcg)	100	94	94.0	6	6.0
Ofloxacin (5mcg)	100	31	31.0	69	69.0
Piperacillin/Tazobactam (100/10mcg)	100	5	5.0	95	95.0

(n)= no. of sensitive or resistance

On the other hand, 12 of the 31 isolates of *K. pneumoniae* from catheter tip culture, urine and pus aspirate showed 100% sensitivity towards Nitrofurantoin

Table 8: Antibiotic sensitivity pattern exhibited by *K. pneumoniae* isolated from urine, catheter tip, Foley's catheter tip and pus aspirate

Antibiotics	Total *K. pneumoniae* (N)	Sensitive		Resistance	
		n	%	n	%
Ampicillin (10mcg)	12	0	0.0	12	100
Amikacin (10mcg)	12	5	41.67	7	58.3
Ciprofloxacin (5mcg)	12	4	33.33	8	66.7
Cefotaxime (30mcg)	12	0	0.0	12	100
Cefoperazone/Sulbactam (75/30µg)	12	5	41.67	7	58.3
Cotrimoxazole (25mcg)	12	0	0.0	12	100
Chloramphenicol (30mcg)	12	8	66.67	4	33.3
Gentamicin (10mcg)	12	0	0.0	12	100
Imipenem (10mcg)	12	9	75.0	3	25.0
Ofloxacin (5mcg)	12	1	8.33	11	91.7
Piperacillin /Tazobactam (100/10mcg)	12	0	0.0	12	100
Nalidixic acid (30mcg)	12	0	0.0	12	100
Norfloxacin (10mcg)	12	0	0.0	12	100
Nitrofurantoin (100mcg)	12	12	100	0	0.0

The AST pattern of *Acinetobacter* spp. isolated only from 22.77% (N=92) of culture positive clinical specimens (only isolated from tracheal aspirate and not from other type of sample), showed resistance towards Ciprofloxacin (100%), Cotrimoxazole (100%), Chloramphenicol (100%), Gentamicin (100%), Ofloxacin(100%) and Piperacillin/Tazobactam (100%) while only 1.08% (n=1) was sensitive to Amikacin, 3.26% (n=3) were sensitive to Cefoperazone/Sulbactam, 13.04% (n=12) were sensitive to Imipenem. Like *P. aeruginosa*, 86.96% (n=80) of the *Acinetobacter* spp. isolated

from the similar samples exhibited resistance towards most antibiotic used. Nonetheless the isolates were sensitive to Polymyxin B (table 16).

Table 9: Antibiotic sensitivity pattern exhibited by *Acinetobacter* spp.

Antibiotics	Total *Acinetobacter spp.* (N)	Sensitive		Resistance	
		n	%	n	%
Ampicillin(10mcg)	92	0	0.0	92	100
Amikacin(10 mcg)	92	1	1.1	91	98.91
Ciprofloxacin(5mcg)	92	0	0.0	92	100
Cefotaxime(30mcg)	92	0	0.0	92	100
Cefoperazone/Sulbactam (75/30µg)	92	3	3.3	89	96.74
Cotrimoxazole(25mcg)	92	0	0.0	92	100
Chloramphenicol(30mcg)	92	0	0.0	92	100
Gentamicin(10mcg)	92	0	0.0	92	100
Imipenem(10mcg)	92	12	13.1	80	86.96
Ofloxacin(5 mcg)	92	0	0.0	92	100
Piperacillin/Tazobactam (100/10mcg)	92	0	0.0	92	100
Polymyxin B (300units)	92	100	100	0	0.0

The antibiotic sensitivity testing of *E. coli* obtained from 7.67% (n= 31) growth positive clinical specimen showed high sensitivity towards Imipenem (83.87%, n=26) followed by Cefoperazone/Sulbactam (54.84%, n=17). The organism showed complete resistance towards Ampicillin (100%), Cotrimoxazole (100%) and Ofloxacin (100%).

Table 10: Antibiotic sensitivity pattern exhibited by *E. coli*

Antibiotics	Total *E. coli* (N)	Sensitive		Resistance	
		n	%	n	%
Ampicillin (10mcg)	31	0	0.0	31	100
Ciprofloxacin (5mcg)	31	1	3.2	30	96.77
Cefotaxime (30mcg)	31	1	3.2	30	96.77
Cefoperazone/Sulbactam (75/30µg)	31	17	54.8	14	45.16
Cotrimoxazole (25 mcg)	31	0	0.0	31	100
Chloramphenicol (30mcg)	8	0	0.0	8	100
Gentamicin (10mcg)	31	2	6.4	29	93.55
Imipenem (10 mcg)	31	26	83.9	5	16.13
Ofloxacin (5mcg)	31	0	0.0	31	100
Piperacillin/Tazobactam (100/10mcg)	31	1	3.2	30	96.77

Furthermore, 8 of the 31 *E. coli* isolates from urine, gastrostomy site aspirate and Foley's catheter tip culture showed 100% sensitivity towards Nitrofurantoin.

Table 11: Antibiotic sensitivity pattern exhibited by *E. coli* isolates from urine, catheter tip, Foley's catheter tip and gastrostomy site aspirate

Antibiotics	Total *E. coli* (N)	Sensitive		Resistance	
		n	%	n	%
Ampicillin (10 mcg)	8	0	0.0	8	100
Ciprofloxacin (5mcg)	8	1	12.5	7	87.5
Cefotaxime (30mcg)	8	1	12.5	7	87.5
Cefoperazone/ Sulbactam (75/30µg)	8	8	100	0	0.0
Cotrimoxazole (25 mcg)	8	0	0.0	8	100
Gentamicin (10 mcg)	8	1	12.5	7	87.5
Imipenem (10mcg)	8	0	0.0	8	100
Ofloxacin (5mcg)	8	0	0.0	8	100
Piperacillin/ Tazobactam (100/10mcg)	8	1	12.5	7	87.5
Nalidixic acid (30mcg)	8	1	12.5	7	87.5
Norfloxacin (10mcg)	8	1	12.5	7	87.5
Nitrofurantoin (100mcg)	8	8	100	0	0.0

(n)= no. of sensitive or resistance

On antimicrobial susceptibility testing for *K. oxytoca*, it was found that isolates of *K. oxytoca* from 1.24% (n=5) of the total growth positive culture specimens showed 100% sensitivity towards Amikacin, Cefoperazone/Sulbactam and Imipenem and showed 100% resistance towards Ampicillin and Piperacillin/Tazobactam.

Table 12: Antibiotic sensitivity pattern exhibited by *K. oxytoca*

Antibiotics	**Total *K. oxytoca* (N)**	**Sensitive**		**Resistance**	
		n	**%**	**n**	**%**
Ampicillin (10 mcg)	5	0	0.0	5	100
Amikacin (10mcg)	5	5	100	0	0.0
Ciprofloxacin (5mcg)	5	3	60.0	2	40.0
Cefotaxime (30mcg)	5	3	60.0	2	40.0
Cefoperazone/Sulbactam (75/30µg)	5	5	100	0	0.0
Cotrimoxazole (25 mcg)	5	3	60.0	2	40.0
Chloramphenicol (30mcg)	5	3	60.0	2	40.0
Gentamicin (10mcg)	5	3	60.0	2	40.0
Imipenem (10 mcg)	5	5	100	0	0.0
Ofloxacin (5mcg)	5	3	60.0	2	40.0
Piperacillin/Tazobactam (100/10mcg)	5	0	0.0	5	100

M. catarrhalis isolated only from the tracheal aspirate (0.74% of the total growth positive specimen) in this study have shown sensitivity towards all antibiotics used which included Ampiciilin (100%), Amikacin (100%), Ciprofloxacin (100%), Cephotaxime (100%), Cefoperazone/Sulbactam (100%), Cefepime/Tazobactam (100%), Gentamicin (100%), Ofloxacin (100%) and Piperacillin/Tazobactam (100%).

Table 13: Antibiotic sensitivity pattern exhibited by *M. catarrhalis*

Antibiotics	Total *M. catarrhalis* (N)	Sensitive		Resistance	
		n	%	n	%
Ampicillin (10 mcg)	3	3	100	0	0
Amikacin(10 mcg)	3	3	100	0	0
Ciprofloxacin (5mcg)	3	3	100	0	0
Cefotaxime (30mcg)	3	3	100	0	0
Cefoperazone/Sulbactam (75/30µg)	3	3	100	0	0
Cefepime/Tazobactam (30 /10mcg)	3	3	100	0	0
Gentamicin (10mcg)	3	3	100	0	0
Imipenem (10 mcg)	3	3	100	0	0
Ofloxacin (5mcg)	3	3	100	0	0
Piperacillin/Tazobactam (100/10mcg)	3	3	100	0	0

The bacterium *P. mirabilis* isolated from 1.24% (n=5) of the total culture positive specimen, when tested for different types of antibiotics, showed 100% sensitivity towards Ciprofloxacin, Cefoperazone/Sulbactam and Chloramphenicol while showed 100% resistance towards Ampicillin, Cefotaxime, Cotrimoxazole, Gentamicin, Ofloxacin and Piperacillin/Tazobactam.

Table 14*:* Antibiotic sensitivity pattern of *P. mirabilis*

Antibiotics	Total *P. mirabilis* (N)	Sensitive		Resistance	
		n	%	n	%
Ampicillin (10 mcg)	5	0	0	5	100
Ciprofloxacin (5mcg)	5	5	100	0	0
Cefotaxime (30mcg)	5	0	0	5	100
Cefoperazone/Sulbactam (75/30µg)	5	5	100	0	0
Cotrimoxazole (25 mcg)	5	0	0	5	100
Chloramphenicol (30mcg)	5	5	100	0	0
Gentamicin (10mcg)	5	0	0	5	100
Imipenem (10 mcg)	5	5	100	0	0
Ofloxacin (5mcg)	5	0	0	5	100
Piperacillin/Tazobactam (100/10mcg)	5	0	0	5	100

Similarly, antibiotic sensitivity pattern of *S. aureus* isolated from 4.95% (n=20) of the total growth positive specimens demonstrated 100% sensitivity to Vancomycin while 100% resistance to Ampicillin. On the other hand, most of the *S. aureus* isolates were resistance to Piperacillin/Tazobactam (95.0%, n=19), Cloxacillin (90.0%, n=18), Gentamicin (90.0%, n=18), Erythromycin (90.0%, n=18) and Ofloxacin (90.0%, n=18). Since most of the *S. aureus* isolates were resistant to Ampicillin and Cloxacillin (a drug of class similar to Oxacillin), it might be MRSA strains.

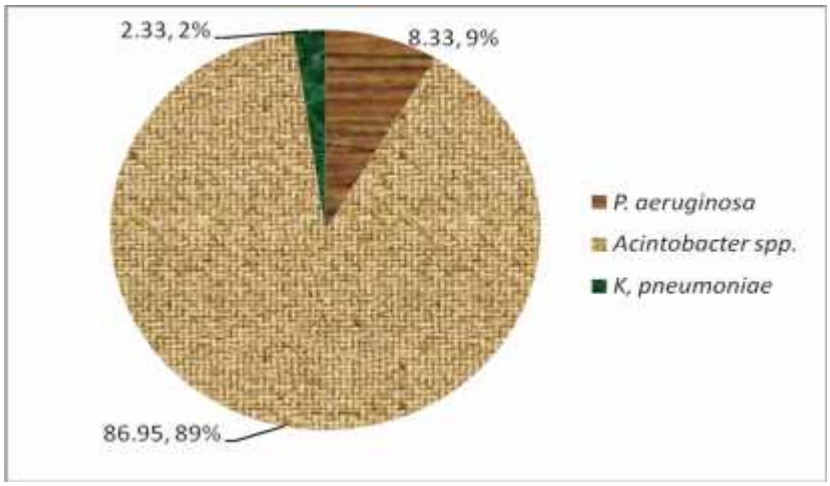
Table 15: Antibiotic sensitivity pattern of *S. aureus*

Antibiotics	Total *S. aureus* (N)	Sensitive		Resistance	
		n	%	N	%
Ampicillin (10 mcg)	20	0	0.0	20	100
Ciprofloxacin (5mcg)	20	3	15.0	17	85.0
Cloxacillin (5mcg)	20	2	10.0	18	90.0
Cotrimoxazole (25 mcg)	20	4	20.0	16	80.0
Gentamicin (10mcg)	20	2	10.0	18	90.0
Ofloxacin (5mcg)	20	14	70.0	6	30.0
Piperacillin/Tazobactam (100/10mcg)	20	1	5.0	19	95.0
Erythromycin(10mcg)	20	2	10.0	18	90.0
Vancomycin (30mcg)	20	20	100	0	0.0

5.1.2 Multidrug resistance among the isolates

Isolates of P. aeruginosa (19, 8.33%), *Acinetobacter* spp. (80, 86.95%) and *K. pneumoniae* (2, 2.33%) recovered consistently from consecutive tracheal aspirate of some patients, later showed resistance to all antibiotics tested. Those when tested with Polymyxin B, found sensitive indicating development of MDR strains.

Figure 6: Pattern of multidrug resistance among the isolates



5.2 Microbial investigation of environmental samples (Animate and inanimate)

Out of 677 environmental samples investigated, a total of 562 were found to be growth positive. Results of individual sample types are discussed in the following paragraphs.

5.2.1 Air sample

A total of 30 air samples collected from the different corners and isolation room of ICU by plate exposure method for a specified time period (30mins), revealed the growth positivity in all the samples (100%). It was found that CoNS (83.33%) were most predominant followed by *P. aeruginosa*, (80.0%), *S. aureus* (43.33%), Micrococci (30.0%), Enterococci (26.67%), *Acinetobacter* spp. (20.0%), *Bacillus* spp. (23.33%), fungi (23.33%), *K. pneumoniae* (16.67%) and *E. coli* (6.67%).

Table 16: Microbial isolates in the sample of air circulated in ICU

Types of microbial isolates in the air of ICU room	**Prevalence**	
	Frequency (n)	***Percentage (%)***
A. Gram positive cocci:		
S. aureus	13	43.33
CoNS	25	83.33
Enterococci	8	26.67
Micrococci	9	30.0
Isolate type	***4***	***40.0***
B. Gram negative bacilli:		
P. aeruginosa	24	80.0
Acinetobacter spp.	6	20.0
E. coli	2	6.67
K. pneumonia	5	16.67
Isolate type	***4***	***40.0***
C. Gram positive bacilli:		
Bacillus spp.	7	23.33
Isolate type	***1***	***10.0***
D. Fungal isolate	7	23.33
Isolate type	***1***	***10.0***
Total isolate type	**10**	**100**
Sample with no growth in all plate	**0**	**0.0**

(***n) = frequency of positive samples in which a particular pathogen occurs***

5.2.2 Water sample

A total of 24 water samples were collected aseptically in sterilized containers from different reservoirs of water and from the drinking water tap (on the terrace of the hospital) and from the taps inside ICU. The samples were then examined by MPN procedure for presumptive coliform count followed by standard microbiological culture method and biochemical tests for identification of the individual organisms in coliform group. On the other hand, noncoliform groups were identified simply by standard conventional culture technique using Mac Conkey Agar and Blood Agar followed by tests with a set of batteries of biochemical media and reagents. Twelve of the 24 samples were collected prior to cleaning and maintenance of the water reservoirs while rests of the other 12 samples were collected after cleaning and maintenance of the water reservoirs. Bacteriological examination based on MPN count for chlorinated water in 100 ml of sample, before cleaning and maintaining the reservoirs, revealed that 66.66% of samples (n=8) did not meet WHO standards of quality of treated water samples (≤ 5 *E. coli* count/100ml) while 83.33% of them were found to be contaminated with *P. aeruginosa* and *Acinetobacter* spp.. However the water sample from the drinking water tap was found to be free of all kinds of microorganisms. On the other hand, after cleaning and maintaining the reservoirs, MPN count in 100 ml of water sample revealed that 50.0% of the samples (n=6) met WHO standards of quality of water samples and none of the samples were found to be free of contamination from *P. aeruginosa* and *Acinetobacter* spp. (83.33% of the water samples contained both of these organisms) with exception of drinking water that was found to be free from all sorts of microbes.

Table 17: Microbial isolates in the water samples supplied to the taps inside ICU

Source or site of sample collection	Time of sample collection					
	Before cleaning and maintenance of water reservoirs			*After cleaning and maintenance of water reservoirs*		
	No. of samples collected	Types of microorganism isolated	Mean MPN/100 ml of water (only for coliforms)	No. of samples collected	Types of microorganism isolated	Mean MPN/100 ml of water (only for coliforms)
Large reservoir (chemically treated but unfiltered	2	Coliforms *P. aeruginosa, Acinetobacter* spp.	3+3/2=3	2	Coliforms (absent) *P. aeruginosa, Acinetobacter* spp.	0
Small reservoir(chemically treated and normal filtered	2	Coliforms *P. aeruginosa, Acinetobacter* spp.	18+16/2=17	2	Coliforms *P. aeruginosa, Acinetobacter* spp.	2+2/2=2
Large collecting tank (water is received here from small reservoir and distributed).	2	Coliforms *P. aeruginosa, Acinetobacter* spp.	16+16/2=16	2	Coliforms *P. aeruginosa, Acinetobacter* spp.	6+9/2=7.5
ICU Hand washing tap (used by Visitors and HCWs in ICU)	2	Coliforms *P. aeruginosa, Acinetobacter* spp.	16+16/2=16	2	Coliforms *P. aeruginosa, Acinetobacter* spp.	16+16/2=16
ICU restroom tap (used only by HCWs in ICU)	2	Coliforms *P. aeruginosa, Acinetobacter* spp.	18+16/2=17	2	Coliforms *P. aeruginosa, Acinetobacter* spp.	9+6/2=7.5
Drinking water tap (used by all)	2	No microorganism found	0	2	No microorganism found	0

5.2.3 Hand imprints samples of HCWs in working in ICU

A total of 100 hand imprint samples (50 samples before hand washing and 50 samples after hand washing) were collected from health care workers including doctors, physiotherapist, nurses and housekeepers on their regular duty hour in ICU. A total of 46 (92.0%) samples before hand wash were found to be positive for presence of microorganisms while 8.0% (n=4) of the total samples were found to show no growth of microorganisms. On the other hand, a total of 40 (80.0%) samples were found to be still carrying microorganisms even after hand wash with medicated soap (Lifebuoy) and water and then with liquid soap (Cholorohexidine/alcohol solution) and with water and then dried by the use of common towel followed by drier. The positive samples included even of those whose hand imprint showed no growth before wash but microbial growth was observed in the imprint taken after they washed their hands. Among the 46 positive samples collected before hand wash, *P. aeruginosa* (71.74%) and CoNS (71.74%) were found to be predominant organisms followed by *S. aureus* (69.57%), *E. coli* (28.26%), *K. pneumoniae* (23.91%), Micrococci (15.22%), *Bacillus* spp. (13.04%), Enterococci (10.87%), fungal isolates (6.52%) and *Acinetobacter* spp. (4.35%). Similar organisms were isolated with similar pattern from the 40 positive samples collected after hand wash. So it was observed that there was no detectable reduction in the number of samples with positive microbial growth in the culture plates in even in the after wash hand imprints.

Table 18: Microbial isolates in the Hands of HCWs regularly working in ICU

Types of microbial isolates from hands of HCWs	Prevalence at different time of sample collection			
	Before hand wash		After hand wash	
	Frequency (n)	***Percentage (%)***	***Frequency (n)***	***Percentage (%)***
A. Gram positive cocci:				
S. aureus	32	69.57	21	52.5
CoNS	33	71.74	19	47.5
Micrococci	7	15.22	5	12.5
Enterococci	5	10.87	2	5.0
Isolate type	***4***	***40.0***	***4***	***40.0***
B. Gram negative bacilli:				
P. aeruginosa	33	71.74	27	67.5
Acinetobacter spp.	2	4.35	2	5.0
E. coli	13	28.26	6	15.0
K. pneumoniae	11	23.91	6	15.0
Isolate type	***4***	***40.0***	***4***	***40.0***
C. Gram positive bacilli:				
Bacillus spp.	6	13.04	2	5.0
Isolate type	***1***	***10.0***	***1***	***10.0***
D. Fungal isolate	3	6.52	2	5.0
Isolate type	***1***	***10.0***	***1***	***10.0***
Total Isolate Type	**10**	**100**	**10**	**100**
Sample with no growth in all plate	**4**	**8.0**	**10**	**20.0**

(n) = frequency of positive samples in which a particular pathogen occurs

5.2.4 Nasal samples of HCWs in ICU

Among the total of 55 nasal samples taken by swabbing both the anterior nares of the HCWs, all of the samples (100%) were found to be growth positive, *S. aureus* being the

most predominant occurring in 52.73% (n=29) of the samples followed by CoNS observed in 47.27% (n=26) of the samples. *K. pneumoniae* and *E. coli* were also isolated from 5.45% and 1.82% of the nasal samples respectively.

Table 19: Microbial isolates in the anterior nares of HCWs

Types of microbial isolates from Anterior nares of HCWs	**Prevalence**	
	Frequency (n)	***Percentage (%)***
A. Gram positive cocci:		
S. aureus	29	52.73
CoNS	26	47.27
Isolate type	***2***	***50.0***
B. Gram negative bacilli:		
E. coli	1	1.82
K. pneumonia	4	7.27
Isolate type	***2***	***50.0***
Total Isolate Type	**4**	**100**

(n) = frequency of positive samples in which a particular pathogen occurs

5.2.4.1 Antibiotic sensitivity testing of nasal swab isolates of HCWs

Antimicrobial susceptibility testing of the nasal isolates was performed. The AST pattern of CoNS isolated from 47.27% (n= 26) of the total positive samples were observed to be resistant to Ampicillin (100%) followed by Erythromycin (88.46%), Cotrimoxazole (34.62%), Cloxacillin (15.38%), Ciprofloxacin (11.54 %) and Gentamicin (7.69%) but all of them were sensitive to Vancomycin. Similarly, the AST pattern of CoPS recovered from 29 nasal samples were sensitive to Vancomycin (100%) while were resistant to Ampicillin (96.55%) followed by Erythromycin (62.05%) , Cotrimoxazole (41.38%), Ciprofloxacin (6.90%), Gentamicin (6.90%) and Cloxacillin

(3.45 %). Isolates resistance to Cloxacillin and Ampicillin suggest that there may be prevalence of MRSA strains of *S. aureus*.

Table 20: Antibiotic sensitivity pattern of Gram positive cocci from nasal sample of HCWs

Antibiotics tested	Antibiotic sensitivity pattern n (%)	Microorganisms	
		S. aureus **(N=29)**	**CoNS (N=26)**
Ampicillin (10 mcg)	**Sensitive**	1 (3.45%)	0 (0.0%)
	Resistance	28 (96.55%)	26 (100%)
Ciprofloxacin (5mcg)	**Sensitive**	27 (93.10%)	23 (88.46%)
	Resistance	2 (6.90%)	3 (11.54%)
Cotrimoxazole (25mcg)	**Sensitive**	17 (58.62%**)**	17 (65.38%)
	Resistance	12 (41.38%)	9 (34.62%)
Erythromycin (10mcg)	**Sensitive**	11 (37.93%)	3 (11.54%)
	Resistance	18 (62.07%)	23 (88.46%)
Cloxacillin (5mcg)	**Sensitive**	28 (96.55%)	22 (84.62%)
	Resistance	1 (3.45%)	4 (15.38%)
Gentamicin (10mcg)	**Sensitive**	27 (93.10%)	24 (92.31%)
	Resistance	2 (6.90%)	2 (7.69%)
Vancomycin (30mcg)	**Sensitive**	29 (100%)	26 (100%)
	Resistance	0 (0%)	0 (0.0%)

(N)= Total number of organism tested

Similarly, the AST pattern of *K. pneumoniae* showed that 100% of them were resistant to Ampicillin, Cotrimoxazole and Piperacillin/Tazobactam while 33.33% of them were resistant to Cephotaxime, Amikacin and Gentamicin but 100% of them were found to be sensitive to Ciprofloxacin and Chloramphenicol. The AST pattern of *E. coli* isolated from only one nasal sample showed that the organism was resistant to Ampicillin, Piperacillin/Tazobactam, Cotrimoxazole and Gentamicin while was sensitive to Amikacin, Ciprofloxacin, Cephotaxime and Chloramphenicol.

Table 21: Antibiotic sensitivity pattern of Gram negative bacilli from nasal sample of HCWs

Antibiotics tested	Antibiotic sensitivity pattern n (%)	Microorganisms	
		K. pneumoniae (N=3)	***E. coli* (N=1)**
Ampicillin (10 mcg)	**Sensitive**	0 (0.0%)	0 (0.0%)
	Resistant	3 (100%)	1 (100%)
Cefotaxime (30mcg)	**Sensitive**	2 (66.67%)	1 (100%)
	Resistant	1 (3.33%)	0 (0.0%)
Ciprofloxacin (5mcg)	**Sensitive**	0 (100%**)**	1 (100%)
	Resistant	3 (100%)	0 (0.0%)
Cotrimoxazole (25mcg)	**Sensitive**	3 (100%)	0 (0.0%)
	Resistant	0 (0.0%)	1 (100%)
Gentamicin (10mcg)	**Sensitive**	2 (66.67%)	0 (0.0%)
	Resistant	1 (3.33%)	1 (100%)
Amikacin (10mcg)	**Sensitive**	2 (66.67%)	1 (100%)
	Resistant	1 (3.33%)	0 (0.0%)
Chloramphenicol (30mcg)	**Sensitive**	3 (100%)	1 (100%)
	Resistant	0 (0.0%)	0 (0.0%)
Piperacillin/Tazobactam (100/10mcg)	**Sensitive**	0 (0.0%**)**	0 (0.0%)
	Resistant	3 (100%)	1 (100%)

(N)= Total number of organism tested

5.2.5 Different fabrics/clothing samples

A total of 217 samples of clothes or fabrics being used by HCWs, Visitors and Patients and that are suspected to carry some forms of microorganisms were sampled with the help of sterile cotton swabs and processed at the same day followed by identification of organisms the next day by microbiological techniques. Altogether 87.56% (n=190) of the total samples were found to be culture positive.

5.2.5.1 HCWs' gown

A total of 62 gown samples (sky blue colored) of HCWs were taken by swabbing with pre moistened and sterilized cotton swabs. Out of 62 samples, 82.26% (n=51) of the gown samples were found to be carrying microorganisms whereby the most predominant being CoNS accounting for 66.7% followed by *S. aureus* (50.98%), *P. aeruginosa* (39.22%), *Acinetobacter* spp. (13.73%), Enterococci (9.80%), *K. pneumoniae* (3.92%), *E. coli* (2.0%), *Bacillus* spp. (1.96%) and fungi(1.96%).

5.2.5.2 Visior's gown

A total of 22 green colored visitor's gown samples placed outside the internal entry door of ICU that were used by visitor of patients, were collected and microbiologically examined. The observation showed 90.90% (n=20) samples to be positive for microbial growth with predominance of CoNS and *P. aeruginosa* accounting for 60.0% each followed by *S. aureus* (40.0%), Enterococci (25.0%), *Acinetobacter* spp. (15.0%), *E. coli* (10.0%) and *K. pneumoniae* (5.0%).

5.2.5.3 Patient's gown

Totally 43 samples of gown (light blue colored) that were worn by the patients during their ICU stay were collected and microbiologically examined. The observation showed the presence of microorganisms in 83.72% (n=36) of the samples collected. Altogether 6 types of microbial isolates were found in the samples, the highest number being of the *S. aureus* (61.11%) followed by CoNS (33.33%), *P. aeruginosa* (19.44%), *Acinetobacter* spp. (19.44%), *K. pneumoniae* (11.11%) and Enterococci (5.55%).

5.2.5.4. Patient's bed sheet:

A total of 50 bed sheet that were being used in the beds of patients were sampled by swabbing with pre moistened and sterilized cotton swabs. Microbiological examination of these samples revealed that 98.0% (n=49) of them were contaminated with microorganisms. The most prevalent organism was found to be *S. aureus* which was isolated and identified from 28 (57.14%) samples. Next to CoPS was CoNS (46.94%) followed by *P. aeruginosa* (28.57%), *Acinetobacter* spp. (16.33%), *K. pneumoniae* (16.33%), Enterococci (12.24%) and *E. coli* (4.08%).

5.2.5.5 Privacy curtains

Altogether 40 privacy curtains that were being used around the ICU patients were sampled to find out the presence of any microorganisms. Luckily, it was found that 85% (n=34) of the sampled curtains showed positive result for the growth of microorganisms. The microorganisms isolated and identified include: CoNS (67.6%) being predominant followed by *S. aureus* (58.82%), *P. aeruginosa* (23.53%), *Acinetobacter* spp. (17.65%), Enterococci (5.88%), *K. pneumoniae* (2.94%) and *E. coli* (2.94%).

Table 22: Microbial isolates in different types of Fabrics/Clothes being used in ICU

Types of microbial isolates	**Prevalence in different types of fabrics/ clothes sample**				
	HCW's gown, n (%)	***Visitor's gown, n (%)***	***Patient's gown, n (%)***	***Patient's bed sheet, n (%)***	***Privacy curtains, n (%)***
A. Gram positive cocci:					
S. aureus	26 (50.98)	8(40.0)	22(61.11)	28(57.14)	20(58.82)
CoNS	34(66.66)	12(60.0)	12(33.33)	23(46.94)	23(67.65)
Enterococci	5(9.80)	5(25.0)	2(5.55)	6(12.24)	2(5.88)
Isolate type	***3(33.33)***	***3(42.86)***	***3(50.0)***	***3(42.86)***	***3(42.86)***
B. Gram negative bacilli:					
P. aeruginosa	20(39.22)	12(60.0)	7(19.44)	14(28.57)	8(23.53)
Acinetobacter spp.	7(13.73)	3(15.0)	7(19.44)	8(16.33)	6(17.65)
E. coli	1(1.96)	2(10.0)	-	2(4.08)	1(2.94)
K. pneumoniae	2(3.92)	1(5.0)	***4***(11.11)	8(16.33)	1(2.94)
Isolate type	***4(44.45%)***	***4(57.54)***	***3(50.0)***	***4(57.14)***	***4(57.14)***
C. Gram positive bacilli:					
Bacillus spp.	1(1.96)	-	-	-	-
Isolate type	***1(11.11)***				
D. Fungal isolate	1(1.96)	-	-	-	-
Isolate type	***1(11.11)***				
Total Isolate Type	**9(100)**	**7(100)**	**6 (100)**	**7(100)**	**7(100)**
Sample with no growth in all plate	**11(17.74)**	**2(9.09)**	**7(16.27)**	**1(2.0)**	**6(15.0)**

5.2.6 Different inanimate surfaces around ICU patient

A total of 251 swab samples of variety of inanimate surfaces including Bed rails, Bedside table, Bed side monitor, Report writing table, Ventilator (air filter), Door handle/knob and Stethoscope carried by Medical personnel, were collected and microbiologically investigated for presence or absence of microorganisms. Out of the total, 72.11% (n=181) samples were identified to carry certain microorganisms. The findings of investigation showed *S. aureus* and CoNS to be the predominant organisms in most of the samples of inanimate surfaces except in the Ventilator (air filter) and Door handle/knob where the predominant organism was *P. aeruginosa.* Along with these, *Acinetobacter* spp., *K. pneumoniae, E. coli,* Enterococci, *Bacillus* spp. and fungi were also isolated from these surfaces.

5.2.6.1 Bed rails

Altogether 50 samples from 50 Bed rails were swabbed and processed in the Microbiology laboratory. Out of 50, 82.0% (n=41) were found to be culture positive. Among the isolated organism, CoNS accounted for 63.41% of the total positive culture followed by *S. aureus* (51.22%), *P. aeruginosa* (12.19%), *Acinetobacter* spp. (12.19%), *K. pneumoniae* (12.19%), Enterococci (4.88%), *E. coli* (2.44%) and *Bacillus* spp. (2.44%).

5.2.6.2 Bedside table

Out of a total of 50 surface swab samples collected from the bedside tables (where medicines, IV fluids are placed) near to patients, altogether 80.0% (n=40) samples were found to show the growth of microbial population. Among the microbial population isolated, the higher prevalence was of CoNS showing growth in 60% (n=24) of the total sample followed by *S. aureus* (55.0%, n=22), *P. aeruginosa* (45.0%, n=18) and *K. pneumoniae* (15.0%, n=6). The least prevalent were fungi (2.5%, n=1), *Acinetobacter* spp. (5.0%, n=2), *E. coli* (7.5%, n=3) and Enterococci (7.5%, n=3).

5.2.6.3 Report writing table

From the total of 50 swab samples of report writing tables used by HCWs to keep daily the clinical record of the patients, 88.0% (n=44) were identified to possess microorganisms. Among those microbial population, *S. aureus* was found to occur in

more than 50% of the samples (i.e. 61.36%, n=27). Similarly, CoNS occurred in 43.18% (n=19), *P. aeruginosa* in 29.54% (n=13), *Acinetobacter* spp. 13.63% (n=6), E. coli 11.36% (n=5), *K. pneumoniae* 11.36% (n=5), Enterococci 2.27% (n=1), *Bacillus* spp. 2.27% (n=1) and fungi 2.27% (n=1).

5.2.6.4 Door handle/knob

Out of 16 door handle/knob samples collected from the doors of ICU room, only 50% (n=8) samples showed microbial growth. Among the positive samples, 50% (n=4) of them showed the presence of *P. aeruginosa* while presence of CoNS and *S. aureus* were shown by 37.50% (n=3) and 12.50% (n=1) of the positive door handle/knob samples respectively.

Table 23: Microbial isolates from different types of inanimate surfaces in ICU

Types of microbial isolates	**Prevalence in different inanimate surface sample**			
	Bed rail n (%)	***Bedside table n (%)***	***Report writing table n (%)***	***Door handle/knob n (%)***
A. Gram positive cocci:				
S. aureus	21(51.22)	22(55.0)	27(61.36)	1(12.50)
CoNS	26(63.41)	24(60.0)	19(43.18)	3(37.50)
Enterococci	2(4.88)	3(7.5)	1(2.27)	-
Isolate type	***3(37.5)***	***3(37.5)***	***3(33.33)***	***2(66.67)***
B. Gram negative bacilli:				
P. aeruginosa	5(12.19)	18(45.0)	13(29.54)	4(50.0)
Acinetobacter spp.	5(12.19)	2(5.0)	6(13.63)	-
E. coli	1(2.44)	3(7.5)	5(11.36)	-
K. pneumoniae	5(12.19)	6(15.0)	5(11.36)	-
Isolate type	***4(50.0)***	***4(50.0)***	***4(44.45)***	***1(33.33)***
C. Gram positive bacilli:				
Bacillus spp.	1(2.44)	-	1(2.27)	-
Isolate type	***1(12.5)***	-	***1(11.11)***	
D. Fungal isolate	-	1(2.5)	1(2.27)	-
Isolate type	-	***1(12.5)***	***1(11.11)***	-
Total Isolate Type	**8(100)**	**8(100)**	**9(100)**	**3(100)**
Sample with no growth in all plate	**9(18.0)**	**10(20.0)**	**6(12.0)**	**8(50.0)**

5.2.6.5 Bedside monitor

Altogether 50 bedside monitors connected to each patient in ICU which comes in frequent contact with hands of HCWs was sampled with swab. Out of the total, 70% (n=35) of the samples exhibited culture positive result. CoNS were isolated as the most predominant pathogen showing growth in 68.57% (n=27) of the total positive samples followed by *S. aureus* 48.57% (n=17), *P. aeruginosa* 20.0% (n=7) and *Acinetobacter* spp. 20.0% (n=7). Other isolates prevalent in only few samples include *K. pneumoniae* 5.71% (n=2), *Bacillus* spp.5.71% (n=2), fungi 5.71% (n=2) and *E. coli* 2.82% (n=1).

5.2.6.6 Ventilator (Air filter)

A total of about 17 air filters of Ventilators that were being used in patients at that moment, were sampled by swabbing with sterile cotton swab. Among the total, 58.82% (n=10) were found to show positive result for the growth of microorganisms. Altogether 3 different types of microbial isolates were identified, in which *P. aeruginosa* was the most predominant organism occurring in 60.0% (6 out of 10) of the positive samples followed by *Acinetobacter* spp. (50.0%, 5 out of 10) and *K. pneumoniae* (30.0%, 3 out of 10).

5.2.6.7 Stethoscope

To determine the prevalence of microorganisms in the Stethoscope carried by Medical personnel while working in ICU, a total of 18 swab samples of Stethoscopes were collected and processed microbiologically. It was observed that only 16.67% (n=3) of the samples exhibited presence of microorganisms. All the positive samples (100%, n=3) contained *S. aureus* whereas only 1 sample (33.33%) showed the presence of *P. aeruginosa*.

Table 24: Microbial isolates in surface samples of different types of equipments/instruments

Types of microbial isolates	**Prevalence in surface samples of different equipments/instruments**		
	Bedside monitor n (%)	***Ventilator (air filter) n (%)***	***Stethoscope n (%)***
A. Gram positive cocci:			
S. aureus	17(48.57%)	-	3(100%)
CoNS	24(68.57%)	-	
Isolate type	***2(25.0%)***		***1(50.0%)***
B. Gram negative bacilli:			
P. aeruginosa	7(20.0%)	6(60.0%)	1(33.33%)
Acinetobacter spp.	7(2.00%)	5(50.0%)	-
E. coli	1(2.86%)	-	-
K. pneumonia	2(5.71%)	3(30.0%)	-
Isolate type	***4(50.0%)***	***3(100%)***	***1(50.0%)***
C. Gram positive bacilli:			
Bacillus spp.	2(5.71%)	-	-
Isolate type	***1(12.5%)***	**-**	**-**
C. Fungal isolate	2(5.71%)	-	-
Isolate type	***1(12.5%)***	-	-
Total Isolate Type	**8(100%)**	**3(100%)**	**2(100%)**
Sample with no growth in all plate	**15(30%)**	**7(41.17%)**	**15(83.33%)**

5.2.6.8 Antibiotic sensitivity pattern of organisms isolated from different growth positive environmental samples

A total of 43 environmental isolates were tested for antibiotic sensitivity pattern. Gram negative isolates from environmental samples were mostly sensitive to Imipenem including *P. aeruginosa* (100%), *Acinetobacter* spp. (85.71%), *K. pneumoniae* (100%) and *E. coli* (100%). On the other hand, the only gram positive isolates common to clinical isolate i.e. *S. aureus* expressed 100% sensitivity to Vancomycin. Similar to clinical isolates, most of the environmental isolates of *Acinetobacter* spp. also expressed

high degree of resistance (100% insusceptibilty) to common antibiotics such as Cefotaxime, Cotrimoxazole, Gentamicin, Ofloxacin, Piperacillin/Tazobactam indicating presence of MDR *Acinetobacter* spp. strain in the surrounding ICU environment. Likewise, *K. pneumoniae* and *E. coli* also exhibited 100% resistance each to several antibiotics including Ampicillin, Cotrimoxazole, Piperacillin/Tazobactam, 80.0% of each were resistant to Gentamicin and 100% and 60.0% showed resistance to Ofloxacin respectively, thus reflecting the existence of MDR organisms.

Table 25: Antibiotic sensitivity pattern of environmental isolates common to clinical isolates

Antibiotics used	**Environmental isolates common to clinical isolates**				
	P. aeruginosa	***Acinetobacter spp.***	***K. pneumoniae***	***E. coli***	***S. aureus***
	Total=7	**Total=7**	**Total=5**	**Total=5**	**Total=19**
	% resistant	**% resistant**	**% resistant**	**% resistant**	**% resistant**
Ampicillin()	-	100	100	100	89.47
Amikacin()	28.57	85.71	40.0	20.0	-
Cefotaxime()	28.57	100	60.0	40.0	-
Cefoperazone/Sulbactam (75/30µg)	0.0	57.14	0.0	0.0	-
Ciprofloxacin()	14.29	85.71	20.0	20.0	68.42
Chloramphenicol()	-	71.42	20.0	0.0	-
Cotrimoxazole()	-	100	100	100	89.47
Cefepime/ Tazobactam()	100	-	-	-	-
Cloxacillin()	-	-	-	-	57.89
Erythromycin()	-	-	-	-	68.42
Gentamicin()	42.86	100	80.0	80.0	78.94
Imipenem()	0.0	14.29	0.0	0.0	-
Ofloxacin()	42.86	100	100	60.0	78.94
Piperacillin/Tazobactam()	42.86	100	100	100	84.21
Polymyxin B()	0.0	0.0	0.0	-	-
Vancomycin()	-	-	-	-	0.0

5.3 Analysis of distribution and association between clinical and environmental isolates

In the table (27), prevalence of five common isolates in growth positive environmental samples and clinical specimens of patients from ICU was determined

Table 26: Distribution of five different isolates common in both clinical specimen of patients and their surrounding environment in ICU

Organisms common in clinical & environmental sample	occurrence pattern in clinical specimen (404) n (%)	Occurrence pattern in environmental sample (562)							
		Air (30) n (%)	Water(20) n (%)		Hand imprint (86) n (%)		Nasal swab (55) n (%)	Fabrics/ clothes (190) n (%)	Inanimate surfaces (181) n (%)
			B.M(10)	A.M(10)	B.W(46)	A.W(40)			
P. aeruginosa	248 (61.39)	24 (80.0)	10 (100)	10 (100)	33 (71.7)	27 (67.5)	_	61 (32.11)	54 (29.83)
K. pneumoniae	100 (24.75)	5 (16.67)	10 (100)	10 (100)	11 (23.91)	6 (15)	3 (5.45)	16 (8.42)	21 (11.60)
***Acinetobacter* spp.**	92 (22.77)	6 (20)	10 (100)	10 (100)	2 (4.35)	2 (5.0)	_	31 (16.32)	25 (13.81)
E. coli	31 (7.67)	2 (6.67)	10 (100)	10 (100)	13 (28.26)	6 (15.0)	1 (1.82)	6 (3.16)	10 (5.52)
S. aureus	20 (4.95)	13 (43.33)	_	_	32 (69.57)	21 (52.5)	29 (52.73)	104 (54.74)	91 (50.28)

(n) = frequency of positive samples in which a particular pathogen occurs, B.M= Before Maintenance, A.M= after maintenance, B.W= before wash, A.W=after wash

In total, Out of 404 culture positive clinical specimens, *P. aeruginosa* tend to occur in more than half (61.39%, 248 out of 404) of them. Simultaneously, *P. aeruginosa* was also isolated as the frequent isolate from 562 culture positive environmental samples accounting for 80.0% (n=24) of air samples, 100% (n=10) of water samples each before maintenance and after maintenance, 71.74% (n=33)and 67.5% (n=27) of Hand imprint

samples each respectively for before wash and after wash, similarly, 32.11% (n=61) of fabrics/clothing samples and 29.83% (n=54) of inanimate surface samples. However, it did not show growth in Nasal sample of HCWs. Statistical analysis showed that there existed significant association between *P. aeruginosa* isolated from various clinical specimens and environmental samples ($p<0.05$) except the hand imprints of HCWs ($p>0.05$)

Similarly, *K. pneumoniae* occurred in 24.75% (n=100) of the culture positive clinical specimens while from environment, it was isolated from 16.67% (n=5) of culture positive air samples, 100% (n=10) of culture positive water samples each before maintenance and after maintenance, 23.91% (n=11) and 15% (n=6) of culture positive Hand imprint samples each for before wash and after wash respectively. Likewise, it was also isolated from 5.45% (n=3) of culture positive nasal swab of HCWs, 8.42% (n=16) of culture positive fabrics/clothing samples and 11.60% (n=54) of growth positive inanimate surface samples. Statistical analysis showed the association of clinical *K. pneumoniae* isolates with that of water, nasal samples o HCWs, fabrics/clothes and inanimate surfaces ($p<0.05$). However, the analysis revealed that *K. pneumoniae* isolated clinical specimens had no significant association with the K. pneumoniae isolated from air and hand imprints of HCWs.

In total, *Acinetobacter* spp. occurred in 22.77% (n=92) of the culture positive clinical specimens. Side by side, this organism was also isolated from 20.0% (n=6) of culture positive air samples, 100% (n=10) of culture positive water samples each before maintenance and after maintenance, 4.35% (n=2) and 5% (n=2) of culture positive Hand imprint samples each for before wash and after wash respectively. Likewise, the isolate was also obtained from 16.32% (n=31) of culture positive fabrics/clothing samples and 13.81% (n=25) of growth positive inanimate surface samples. Though isolated from most of the environmental samples, however the clinical isolates of *Acinetobacter* spp.

was found to be associated only with the isolates from water, hand imprints of HCWs and fabrics/clothes ($p<0.05$) but not with those isolated from air and inanimate surfaces ($p>0.05$)

Further, in the present study, 7.67% (n=31) of the total positive culture specimen was positive for the presence of *E. coli* and a total of 6.67%, 100%, 28.26% & 15.0%, 1.82%, 3.16% and 5.52% of different environment samples respectively including air, water (each from before and after maintenance), HCW's hand imprint before & after wash, anterior nares of HCWs, fabrics/ clothes and inanimate surfaces exhibited the presence of *E. coli*. Statistical analysis showed that there was significant association of clinical isolates of E. coli with the similar isolates from water, hand imprints of HCWs and fabrics/clothes ($p<0.05$) and no significant association with the isolates from air, nasal samples of HCWs and inanimate surfaces ($p>0.05$).

Likewise, 4.95% (n=20) of the total culture positive clinical specimens revealed the presence of *S. aureus*. Subsequent culture positive samples of environment also demonstrated the presence of *S. aureus* viz., air (43.33%), hand imprints before hand wash (69.57%) and after hand wash (52.5%), nasal samples of HCWs (52.73%), fabrics/clothes (54.74%) and inanimate surfaces (50.28%). No *S. aureus* isolates from water was observed. Finding of statistical analysis revealed that there was significant association between the *S. aureus* from clinical specimens and from various environmental samples including both animate and inanimate samples ($p<0.05$).

Table 27: Statistical analysis showing association between the clinical and isolates environmental isolates

Sample type	*P. aeruginosa*		*K. pneumoniae*		*Acinetobacter* spp.		*E. coli*		*S. aureus*	
	n (%)	**p-value**	**n (%)**	**p-value**	**n (%)**	**p-value**	**n (%)**	**p-value**	**n (%)**	**p-value**
Clinical (N=404)	248 (61.39)	**<0.05**	100 (24.75)	**>0.05**	92 (22.77)	**>0.05**	31 (7.67)	**>0.05**	20 (4.95)	**<0.05**
Air (N=30)	24 (80.0)		5 (16.67)		6 (20.0)		2 (6.67)		13 (43.33)	
Clinical (N=404)	248 (61.39)	**<0.05**	100 (24.75)	**<0.05**	92 (22.77)	**<0.05**	31 (7.67)	**<0.05**	-	
Water (N=20)	20 (100)		20 (100)		20 (100)		20 (100)		-	
Clinical (N=404)	248 (61.39)	**>0.05**	100 (24.75)	**>0.05**	92 (22.77)	**<0.05**	31 (7.67)	**<0.05**	20 (4.95)	**<0.05**
Hand imprint (N=86)	60 (69.77)		17 (19.77)		4 (4.65)		13 (22.1)		53 (61.63)	
Clinical (N=404)	-	-	100 (24.75)	**<0.05**	-	-	31 (7.67)	**>0.05**	20 (4.95)	**<0.05**
Nasal (N=55)	-		3 (5.45)		-		1 (1.82)		29 (52.73)	
Clinical (N=404)	248 (61.39)	**<0.05**	100 (24.75)	**<0.05**	92 (22.77)	**>0.05**	31 (7.67)	**<0.05**	20 (4.95)	**<0.05**
Fabrics/clothes (N=190)	61 (32.11)		16 (8.42)		31 (16.32)		6 (3.16)		104 (54.74)	
Clinical (N=404)	248 (61.39)	**<0.05**	100 (24.75)	**<0.05**	92 (22.77)	**<0.05**	31 (7.67)	**>0.05**	20 (4.95)	**<0.05**
Inanimate surface (N=181)	54 (29.83)		21 (11.60)		25 (13.81)		10 (5.52)		91 (50.28)	

CHAPTER VI
DISCUSSION AND CONCLUSION

6.1 Discussion

Intensive care unit (ICU) or critical care unit (CCU) or intensive treatment unit (ITU) is a special and focused area of a hospital where critically ill patients requiring intensive care are treated. These critically ill patients are at a higher risk of acquiring nosocomial infection due to multiple reasons including distruption of barrier to infection by invasive instrumentational procedures such as endotracheal intubation and tracheostomy, urinary bladder catheterization, central venous catheterization etc. (Shannon, 2005 and Shaikh *et al.,* 2008). The nosocomial infections are caused by pathogens prevalent in hospital, several of which are often resistant to many antimicrobials because of the selective pressure due to extensive use of broad-spectrum antibiotics in ICU patients (Hassanzadeh *et al.,* 2008; Fridkin, 2001 and Kollef *et al.,* 2001). Most of these pathogens may remain viable in the environment of hospitals like air, dust, clothes and in inanimate surfaces and equipments which can serve as important source of pathogens (Neely *et al.,* 2000 and 2001; Hota, 2004; Kramer *et al.,* 2006) to the immunocompromised ICU patients. Therefore, the prevention of ICU acquired nosocomial infection 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 like the United States and Europe, 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 ICU patients. ICUs of all hospitals follow various standard international guidelines to control the growth of microorganisms in that unit and so does ICU of NINAS hospital. But despite following

all the norms, persistence of organisms is possible; hence, it is essential to check the efficacy of such guidelines followed. This cross sectional study was thus carried out to determine the prevalence of nosocomial microorganisms in the environment of ICU and their association with the ones isolated from the clinical specimens of indoor ICU patients suspected of infection. But it was beyond the scope of this thesis to determine whether the isolates from clinical specimens of patients caused infection or only reflected the colonization of the critically ill. The current study also examined the association between the clinical and environmental isolates of ICU on the basis of similarity in characters determined by conventional microbiological techniques (cultural, biochemical tests and antibiotic sensitivity pattern) and by statistical analysis. Though the study was carried out in ICU of 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 in ICU.

A total of 687 clinical specimens (including 26 clinical devices that were being used by patients) were collected from 57 ICU patients and were sent for microbological investigation by the clinicians attending the ICU. Out of 687 specimens investigated, significant growth was obtained only in 58.81% (n=404). The percentage of microbial isolation is different in various studies. Study by Singh *et al.* (1996) showed 60.6% of microbial isolation while Deep *et al.* (2004) showed 40.3% (as compared to 58.81% in this study). Such variation in the percentage may have occurred due to the difference in the prevalence of prevailing local ICU flora.

Out 26 specimens of different tip cultures of clinical devices being inserted into the patients, 10 of them showed positive growth with higher occurrence of *P. aeruginosa* (5/68, 83.33%) followed by *K. pneumoniae* (2/6, 33.33%) and *E. coli* (1/6, 16.67%). A study carried out by Deep *et al.* (2004) isolated *S. aureus*, Coagulase negative staphylococci, *Klebsiella* and *Pseudomonas* as predominant organism from 95 catheter tip cultures of which 43.1% showed IV catheter related infection and only 10.5% (n = 10) showed definite catheter related bacteremia. Studies report that intubation and

mechanical ventilation, tracheostomy and nasogastric tube are associated with a risk of developing nosocomial pneumonia. Similarly, urinary catheterization is associated with nosocomial UTI and central venous catheterization is associated with development of nosocomial BSI (Habibi *et al.,* 2008; Tennant *et al.,* 2005 and Caglayan *et al.,* 2005). Therefore presence of these isolates in the tip cultures of medical devices indicate the possible source of nosocomial pathogens in ICU patients which can cause infections in the patients at the respective site of insertion. Isolates of tip cultures were similar to those from the specimen collected from the site of insertion of such medical devices. These tip cultures may have become colonized with the pathogens present within the patients or may have become contaminated via the HCWs hands or by the means of water through which it was rinsed.

The overall clinical isolates in the present study were of seven different genuses. Of these seven genus, the most predominating (6/7, 85.71%) were gram negative ones followed by gram positive (1/7, 14.29%). Similar results showing greater prevalence of gram negative bacteria followed by gram positive bacteria in the ICU patients has also been reported in many other studies *(Hassanzadeh et al., 2009*; Shehabi *et al*., 1996; Nikodemski 1999; Tennant *et al,*. 2005 and Vincent *et al,*. 1995 & 2009). However the distribution and types of gram positive and gram negative bacterial isolates vary among these different ICU studies.

In the present study, the most frequent gram negative isolates were *P. aeruginosa* (61.39%) followed by *K. pneumoniae* (24.75%) and *Acinetobacter* spp. (22.77%). Furthermore, *E. coli* (7.67%), *K. oxytoca* (1.24%), *P. mirabilis* (1.24%) and *M. catarrhalis* (0.74%) were also isolated. Similar reports were shown in national surveillance study of Russian ICUs (Reshedko *et al.,* 2007) Similar, predominating bacteria were also reported from the two ICUs of hospital in Kuwait and Jeddah (Rotimi *et al*., 1998) and from the ICU of hospitals in Turkey (Aksaray *et al*., 2000) and in West Indies (Tennant *et al.,* 2005).On the other hand some study report Enterobacteriaceae as the most predominating ones followed by other gram negative bacilli including *P.*

aeruginosa, *Acinetobacter* spp., etc. (Vincent *et al.*, 1995 and de Leon-Rosales *et al.*, 2000). Contrastingly, some studies report *S. aureus* to be the most frequent isolates followed by other bacteria (Shehabi *et al.*, 1996; Fluit *et al.*, 2001; Fridkin *et al.*, 2001 and Zhanel *et al.*, 2008). The only gram positive bacteria isolated from the clinical specimen during this study were *S. aureus* with comparatively low prevalence (4.95%). However, various studies have reported several other gram positive bacteria such as CoNS, *S. pneumoniae, E. faecalis* including *S. aureus* (Fluit *et al.*, 2001; Fridkin *et al.*, 2001; Tennant *et al.*, 2005 and Zhanel *et al.*, 2008). The presence and predominance of particular potential pathogens may have varied as a result of factors such as patient case mix, device utilization rates, teaching affiliation and empirical antibiotic usage patterns among different ICU types and among similar types. Furthermore, prevailing ICU flora in different types of ICU, location and surrounding environment of hospital and presence of suitable ecological niche for a particular pathogen could be other factors that have contributed for the predominancy. Additionally, effectiveness of the control strategies could be one of most important factor that might have indispensably contributed to the existence of microorganisms in an ICU of a particular hospital. Most of the isolates in this study were especially from the respiratory specimen i.e. tracheal aspirate (88.12%) which is in accordance to several other studies (Zhanel *et al.*, 2008; Reshedko *et al.*, 2007)

Many of the references considered in this study represented the clinical isolates as major cause of infection but isolates of the current study might not necessarily have represented the cause of the infection. Instead they may have only reflected the possible contamination of the specimen or the process of colonization. But nevertheless the isolates were considered etiological agent of infections when the attending physician suspected infection based on systemic signs, and/or local and when the etiological agent occurred repeatedly in culture of consecutive samples of the same patient.

Intensive care units (ICUs) are generally considered epicenters of antibiotic resistance and the principal sources of outbreaks of multi-resistant bacteria. The most important

risk factors are excessive consumption of antibiotics exerting selective pressure on bacteria, the frequent use of invasive devices and relative density of a susceptible patient population with severe underlying diseases (Kumar, 2006; Hassazadeh *et al.,* 2009). Thus, knowledge of the antibiotic susceptibility of the organisms isolated in the ICU helps to formulate an antibiotic policy for the ICU. This also avoids unnecessary use of broad spectrum empirical antibiotics and prevents emergence of drug resistant bacterial strains. In the present study, multiple resistant bacteria with high resistance rates to common antibiotics have been isolated which are described in detail in the following paragraphs.

The AST of gram negative isolates isolated in this study, in overall showed higher sensitivity towards Imipenem followed by Cefoperazone/Sulbactam and to a lesser extent to Ciprofloxaxin and Amikacin whereas higher resistance was observed towards Ampicillin, followed by Cefotaxime and Piperacillin/Tazobactam. On the other hand, gram positive isolates showed higher sensitivity towards Vancomycin with lowest sensitivity towards Piperacillin/Tazobactam and complete resistance towards Ampicillin. Similar type of results was reported by Shehabi *et al*. (2005) however they reported excellent activity of Imipenem and ciprofloxacin against all Gram-negative isolates and Vancomycin against all staphylococci. 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. Such variations in the antimicrobial sensitivity pattern among different studies have differed may be due to the variation in duration and dose of antibiotics used, spectrum of antibiotics used, differeing antibiotic policies among ICUs of different hospitals, etc.

Imipenem is the broadest-spectrum parenteral antimicrobial agent that is commercially available and has remained a useful drug for gram-negative bacilli. In this study as well, Imipenem was consistently the most active agent against most of the gram negative isolates. But in contrary, Japoni *et al.* (2009) have shown Imipenem to be most active against gram positive bacteria. Since imipenem has not been tested against gram

positive bacteria in this study, therefore comparision was difficult to be made. Despite of its effectiveness, resistant pattern for Imipenem during the study duration was observed to be in 2.33% isolates of *K. pneumoniae*, 8.33% of *P. aeruginosa* and 86.96% of *Acinetobacter* spp. **H**igh rates of Imipenem non susceptible *P. aeruginosa* (15.0%) and *Acinetobacter* spp. (22.0%) has also been shown in the study undertaken by Hsueh *et al*. (2004) in ICUs of Taiwan which was comparatively lower rate than in our study. But nonetheless those isolates including *P. aeruginosa, Acinetobacter* spp. and *K. pneumoniae* that were resistant to Imipenem and all other antibiotics used in this study were found to be susceptible to Polymyxin B.

Ciprofloxacin and Amikacin were found effective against 46.78% and 47.18% of the isolates of *P. aeruginosa* respectively followed by 41.0% & 75.0% isolates of *K. pneumoniae* and 60.0% & 100% of *K. oxytoca*. Similarly, *M. catarrhalis* was fully susceptible to both of these antibiotics. All the isolates of *P. mirabilis* were susceptible to Ciprofloxacin while 96.77% isolates of *E. coli* were found to be resistant to Ciprofloxacin. Likewise 100% isolates of *Acinetobacter* spp. were found to be resistant to ciprofloxacin and 98.91% to Amikacin.

In the study all the isolates of *Acinetobacter* spp. and *P. mirabilis* were found to be unaffected by the action of Gentamicin and Ofloxacin nevertheless these antibiotics were effective against all the isolates of *M. catarrhalis*, 60.0% isolates of *K. oxytoca* and about 48.0% isolates of *P. aeruginosa*. Cefotaxime and Cotrimoxazole were found to be resistant to almost all types of bacteria isolates except *M. catarrhalis* and *K. oxytoca* respectively. Piperacillin /Tazobactam and Cefepime/Tazobactam were also found to be not so effective against several isolates however the isolates of *M. catarrhalis* were found to be susceptible. Except for the *Acinetobacter* spp., the antimicrobial action of Cefoperazone /Sulbactam was observed to be higher towards most of the clinical isolates.

The rise in resistance of organisms to Cefotaxime, Ciprofloxacin, Amkacin and Imipenem in this study may be due to increased consumption of spectrum of

cephalosporins, β lactam-beta-lactamase inhibitor combinations (Cefepime/Tazobactam, Piperacillin/Tazobactam and Cefoperazone/Sulbactam), Imipenem, fluoroquinolones and aminoglycosides in the hospital. In present study enterobacteriaceae (*E. coli and K. pneumonia*) isolates with decreased susceptibility to Cefotaxime and/or Cefepime (in combination with Tazobactam) were defined as extended spectrum β-lactamase (ESBL) phenotypes whereas multidrug resistance *P. aeruginosa* was characterized by pattern of resistance to a number of antibiotics tested especially aminoglycoside, Cefepime/tazobactam, Cefotaxime, Ciprofloxacin and Imipenem to which most of isolates of a species were susceptible (Zhanel *et al.,* 2008 and Lokhart *et al.,* 2007). Resistance to β-lactam antibiotics has emerged mainly due to the production of β-lactamase as well as through alterations in the targets of the drugs, the penicillin-binding proteins, and through alterations in outer membrane permeability of the organisms to the drugs. The development of extended spectrum β-lactamases has been explosive and since 1983 more than two dozen beta-lactamases among gram-negative bacilli have been described (Philippon *et al*., 1989). Gupta *et al*. (2006) reported that resistance to Meropenem and Imipenem was seen in various clinical isolates of Gram-negative bacteria that were ESBL-positive. Such β- lactamase producing clinical isolates with resistance to Imipenem were a problem even in the ICU of NINAS hospital surveyed during this dissertation period.

Similarly, *Acinetobacter* spp. showing resistance to Gentamicin, Cefotaxime, Ciprofloxacin, Chloramphenicol, Cotrimoxazole and Imipenem were defined as multidrug resistant in this study. Isolates of *Acinetobacter* spp. in the study were resistant to most antibiotic tested even the Imipinem, which may be due to acquisition of genes encoding resistant determinants (such as genes encoding for β lactamases, aminoglycosides modifying enzymes, carbapenemases, etc.) and due to prolonged ICU stay of patients with prolonged use of such antibiotics. Hence in the present study the most important resistance problems are encountered in Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter* spp., with increasing trends observed for all major anti-gram-negative agents (β-lactams, fluoroquinolones, aminoglycosides and Imipenems).

The emergence of new β-lactamases such as the extended-spectrum β-lactamases (ESBLs) and the carbapenemases that are capable of degrading the expanded-spectrum cephalosporins and/or carbapenems, might have aided in the determination of resistance to non-β-lactam agents such as aminoglycosides and fluoroquinolones. Therefore it is noteworthy to say that widespread use of broad-spectrum antibiotics leads to the emergence of antibiotic-resistant strains of many Gram-negative organisms which was also indicative even in this study. So, for such multiple drug resistant pathogens like *P. aeruginosa, Acinetobacter* spp. *and K. pneumoniae,* Polymyxin B (that acts on cell membrane of the organism) was the only remaining antibiotic drug class exhibiting fairly consistent antimicrobial activity or efficacy in the present investigation. Thus this study highlights the increasing trends of multidrug resistance among gram negative isolates towards Imipenem and some only susceptible to last line drugs Polymyxins, therefore indicating an alarm of threat of emergence of all drug resistant pathogens.

Current study showed Vancomycin as the most effective antibiotic against *S. aureus* isolated from tracheal aspirate and so was in the study carried out by Koirala (2009) in the same hospital. *S. aureus* isolates from other specimens such as blood, pus and wound swab of ICU patients also showed higher sensitivity to Vancomycin. Ofloxacin was another effective drug against *S. aureus* (70.0%) after Vancomycin but many of the other antibiotics tested for *S. aureus* were found to be less effective. In this study, the susceptibility pattern of *S. aureus* against Methicillin has not been tested due to immediate unavailability of the antibiotics which would otherwise have helped to trace out Methicillin resistant (MRSA) or Methicillin sensitive (MSSA) strain of S. *aureus*. Though not tested with Oxacillin and methicillin discs, however exhibition of 100% reistance to Ampicillin and 90.0% resistance to Cloxacillin by *S. aureus* in this study led us to suspect that those strains could be MRSA ones. So, indicating again a great problem for the patients and hospital as well.

The hospital environment may serve as a potential source of nosocomial bacterial or fungal strains in hospital. Ambient environmental factors such as air circulating inside

the ward; water supplied through taps in the ward; hands and nasal carriage of health care workers; different types of fabrics or clothing material used by the health care workers, visitors and patients; different inanimate surfaces, e.g., surfaces of medical devices, door handle, bed rails, tables etc. may harbor some forms of microorganisms through contamination from exogenous or endogenous sources. When these already present hospital pathogens in the environment get a route to enter into a susceptible host, they may cause infections to the ICU patients who are extremely immune suppressed.

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 the critically ill patients. 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 in Alwehda educational hospital, in Thamar. 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 of the MRSA strains of *S. aureus.* Although in this study, the prevalence of MRSA was not determined using guidelines, however identical and closely related isolates of *S. aureus* in ICU patients and their environment such as air, fabrics and fomites (inanimate surfaces and equipments) was indicative of the fact that there might be association between the clinical and environmental *S. aureus* isolates.

Therefore, investigating the occurrence of the environmental hospital pathogens around the patient so as to correlate it with clinical isolates was the side by side work in the present study, while performing the culture and sensitivity testing of the clinical specimens of suspected ICU patients. Doing so in this dissertation work it was observed that many isolates of *P. aeruginosa*, *Acinetobacter* spp., *K. pneumoniae, E. coli* and *S. aureus* did not appear in first few eligible consecutive specimens of the same patient.

But after few days, as the patients length of stay in ICU increased, a single type of organism continuously appeared in the specimens signifying the possibility of exogenous or environmental source of the hospital pathogen. So, abruptly after identification and sensitivity testing of the clinical isolates from eligible consecutive samples of ICU patients, the sources of the pathogen in the ICU environment around the patients was sinvestigated. Hands and nares of HCWs in ICU, air of ICU room, water from the taps inside ICU and its reservoirs in the collecting tanks, different clothing materials used by the HCWs, visitors and patients and as well as various inanimate surfaces in and around the patients were randomly sampled as soon as a patient's clinical specimen was found to be infected with same bacterium during his/her stay in ICU.

A total of 677 duplicate environmental samples (including both animate and inanimate objects) were collected and studied. Out of the total, 562 samples were found to be culture positive. The most predominating Gram negative bacteria in the environmental samples were *P. aeruginosa* followed by *Acinetobacter* spp., *K. pneumoniae* and *E. coli* whereas the most predominant gram positive bacteria include CoNS, *S. aureus* , Enterococci, *Bacillus* spp. and Micrococci. In many samples fungi were also isolated however their identification was not done which was one of the limitations of this study. Study carried out by Panta (2006) showed gram negative rods were the least occurring microorganisms and gram positive cocci were the most frequent. However in the present study, gram negative environmental isolates were as frequent as gram positive cocci, infact some gram negative rods such as *P. aeruginosa* tends to occur in most of the environmental sample processed. In another study, *S. aureus* have been reported as the most frequent isolates from the environment samples, including the environment of ICU (Pokhrel *et al*., 1993). The greater prevalence of both gram negative rods as well as gram positive cocci in this study may be presumably due to difference in the study site (ICU was only the site in this study).

Isolation and identification of bacteria such as *P.aeruginos, Acinetobacter* spp., *K. pneumoniae*, *E. coli* and *S. aureus* from the environmental samples collected several times during the study period of ICU was indicative of the viability of these pathogens in the environment that may have invaded and colonized the susceptible patients in ICU, isolated and identified from the clinical specimens.

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. In this study, microbiological examination of indoor air of ICU revealed CoNS as the most predominant organism followed by, *S. aureus,* Micrococci, Enterococci, *Bacillus* spp., Fungal isolates, in the decreasing order of frequency of 83.33%, 80 %, 43.33%, 30%, 26.67%, 23.33% , 23.33% , 20%, 16.67% and 6.67% respectively. Similar results were reported in the study by Sharma (2006) where he showed the higher prevalence of CoNS followed by *S. aureus,* Micrococci, *Bacillus* spp., gram negative rods, gram negative cocci and a few yeast in the circulating air inside ICU of a TU teaching Hospital in Nepal. However other studies reported *S. aureus* as the most prevalent air flora followed by CoNS in the ICU of hospitals in Nepal (Panta 2006; Banjara 2002).

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 (Croft *et al.*,1986), and 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, although air

flowing inside ICU was HEPA filtered, however showed 100% result i.e. all samples revealed high prevalence of organisms in the air. The main reason for the high prevalence of microorganisms in ICU air surveyed may be due to improper functioning of the HEPA filter, inappropriate fumigation of the ICU room as well as inappropriate cleaning of the sources around the environment. The second reason may be due to higher flow of HCWs, visitors and even of patients, all of whom in some way, may have contributed for the shedding of pathogens they carried, into the ICU air. Some of those air isolates such as *P. aeruginosa*, *Acinetobacter* spp., *E. coli*, *K. pneumonia* and *S. aureus*, on other hand, were also isolated from clinical specimens of ICU patients signifying that air might have been another critical source of these pathogens in ICU patients. However, in an epidemiological study, Bauer *et al.* (1990) showed that the spectrum of bacteria recovered from patients and air was generally different.

Since water is vital for survival, water distributed and used in the hospitals for hydration and hygiene of both patient and health care worker is assumed to be clean and safe. The water in the hospital intended for human consumption must be free of pathogenic and chemical agents, pleasant to taste and usable for domestic purposes. However contamination of the hospital water supply with potentially pathogenic organisms is very common. Much of the ill-health which affects humanity, especially in developing countries can be traced to lack of safe and wholesome water supply. There can be no state of positive health and well being with-out safe water. Tap water in health-care institutions can be a source of nosocomial infections. Study by Anaissie *et al*. (2002) demonstrated that epidemiological and molecular evidence to biofilms (a community of microorganisms growing as a slimy layer on surfaces immersed in a liquid) in water storage tanks, tap water, and water from showers serving in several outbreaks of infection in the hospital.

In NINAS hospital, water to be supplied to different wards including ICU is collected in a big reservoir and treated with standard chemicals (chlorine, alum and caustic soda) and filtered and then collected in big tanks for distribution into taps. Drinking water is

ultrafiltered prior to distribution to tap. During the study period, all the water samples investigated from the taps of ICU and its reservoir collecting tanks before cleaning and maintenance of water supply system showed higher prevalence of *P.aeruginosa, Acinetobacter* spp. and coliform bacteria even they were normally filtered and chemically treated. Unfortunately, there was no detectable reduction in the microbial growth of those isolates from water even after the cleaning and maintenance of the whole water supply system and its reservoir tanks except in the reduction of coliform counts in 50% samples (WHO standards for unacceptable coliform count in chlorinated water supply is <5MPN/100ml). This indicated that the cleaning and maintenance of water supply system was not to the satisfactory level where microorganisms are almost removed. Since the water coming from the taps of ICU are used by all HCWs in ICUs to wash their hands, to wash the feeding dishes such as glasses and bowls used for patients, to rinse medical equipments such as tube feed bags, flexible endoscopes, respiratory equipments, etc. and is also used in hospital laundry to wash different clothing materials such as towels, aprons, gowns used by within the unit, therefore these ICU water taps and its reservoirs need to be carefully treated both physically by proper filtration as well as chemically by chlorine or other chemicals so as to prevent exposure and spread of those pathogens from water to the critically ill patients. However, not even a single microorganisms was detected in the water sample collected from drinking water tap before and after cleaning and maintenance, which was indicative of excellent quality of drinking water that was maintained so may be by ultrafiltration technique which was not applied to other tap water source in ICU and its reservoir in the hospital. So the water from the sources other than drinking water tap may pose a risk of contaminating waterborne microorganisms which can easily infect the ill health patients if mode of transmission is provided.

Many waterborne microorganisms are opportunistic pathogens that can increase the risk of infection in immunocompromised patients. There are many evidential studies to show that nosocomial infections have been traced to water supplies and/or point-of-use water in the hospital such as a molecular level study by Trautman *et al*. (2001) through

Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), showed that strains of *P. aeruginosa* isolated from different infection sites of patient such as blood, lungs, trachea, urine and peritoneum was similar to the strain isolated from water supplies. Similarly, molecular study by Pina *et al.* (1998) demonstrated that the *A. baumanii* involved in skin and wound infection of patients matched with that isolated from water supply system. In another study, tap water from faucets contaminated with *P. aeruginosa* has been shown to play an important role in the propagation of this pathogen among patients in SICU. A high number of transmissions were shown to occur both from faucet to patient and from patient to faucet (Reuter *et al*., 2002).

Therefore water can be the most important potential source of infectious nosocomial pathogens to the patients in ICU with reduced immune status. Though in this study we were unable to perform molecular analysis to show strong association among the isolates from clinical specimens and water sample but nevertheless by observation of similarity in microscopic structure, cultural and biochemical characters of those isolates we can presume that the isolates of *P. aeruginosa, Acinetobacter* spp. and some coliform groups from the water supply system may have association with that recovered from clinical specimens of ICU patients.

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 pathogen. 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., Vancomycin-sensitive and -resistant enterococci (VSE and VRE) and Methicillin-sensitive and -resistant staphylococci (MSSA and MRSA) and as well as fungi such as *Fusarium* ,

Paecilomyces, Mucor, Aspergillus, various Candida species, 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 that fabrics thus can serve as reservoir of various types of nosocomial pathogens. Pilonetto *et al*. (2004) analyzed the microbiota in the gowns of 31 professionals from the general intensive care unit. Among the isolated pathogens 11/18 were *S. aureus,* 2/18 were *A. baumannii,* 2/18 were *K. pneumoniae* and 1/18 were *S. rubidae*. Similar type of result was shown by this study in clothing materials or fabrics including gowns of HCWs, patients and visitors; bed sheet used by patients and privacy curtain around individual patient in ICU.

Gowns are worn by health care workers and visitors before entering the ICU room to protect themselves from drops and splashes as well as to protect the patient from the flora they may carry, have been found to be contaminated with several organisms ,the most prevalent being CoNS accounting for 66.66% and 60.0% in HCWs gown and visitor's gown respectively. The study also report other isolates in the gowns including *S. aureus, P. aeruginosa, Acinetobacter* spp., Enterococci, *K. pneumoniae, E.coli, Bacillus* spp.and fungal isolates. Previous study by Sharma (2006) has also reported the presence of similar type of microorganisms on the apron worn by the healthcare workers. Though these aprons were washed daily with bleaching powder and detergents every day, it was suspected that these gowns in the ICU surveyed, have become contaminated may be due to organisms carried by the visitors or health care workers on their personal clothing or on their exposed body parts (such as hands, neck or skin etc.) from outside environment other than hospital or may be due to irregular and inadequate laundering by use of contaminated water.

This indicates that the gown worn by HCWs and visitors can serve to transmit those pathogens to the susceptible patient through direct contact of gowns with the patient or by the hands of HCWs that may have become contaminated after touching the gowns.On the other hand, sampling of gown worn by patient and bed sheet used by them during their stay in ICU showed Coagulase positive *S. aureus* to be the most

predominant organism, frequency being 61.11% and 57.14% respectively. Other predominant organisms include CoNS, *P.aerugnosa, Acinetobacter* spp., *K. pneumoniae,* Enterococci *and E. coli.* This result was in accordance to result by Sharma (2006) who reported higher presence of *S. aureus* followed by CoNS, gram negative cocci, *Micrococcus, P. aeruginosa, Acinetobacter* spp. *and Klebsiella* spp. Similarly, privacy curtains around the patient were also shown to harbor similar types of isolates as that of other clothing materials. Patients clothing may have become contaminated with such pathogens due to their own body flora or via the contaminated hands of HCWs and their clothes in contact or even due to improper washing of such clothes by the hospital laundry. Whatever is the mode of contamination, but it should be cautiously understood that all these contaminated fabrics or clothes in one or the other way can be an underestimated source of pathogens to the patients requiring intense care.

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%) of which 13.33% were MRSA. In our study, 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, Coaulase positive *S. aureus, P. aeruginosa, Acinetobacter* spp., *K. pneumoniae, E.coli,* Enterococci, *Bacillus* spp. and some fungal isolates. Microbiological reports of inanimate surface samples of this study approximately concurred to the previous report made by Panta (2006) which was however representative of all wards including ICU of a Teaching Hospital in Nepal. The predominant pathogens reported by Panta 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 by Sharma (2006) which was more representative to our study as it included surface samples of inanimate objects from ICU and SICU. Such contaminated fomites lead to nosocomial infections either through direct contact of patients with fomites or indirectly through the hands of health care workers that becomes contaminated after touching contaminated surfaces.

But there are rarely any studies showing association of organisms from inanimate surfaces with that of clinical isolates of patients. However comparing similarity in cultural and biochemical characters of isolates such as *P. aeruginosa*, *Acinetobacter* spp., *K. pneumoniae*, *E. coli* and *S. aureus* from the inanimate surface samples and from the patient clinical specimens, it was presumed that these organisms were associated, hence inanimate surfaces considered to be the potent source of nosocomial pathogens in ICU patient we studied. Since the bed rails, bedside tables, working tables and bed bar handles are the objects of frequent touch, they may have become contaminated through unwashed or contaminated hands of visitors and HCWs.

On the other hand, equipment surfaces such as bedside monitor, ventilator air filter and stethoscopes are the objects that come in frequent contact with HCWs, therefore contaminated hands of clothes of HCWs are probably the source of organisms in such equipments. Existence of these organisms in the inanimate surface samples of ICU indicate poor sanitation and disinfection practices .If by any means these organisms entered the body of susceptible host, will definitely cause infections. Therefore proper disinfection with appropriate chemical disinfectant should be carried out as well as the hand washing practices of both health care workers and visitors should be strictly monitored which if not done would pose a serious threat to those critically ill patients in ICU.

Nasal carriage of hospital strain among health care worker is also an important source of nosocomial infection. About 25.0% of HCWs are regarded as stable nasal carrier (Farzana *et al*., 2008). Especially the NI caused by Staphylococci can be associated with

the nasal carriage of HCWs. Study by Panta (2006) showed that out of 48 nasal samples of health care worker, *S. aureus* was isolated from 44.0% of the samples. *Staphylococci* MRSA is a nasal infectious pathogen which is becoming of significant importance year by year. In a study carried out by Zer *et al.* (2009), a total of 98 samples from health care workers of ICUs were collected out of which 14 (14.3%) samples showed MRSA nasal colonization in the HCWs. Another study by Luzar *et al.* (1990) and Cespedes *et al.* (2002) have demonstrated that occasionally health care workers who carry *S. aureus* in their nares can cause outbreaks of surgical-site infections.

In an investigational study on nasal carriage of HCWs by Farzana *et al*. (2008), 86.8% of the total samples were positive for at least one of the staphylococcal species, nine specimens (6.9%) contained two different species, and one contained *K. pneumoniae* along with staphylococci. Similar type of results was observed in this study as well whereby out of the total of 55 nasal samples, 100% were found to be culture positive, with coagulase positive *S. aureus* (52.73%) being the most predominant organism followed by CoNS (47.27%), *K. pneumoniae* (5.45%) and *E. coli* (1.82%). In this study, the nasal colonization of nosocomial strains in the HCWs in ICU may be associated with the exposure status that is to say HCWs have three fold high exposure to the organisms from several sources including patients, contaminated surfaces etc. than others. Therefore, presence of such notorious organisms especially *S. aureus, K. pneumoniae and E. coli* in the anterior nares of the HCWs may have become an active source of transmission of pathogen among the ICU patient (the clinical specimen of whom grew similar type of pathogens). So it can be said that during this study, some of the pathogens from the anterior nares of HCWs may have infected the ICU patient either via droplet route or via the route of HCWs hands. Therefore it is necessary for them to decolonize the commensal staphylococci as well as appropriately clean their nose and practice safe hygienic and along with good hand washing compliance. On antimicrobial susceptibility testing of nasal isolates, Ampicillin was found to be ineffective against almost all isolates. Vancomycin was the most effective antibiotic towards the gram positive cocci isolates followed by Gentamicin, Cloxacillin and

Ciprofloxacin. For gram negative nasal isolates, Cholramphenicol and Ciprofloxacin were the most effective drugs followed by Amikacin and Cefotaxime.

As it is known that the hospital pathogens can remain viable on several inanimate surfaces, clothing materials, air and water, it can later serve as important source of cross transmission from HCWs to patients or among the patients. 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 health care workers is considered the main route of spread of nosocomial infections. *A. baumanii* strains isolated from the hands of health care workers 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.

In the previous study carried out by Panta (2006), several isolates from the hands of health care workers 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. In the present study, out of the total of 100 hand samples of HCWs in ICU, each 50 samples collected before and after hand wash, showed positive culture in 46 samples of before hand wash and 40 samples after hand wash. Among the 46 positive samples collected before hand wash, *Pseudomonas* spp. and CoNS were found to be predominant organisms followed by *S. aureus, E. coli, K. pneumoniae,* Micrococci, *Bacillus* spp., Enterococci, fungal isolates and *Acinetobacter* spp. Similar organisms were isolated with similar pattern from the 40 positive samples collected after hand wash indicating much less reduction in the number of positive samples for the growth of microorganisms (the load was not measured as it was TMTC and difficult to be counted). This study showed higher prevalence of microorganisms in the hands of health care worker before they wash their hands. If in such state of carriage, the health care staffs comes in direct contact with patients in ICU during regular activities, then it

is for sure that their hands can serve as a potential source of microorganisms to the patients and nonetheless such contaminated hands can also contaminate nearby inanimate surfaces which is again a source of pathogens to nearby 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). However in our study, no satisfactory removal of the microbial flora from the hands of healthcare workers was achieved even after hand washing. Except 10 samples, all grew organisms signifying poor hand washing compliance. Such results aided us to come to one conclusion that hands of HCWs both washed and unwashed could be very frequent source of nosocomial pathogens especially *P. aeruginosa*, *Acinetobacter* spp., *K. pneumoniae* , *E. coli* and *S. aureus* which were the major isolates in the clinical specimens of ICU patients studied. Such presence of high microbial load in this study, even after wash was associated with four main reasons: haphazard hand washing, washing with soap and water without drying, washing and drying only for a few seconds and washing with contaminated water followed by swapping with a common towel which was also found to grow similar microbes as was in the hands of HCWs when its centre and edges were impinged directly into agar plate and followed by identification with biochemical test results.

Hand washing is widely accepted as the cornerstone of infection control in the intensive care unit. Hand washing on its own does not abolish, but only reduces transmission, as it is dependent on the bacterial load on the hand of healthcare workers. Though not satisfactory from compliance point of view, however our study suggests that washing hands helps more or less in diminishing the load of bacterial flora from the hand of health care workers. Depletion in microbial load however depends on the type of antiseptics soap being used, quality of water supplied as well as time taken by the HCWs to wash their hands. It was also observed that only washing hands with soap and

water without drying does not reduce microbial load whereas hand washed with medicated soap (Lifebuoy) and water then with liquid soap (Chlorohexidine/ alcohol solution) and water and then dried properly, helped in reduction of microbial load from the hands which was being performed by only few of the HCWs.

During this dissertation work, there were only five common organisms: *P. aeruginosa*, *Acinetobacter* spp., *K. pneumoniae, E. coli* and *S. aureus*, that were isolated from both clinical as well as environmental samples including hands and anterior nares of HCWs, circulating ICU air, ICU tap waters and its reservoirs, different types of fabrics/ clothing materials and various inanimate hospital surfaces. Analysing all the culture positive environmental samples and clinical specimens simultaneously, it was observed that *P. aeruginosa* which was frequently isolated from clinical specimens was also a common isolate in environmental samples except in the nasal swab of HCWs and similar type of occurrence was observed with *Acinetobacter* spp. Similarly, *S. aureus* that appeared in clinical specimen was also present in almost all environmental samples except from the water. On the other hand, *K. pneumoniae* and *E.coli* were the two bacteria in clinical specimens that were isolated from all environmental samples investigated even from the nasal swab of HCWs in ICU, however were lesser in prevalence than compared to *P. aeruginosa.* Antibiotic sensitivity testing of the environmental isolates also showed similar pattern of resistance and sensitivity as that of clinical isolates to some of the drugs such as Imipenem, Cefoperazone/Sulbactam, Cefotaxime, Ciprofloxacin, Amikacin, etc. indicating probable association between them.

Thus on the basis of extent of occurrence, colony morphology, cultural characteristics, biochemical characteristics, pigmentation, coagulase test results, microscopic observations and antibiotic sensitivity pattern, an association was made between the isolates of Clinical specimens of ICU patient and the isolates from the environment around him or her. In addition, statistical analysis also demonstrated that there occurred significant association between the isolates from clinical and environmental samples. This means, in the present study, the patients whose clinical specimen was found to be

positive for certain kind of microorganisms, may have acquired the organisms from various environmental sources around them. As with accordance to NNIS System of CDC, if the infection or infectious agent is acquired after 48 hours of hospital stay, it is to say that the source might be of hospital origin (Garner *et al*., 1996), therefore finding of this study also revealed that those organisms in clinical isolates were not of endogenous origin as the first few consecutive samples sent on each day of patient stay did not show growth of any microorganisms, however growth of a specific type of organism was observed in the specimens sent on three to fifth day. Thus this observation led to establish a concrete fact that the organisms from the exogenous or environmental sources might have inavaded those immunocompromised patients in ICU, thereafter appearing in respective clinical specimens.

Furthermore, statistical analysis using χ^2 – test also showed the association of clinical isolate with those similar isolated from environmental samples ($p< 0.05$). However, there was no significant association of *P. aeruginosa* from clinical isolate with that isolated from hand imprints of HCWs. Similarly, *K. pneumoniae* isolated from clinical isolates and from air and hand imprints of HCWs did not show any significant association.

Although, not confirmed by antibiogram and advanced molecular analysis due to several limitations, the results of this study obtained by the application of conventional microbiological methods of isolation and identification for the microbes, aided us to establish a relation that isolates in clinical and environmental samples might be the same and the clinical isolates that have infected or colonized ICU patients may have come from the environmental sources in ICU including HCWs hands, Equipments used in patients, ICU air etc. 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.

6.2 Conclusion

From this study of microbiological analysis of both the patients and the ambient environment of ICU allowed to relate the prevalence of nosocomial pathogens in the clinical specimens of ICU patients to that of nosocomial pathogens prevalent in ICU environment. Five of the most common clinical isolates including: *P. aeruginosa*, *K. pneumoniae*, *Acinetobacter* spp., *E. coli* and *S. aureus* were frequently isolated from the environmental samples (both animate and inanimate objects), thus indicating environment as the source of these pathogens responsible for the colonization followed by infection in the critically ill ICU patients. For instance, *P. aerugonosa* that was isolated from a total of 61.39% of the positive specimen was on the other hand subsequently isolated from air (80.0%), Fabrics/Clothes (28.11%), Inanimate surface (54.0%), water (83.33% respectively before and after maintenance) and from 66.0% of the hand imprint samples of HCWs before wash and 54.0% after wash, thus indicating higher prevalence. The organism was found to show similar pigmentation pattern, colony morphology, etc. therefore regarded as related. Similarly other four isolates also were related in this manner. Prevalence of these five common pathogens in the washed hands of HCWs indicated the lack of effective hand washing compliance and lack of good hygienic practices. The study thus called for development and adherence to strict hand washing guidelines followed by regular monitoring and supervision of the practices. High prevalence of *S. aureus* and even some unusual pathogens such as *K. pneumoniae* and *E. coli* in the anterior nares of the HCWs indicated another potent source of these pathogens to the patients coming in frequent contact with them and therefore signaled for the screening of such carriers and their subsequent elimination. Similarly, existence of similar isolates to that of clinical isolates in the inanimate objects around the periphery of patients including equipments such as ventilator and stethoscope, on the other hand, signified another important source of pathogens for the ICU patients and therefore necessitates the accurate decontamination procedure for the removal of isolates from those surfaces. Likewise, presence of the microbes in the air circulated and water distributed in the taps of ICU indicated another important source of

pathogens to the critically ill patients and hence alarms for effective fumigation of ICU room followed by proper treatment of water supplied through the taps of ICU. Finally, from this study it can be concluded that there existed close association between the isolates of clinical specimens from ICU patients and the isolates from environment. That means microbes prevalent within the ICU may have played significant role in colonization and subsequent infection in the severely ill ICU patients. However implementation of strong and timely intervention strategies may help in reducing the prevalence of those microbes in the ICU.

CHAPTER VII
SUMMARY AND RECOMMENDATION

7.1 Summary

1. ICU patients have 2-5 times greater susceptibility of acquiring infection by a pathogens (prevalent around or within the patients) than in patients of other ordinary wards. Hence, this study was conducted to determine the prevalence of microorganisms in ICU and then to determine whether or not these organisms were responsible for causing infection or colonization of ICU patients.
2. Among he different types of clinical specimens sent to the laboratory for microbiological investigation, tracheal aspirate (N=498) was the most regularly sent specimens followed by CSF (n=98) and others .The most frequent isolates from these specimens was *P. aeruginosa* recovered from the 61.39% of the positive specimens followed by *K. pneumoniae* (24.75%), *Acinetobacter* spp. (22.77%), *E. coli* (7.67%), *S. aureus* (4.95%), K. oxytoca (1.24%), *P. mirabilis* (1.24%) and *M. catarrhalis* (0.74%).
3. Antibiotics such as Imipenem, Cefoperazone/Sulbactam and Amikacin had relatively higher affectivity against most of the clinical isolates than other antibiotics tested. Ampicillin and Cefepime/ Tazobactam were the least effective ones against all isolates.
4. Sampling of environment (animate and inanimate) immediately after conforming the clinical isolates to have exogenous origin, revealed presence of those isolates in the environmental samples. There were five organisms common in both clinical and environmental samples viz., *P. aeruginosa, K. pneumoniae, Acinetobacter* spp., *E. coli* and *S. aureus.*
5. *P. aeruginosa*, *Acinetobacter* spp. were isolated from almost all the samples of environment except from the nasal swab of HCWs while *E. coli* and *K. pneumoniae* have been identified from all the samples including nasal swab of

HCWs. On the other hand, except in water, *S. aureus* was highly prevalent in all other samples from the environment of ICU.

6. Out of 50 hand imprints of HCWs sampled before washing the hands, 92% of the sample showed growth whereas 80% of the hand imprint samples taken after hand wash showed growth of microorganisms, thus demonstrating low hand washing practices of some HCWs. *P. aeruginosa* was the most common isolate in both samples taken before and after wash.
7. Presence of *K. pneumoniae* (5.45%) and *E. coli* (1.81%) in the nasal sample of HCWs, both of which were sensitive to Ciprofloxacin and Chloramphenicol, was surprising and was thought to be one of the potent sources of these pathogens in ICU patients. However, there was greater prevalence of Vancomycin sensitive *S. aureus* in most (52.73%) of the nasal samples.
8. Air of ICU sampled by Plate exposure method, was found to possess different microorganisms similar to clinical isolates most predominant being *P. aeruginosa* (80%) followed by *S. aureus* (43.33%), *Acinetobacter* spp. (20%), *K. pneumoniae* (16.67%) and *E. coli* (6.67%), therefore indicating another probable source of organisms in the clinical specimen of ICU patients.
9. Chemically treated water sample (except that sampled from drinking water tap) before cleaning and maintenance revealed unacceptable level of coliforms (>5MPN/100ml) and presence of *P. aeruginosa* and *Acinetobacter* spp.. However the large reservoir showed acceptable level of coliform count (3MPN/100ml) along with the presence of *P. aeruginosa* and *Acinetobacter* spp. At the other point, even after cleaning and maintenance, persistence of the same organisms was recorded in all the samples except from the drinking water tap. All samples showed unacceptable level of coliforms except in the water of large reservoir (0MPN/100ml) and small reservoir (2 MPN/100ml), thus revealing water as another possible source of pathogens.
10. Prevalence of microbes were also observed in clothes/ fabrics used by patients, HCWs and visitors. Common isolates from positive samples include *S. aureus* (47.93%), *P. aeruginosa* (28.11%), *Acinetobacter* spp. (14.29%), *K. pneumoniae*

(7.37%), *E. coli* (2.76%) which were also the common isolates from clinical specimens and several other isolates had also been isolated and identified.

11. AST of environmental samples revealed that all of the gram negative isolates were 100% sensitive to imipenem while gram positive isolate i.e. s. aureus was most sensitive to vancomycin (100%).
12. Association between the common clinical and environmental isolates on the basis of occurrence pattern, microscopic observations, colony characteristics, pigmentation, biochemical characteristics and coagulase test reaction revealed that they are related and thus the fact was developed that the environment might be one of the major sources of pathogens in ICU patients.
13. Statistical analysis made, also reflected the significant association of clinical isolates with that of environmental isolates ($p<0.05$) thereby making the measure of relatedness strong.

7.2 Recommendations

1. Hands of HCWs have been recognized as one of the common mode of cross transmission of nosocomial pathogens to the patients. Therefore strict hand washing guidelines followed by regular monitoring and supervision is highly recommended so as to reduce contact and cross transmission by HCWs.
2. It is recommended to use single use towel rather than common use towel so as to reduce common source transmission.
3. It is recommended, if possible daily otherwise weekly, to carry out microbiological surveillance of the suitability and effectiveness of each and every intervention strategies applied in ICU and the entire hospital to eliminate microorganisms. Proper dissemination of the result of the surveillance among all hospital staffs would help them to become aware of all the facts.

CHAPTER VIII
REFERENCES

Agvald- Ohman C, Wernerman J, Nord CE and Edlund C (2003) Anaerobic bacteria commonly colonize the lower airways of intubated ICU patients. Clin Microbiol Infect 9(5): 397-405.

Aksaray S, Dokuzoguz B, Guvener E,Yucesoy M, Yulug N, Kocagoz S,Unal S, Cetin S,Calangu S, Gunaydin M, Leblebicioglu H,Esen S, Bayar B, Willke A, Findik D, Tuncer I, Baysal B, Gunseren F and Mamikoglu L (2000) Surveillance of antimicrobial resistance among gram negative isolates from intensive care units in eight hospitals in Turkey. J Antimicrob Chemother 45: 695-9.

Alberti C, Brun-Buisson C, Burchardi H, Martin C, Goodman S, Artigas A, Sicignano A, Palazzo M, Moreno R, Boulme R, Lepage E and Le Gall R (2002) Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study. Intensive Care Med 28(2): 108-12.

Ali AM, Abbasi SA, Arif S and Mirza IA (2007) Nosocomial infections due to methicillin resistant *Staphylococcus aureus* in hospitalized patients. Pak J Med Sci 23 (4): 593-596.

Anaissie EJ, Penzak SR and Dignani C (2002) The hospital water supply or a source of nosocomial infection: a plea for action. Arch Intern Med 162: 1483-92.

Appelgren P, Hellstrom I, Weitzberg E, Soderlund V, Bindslev L and Ransjo U (2001) Risk factors for nosocomial intensive care infection: a long-term prospective analysis. Acta Anaesthesiol Scand. 45(6): 710-9.

Archibald L, Phillips L, Monnet D, McGowan JE Jr, Tenover F and Gaynes R (1997) Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit. Clin. Infect. Dis. 24 (2): 211-5.

Arunodoya GR (2001) Infections in neurology and neurosurgery intensive care units. Neurol India 49 (1): 51-9.

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

Barg N (1995) New resistance mechanisms of common bacterial pathogens. Contemp Intern Med 7: 61-69.

Bauer TM, Ofner E, Just HM, Just H and Daschner FD (1990) An epidemiological study assessing the relative importance of airborne and direct contact transmission of microorganisms in a medical intensive care unit. J Hosp Infect. 15(4): 301-9.

Bdareen KZ (2009) Bacterial contamination in the hospital and its aggressive behaviors. BIB 1801: 0000-00-00 (Draft) (S, G).

Beggs CB (2003) The airborne transmission of infection in hospital buildings: Fact or fiction? Indoor Built Environ 12: 9-18.

Bert F, Maubec E, Bruneau B, Berry P and Lambert-Zechovsky N (1998) Multi-resistant Pseudomonas aeruginosa outbreak associated with contaminated tap water in a neurosurgery intensive care unit. J Hosp Infect. 39(1): 53-62.

Blom DW, Lyle EA, Weinstein RA Meyer P and Hayden MK (2000) The relationship between environmental contamination with vancomycin-resistant enterococci *(VRE)* and patient colonization in a medical intensive care unit. Paper presented at: 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Ontario.

Bolyard EA, Tablan OC, Williams WW, Pearson ML, Shapiro CN and Deithman SD (1998) Guideline for infection control in health care personnel. Am J Infect Control 26 (3)*:* 289-354.

Boyce JM, Potter-Bynoe G, Chenevert C, King T (1997) Environmental contamination due to methicillin-resistant Staphylococcus aureus: possible infection control implications. Infection Control and Hospital Epidemiology 18: 622-7.

Brachman PS (1970) Nosocomial infection- airborne or not? Proceedings of the international conference on nosocomial infections. American Hospital Association: 189-92.

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

Brawley RL, Weber DJ, Samsa GP and Rutala WA (1989) Multiple nosocomial infections: an incidencestudy. Am J Epidemiol. 130 (4): 769-80.

Breathnach AS, Jenkins DR and Pedler SJ (1992) Stethoscopes as possible vectors of infection by staphylococci. Br Med J 305: 1573-4.

Brown RB, Hosmer D, Chen HC, Teres D, Sands M, Bradley S, Opitz E, Szwedzinski D and Opalenik D (1985) A comparison of infections in different ICUs within the same hospital. Crit. Care Med 13 (6): 472-6.

Brown SM (2003) Use of an alcohol-based hand rub and quality improvement interventions to improve hand hygiene in a Russian neonatal intensive care unit. Infect Control Hosp Epidemiol 24: 172-9.

Bueno CA, Delgado RM, López LA, Schaffino CS and Gálvez VR (1994) Influence of nosocomial infection on mortality rate in an intensive care unit Crit Care Med. 22(1): 55-60.

Bures S, Fishbain JT, Uyehara CF, Parker JM and Berg BW (2000) Computer keyboards and faucet handles as reservoirs of nosocomial pathogens in the intensive care unit. Am J Infect Control 28: 465-471.

Burke JP and Yeo TW (2004) Nosocomial urinary tract infections. In: Mayhall CG (editor) Hospital epidemiology infection control. 3rd edition. Philadelphia: Lippincott Williams and Wilkins: 267-86.

Caglayan C, Meric M, Willke A and Toker K (2005) Intensive Care Unit- Acquired Infection: Incidence, Risk factors and Associated Mortality in a Turkish University Hospital. Jpn. J. Infect. Dis.58: 297-302.

Catalano M, Quelle LS, Jeric PE, Di Martino A and Maimone SM (1999) Survival of *Acinetobacter baumannii* on bed rails during an outbreak and during sporadic cases. J Hosp Infect 42: 27-35.

CDC (1975) NNIS Site Definitions Manual, Atlanta, GA.

CDC (1988) CDC surveillance update. Centers for Disease Control and Prevention, Atlanta, GA.

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

CDC (1997) Update *Staphylococcus aureus* with reduced susceptibility to Vancomycin. United States MMWR 44: 81.

CDC, Hospital Infections Program: National Nosocomial Infections Surveillance (NNIS) Report (1996) Data summary from October 1986-April 1996. Am J Infect Control 24: 380-8.

Cespedes C, Miller M, Quagliarello B, Vavagiakis P, Klein RS and Lowy FD (2002) Differences between *Staphylococcus aureus* Isolates from Medical and Nonmedical Hospital Personnel. J. Clin. Microbiol. 40: 2594-97.

Chandrasekar PH, Kruse JA and Matthews MF (1986) Nosocomial infection among patients in different types of intensive care units at a city hospital. Crit Care Med. 14:508-10.

Cheesbrough M (2000) District Laboratory Practice in Tropical countries. Cambridge University Press, London. 2: 151-154, 180-265.

Craven DE and Steger KA (1996) Nosocomial Pneumonia in mechanically ventilated adult patients: Epidemiology and Prevention. Semin Respir Infect 11(1): 32-53

Craven DE, Kunches LM, Lichtenberg DA, Kollisch NR, Barry MA, Heeren TC and McCabe WR (1988) Nosocomial infection and fatality in medical and surgical intensive care unit patients. Arch.Intern.Med. 148(5): 1161-1168.

Croft WA, Jarvis BB and Yatawara CS (1986) Airborne outbreak of trichothecene toxicosis. Atmos Environ 20: 549-52.

Darelid J, Bengtsson L, Gastrin B, Hallander H, Lofgren S, Malmvall BE, Olinder-Nielsen AM and Thelin AC (1994) An outbreak of Legionnaires' Disease in a Swedish hospital. Scand J Infect Dis 26 (4): 417-25.

Das I, Lambert P, Hill D, Noy M, Bion J and Elliott T (2002) Carbapenem-resistant *Acinetobacter* and role of curtains in an outbreak in intensive care units. J Hosp Infect 50: 110-14.

Daschner FD, Frey P, Wolff G, Baumann PC and Suter P (1982). Nosocomial infections in intensive care wards: a multi-centre prospective study. Crit Care Med 8: 5-9

Dashner FP, Frey P, Wolff G and Suter P (1982) Nosocomial infections in intensive care wards: a multicenter prospective study. Intensive Care Med 8: 5-9.

de Leon-Rosales SP, Molinar-Ramos F, Dominguez-Cherit G, Rangel-Frausto MS and Vazquez-Ramos VG (2000) Prevalence of infections in intensive care units in Mexico: A multicenter study. Crit Care Med 28(5): 1316-21.

Deep A, Ghildiyal R, Kandian S and Shinkre N (2004) Clinical and Microbiological Profile of Nosocomial Infections in the Pediatric Intensive Care Unit (PICU). Indian J Pediatr 41: 1238-46.

Denton M and Kerr KG (1998) Microbiological and clinical aspects of infection associated with *Stenotrophomonas maltophilia.* Clin Micrbiol Rev 11(1): 57-80.

Denton M, Rajgopal A, Mooney L, Qureshi A, Kerr KG, Keer V, Pollard K, Pekham DG and Conway SP (2003) *Stenotrophomonas maltophilia* contamination of nebulizers used to deliver aerosolized therapy to inpatients with cystic fibrosis. J Hosp Infect 55(3): 180-3.

Doebbeling BN, Stanley GL, Sheetz CT, Pfaller MA, Houston AK, Annis L, Li N, Wenzel RP (1992) Comparative efficacy of alternative hand-washing agents in reducing nosocomial infections in intensive care units. N Engl J Med 327: 88–93.

Donowitz LG (1986) High risk of nosocomial infection in the pediatric critical care patient. Crit Care Med 14: 26-28.

Donowitz LG, Wenzel RP and Hoyt JW (1982) High risk of hospital acquired infection in the ICU patient. Crit Care Med.10: 355-7.

Ducel G, Nicolle L and Fabry J (2002) Prevention of hospital acquired infections: A practical guide.2nd edition. WHO/CDS/CSR/EPH/2002.12

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

El Shafie SS, Alishaq M and Leni Garcia M (2004) Investigation of an outbreak of multidrug-resistant *Acinetobacter baumannii* in trauma intensive care unit. J. Hosp. Infect. 56: 101-5.

Elliott TSJ and Lambert PA (1999) Antibacterial resistance in the intensive care unit: mechanisms and management. Br Med Bull 55 (1): 259- 76.

Emori TG and Gaynes RP (1993) An Overview of Nosocomial Infections, Including the Role of the Microbiology Laboratory. Clin. Microbiol. Rev. 6 (4): 428-442.

Erbay H, Yalcin AN, Serin S, Turgut H, Tomatir E, Cetin B and Zencir M (2003) Nosocomial infections in intensive care unit in a Turkish university hospital: a 2-year survey. Intensive Care Med 29(9): 1482-88.

Esen S and Leblebicioglu H (2009) Prevalence of nosocomial infections at intensive care units in Turkey- multi center 7day-point prevalence study. Scand. J. Infect. Dis.36: 144-8.

Ewans TM, Ortiz CR and La Force FM (1999) Prevention and control of nosocomial infection in the intensive care unit.p.1074-1080. In Rs Irwin, FB Cerra and JM Rippe (eds.). Intensive Care Medicine. 4th ed. Lippincot- Raven, New York.

Fagon JY, Chastre J, Vuagnat A, Trouillet JL, Novara A and Gilbert C (1996) Nosocomial pneumonia and mortality among patients in intensive care units. JAMA 275: 866-69.

Farzana K, Rashid Z, Akhtar N, Sattar A, Khan JA and Nasir B (2008) Nasal carriage of Staphylococci in Health care workers: Antimicrobial susceptibility profile. Pak. J. Pharm. Sci. 21(3): 290-4.

Fernandez-Crehuet R, Diaz-Molina C, de Irala J, Martinez-Concha D, Salcedo-Leal I and Masa-Calles J (1997) Nosocomial infection in an intensive-care unit: identification of risk factors.Infect Control Hosp Epidemiol 18(12): 825-30.

Fluit . A, Verhoef J, Schmitz F and The European SENTRY Participants (2001) Frequency of Isolation and Antimicrobial Resistance of Gram-Negative and Gram-Positive Bacteria from Patients in Intensive Care Units of 25 European University Hospitals Participating in the European Arm of the SENTRY Antimicrobial Surveillance Program 1997–1998. Eur J Clin Microbiol Infect Dis 20 (9): 617-25

Forbes AB, Sahm FD and Weissfeld SA (2000) Bailey and Scott's Diagnostic Microbiology. Eleventh edition, CV Mosby- Year book Inc, St. Louis Missouri Baltimore: 68-72.

Forbes AB, Sahm FD and Weissfelt SA (2007) Bailey and Scott's diagnostic Microbiology 12th edition. Mosby publication.

Fraise AP (1997) Epidemiology of resistance in intensive therapy units (ITUs). J Med Microbol 46 : 447-49.

Frebourg NB, Couliez B and Lemeland JF (1999) Evidence for nasal carriage of methicillin-resistant Staphylococci colonizing intravascular devices. J. Clin. Microbiol. 37(4): 1182-85.

Fridkin SK (2001) Increasing prevalence of antimicrobial resistance in intensive care units. Crit Care Med. 29: 64-8.

Fridkin SK and Gaynes RP (1999) Antimicrobial Resistance in Intensive Care Units. Clin. Chest Med. 20 (2): 303-16.

Fridkin SK, Pear SM, Williamson TH, Galgiani JN and Jarvis WR (1996) The role of understaffing in central venous catheter-associated bloodstream infections. Infect Control Hosp Epidemiol 17: 150–8.

Fridkin SK, Steward CD, Edwards JR, Pryor ER, McGowan JE Jr, Archibald LK, Gaynes RP and Tenover FC (1999) Surveillance of antimicrobial use and antimicrobial resistance in United States hospitals: project ICARE phase 2. Project Intensive Care Antimicrobial Resistance Epidemiology (ICARE) hospitals. Clin Infect Dis 29: 245-52.

Fridkin SK, Welbel SF, Weinstein RA (1997) Magnitude and prevention of nosocomial infections in the intensive care unit. Infect Dis Clin North Am 11: 479–96.

Garner JS and The Hospital Infection Control Practices Advisory Committee (HICPAC) (1996) Guideline for isolation precautions in hospitals. Infect Control Hosp Epidemiol 17(1): 53–80 and Am J Infect Control 24(1): 24–52.

Garner JS, Jarvis WR, Emori TG, Horan TC and Hughes JM (1988) CDC definitions for nosocomial infections. Am J Infect Control 16(3): 128-40.

Gaya H (1976) Infection control in Intensive Care Unit. Br. J. Anaesth. 48(9).

Gelber SE and Ratner AJ (2002) Hospital-acquired viral pathogens in the neonatal intensive care unit. Semin Perinatol 26 (5): 346-56.

Girou E, Legrand P, Soing-Altrach S, Lemire A, Poulain C, Allaire A , Tkoub-Scheirlinck L, Ho Tam Chai S, Dupeyron C and Loche CM (2006) Association between hand hygiene compliance and methicillin-resistant *Staphylococcus aureus* prevalence in a French rehabilitation hospital. Infection Control and Hospital Epidemiology 27: 1128-30.

Gold HS and Moellering RC Jr (1996) Antimicrobial drug resistance. N Engl J Med 335:1445-53.

Goldmann DA, Weinstein RA, Wenzel RP, Tablan OC, Duma RJ, Gaynes RP, Schlosser J, Martone WJ, Workshop to Prevent and Control the Emergence and Acar J, Avorn J,Burke J, Boyce J, Carlet J, Garner J, Gilchrist MJ, Larson E, Lee J, Malangoni MA, McSweegan E, McGowan J, Meza AD, Moses J, Patterson C, Perry BC, Russell B, Schentag J, Sheldon AT Jr, Siegel J, Spitalny K, Tenne J, Tenover F, Timperi R and van den Berg JM (1996) Strategies to Prevent and Control the Emergence and Spread of Antimicrobial-Resistant Microorganisms in Hospitals: A Challenge to Hospital Leadership. JAMA 275 (3): 234-40.

Gordin FM, Schultz ME, Huber RA and Gill JA (2005) Reduction in nosocomial transmission of drug-resistant bacteria after introduction of an alcohol-based handrub. Infect Control Hosp Epidemiol 26: 650-3.

Gould D (1991) Nurses' hands as vectors of hospital acquired infection: a review. J Advanced Nursing 16: 1216-25.

Gould FK and Freeman R (1993) Nosocomial infection with microsphere beds. Lancet 342: 241-42.

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

Gupta E, Mohanty S, Sood S, Dhawan B, Das BK. & Kapil A (2006) Emerging resistance to carbapenems in a tertiary care hospital in north India. Indian J Med Res 124: 95-8.

Habibi S, Wig N , Agarwal S, Sharma SK , Lodha R, Pandey RM and Kapil A (2008) Epidemiology of nosocomial infections in medicine intensive care unit at a tertiary care hospital in northern India. Trop Doct 38:233-5.

Haley RP and Bregman DA (1982) The role of understaffing and overcrowding in recurrent outbreaks of staphylococcal infection in a neonatal special-care unit. J Infect Dis 245: 875–85.

Haley RW, Culver DH, White JW, Morgan WM, Emori TG, Munn VP and Hooton TM (1985) The efficacy of infection surveillance and control programs in preventing nosocomial infections in US hospitals. Am J Epidemiol 121(2): 182-205.

Hart CA (1982) Nosocomial gentamicin and multiply resistant enterobacteria at one hospital. Factors associated with carriage. J. Hosp Infect 3: 165-72.

Hassanzadeh P, Motamedifar M and Hadi N (2009) Prevalent bacterial infections in intensive care units of Shiraz University of medical sciences teaching hospitals, Shiraz, Iran. Jpn. J. Infect. Dis. 62: 249-53.

Heyland DK, Cook DJ, Griffith L, Keenan SP and Brun-Buisson C (1999) The attributable morbidity and mortality of ventilator-associated pneumonia. Am J Respir Crit Care Med. 159: 1249-56.

Hilker R, Poetter C, Findeisen N, Sobesky J, Jacobs A, Neveling M and Heiss WD (2003) Nosocomial pneumonia after acute stroke: implications for neurological intensive care medicine. Stroke 34: 975-81.

Hirai Y (1991) Survival of bacteria under dry conditions from a viewpoint of nosocomial infection. J. Hosp. Infect. 19: 191-200.

Hiramatsu K, Yabuta K, Ino T, Hanaki H, Tenover FC and Oguzi T (1997) Methicillin-Resistant *Staphylococcus aureus* clinical strains with reduced Vancomycin susceptibility. J Antimicrob Chemother 40: 135-136.

Hota B (2004) Contamination, disinfection and cross colonization: Are hospital surfaces reservoirs for nosocomial infection? Clin Infect Dis 39 (8): 1182-9.

Hsueh Po-Ren, Liu Yung-Ching, Yang Dine, Yan Jing-Jou, Wu Tsu-Lan, Huang, Jiunn-Jong Wu Wen-Kuei, Ko Wen-Chien, Leu Hsieh-Shong, Yu Chong-Ren and Luh Kwen-Tay (2004) Microbial Drug Resistance: Multicenter Surveillance of Antimicrobial Resistance of Major Bacterial Pathogens in Intensive Care Units in 2000 in Taiwan. Marry Ann Liebert, Inc. 7 (4).

Hugonnet S, Chevrolet JC and Pittet D (2007) The effect of workload on infection risk in critically ill patients. Crit Care Med 35(1): 76-81.

Jacobs RR (1989) Airborne endotoxins: an association with occupational lung disease. Applied Ind. Hyg. 4: 50-56.

Japoni A, Vazin A, Hamedi M, Davarpanah MA, Alborzi A and Rafaatpour N (2009) Multidrug-resistant bacteria isolated from intensive-care-unit patient samples. Braz J Infect Dis.13(2).

Jarvis WR and Martone WJ (1992) Predominant pathogen in hospital infections. J Antimicrob Chemother 29 Suppl: 64-8.

Jarvis WR, Edwards JR, Culver DH, Hughes JM, Horan T, Emori TG, Banerjee S, Tolson J, Henderson T and Gaynes RP (1991) Nosocomial infection rates in

adult and pediatric intensive care units in the United States. Am J Med 91 (3): 185-191.

Jarvis WR, Temprado CS and Robles B (1996) Prevention of Nosocomial Blood Stream Infections: A National and International Priority. Infect Control Hosp Epidemiol 17: 272-4.

Jiménez P, Torres A, Rodriguez-Roisin R, Bellacasa JP, Aznar R, Gatell JM, Agusti-Vidal A (1989) Incidence and etiology of pneumonia acquired during mechanical ventilation. Crit Care Med. 17**:** 882-6.

Jones JS, Hoerle D and Rickse R (1992) Stethoscopes: a potential vector of infection? Ann Emerg Med 26: 296-9.

Klingspor L, Tornqvist E, Johansson A, Petrini B, Forsum U and Hedin G (2004) A prospective epidemiological survey of candidaemia in Sweden. Scand J Infect Dis 36(1): 52-55.

Koirala P (2009) Bacteriological profiles of tracheal aspirates of patients attending National Institute of Neurological and Allied Science. A dissertation submitted to Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal.

Kollef MH and Fraser VJ (2001) Antibiotic resistance in intensive care unit. Ann Intern Med.134: 298-314.

Kollef MH, Silver P, Murphy DM and Trovillion E (1995) The effect of late-onset ventilator-associated pneumonia in determining patient mortality. Chest 108: 1655-62.

Kominos SD, Copeland CE and Grosiak B (1972) Mode of Transmission of *Pseudomonas aeruginosa* in a Burn Unit and an Intensive Care Unit in a General Hospital. Appl Microbiol 23(2): 309-12.

Kramer A, Schwebke I and Kampf G (2006) How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect. Dis. 6: 130.

Kucukates E (2005) Antimicrobial resistance among gram negative bacteria isolated from intensive care unit in a cardiology institute in Istanbul, Turkey. Jpn. J. Infect. Dis. 58: 228-31.

Kumar A (2006) Multi Drug Resistance: Another Fatal ICU Concern. Pulmon 8(3): 84-8

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

Laupland KB, Zygun DA, Davies HD, Church DL, Louie TJ and Doig CJ (2002) Incidence and risk factors for acquiring nosocomial urinary tract infection in the critically ill. J Crit Care 17(1): 50-57.

LeClercq R, Derlot E, Duval J and Couvalin P (1988) Plasmid-mediated resistance to vancomycin and teichoplanin in *Enterococcus faecalis*. N. Engl. J. Med. 319: 157-59.

Legras A and Robert R (1999) Nosocomial infection: prospective survey of incidence in 5 French ICUs. Intensive Care Med 24: 1040-46.

Legras A, Malvy D, Quinioux AI, Villers D, Bouachour G, Robert R and Thomas R (1998) Nosocomial infections: prospective survey of incidence in five French intensive care units. Intensive Care Med 24 (10): 1040-46.

Lemmen SW, Hafner H, Zolldann D, Stanzel S and Lutticken R (2005) Distribution of multi-resistant Gram-negative versus Gram-positive bacteria in the hospital inanimate environment. J Hosp Infect. 59(2): 164-5.

Lockhart SR, Abramson MA, Beekman SE, Gallagher G, Riedel SR, Diekma DJ, Quinn JP, and Doern GV (2007) Antimicrobial resistance among gram-negative bacilli as causes of infections in intensive care unit patients in the United States between 1993 and 2004. J. Clin. Microbiol.45: 3352-9.

Luzar MA, Coles GA, Faller B, Slingeneyer A, Dah GD, Briat C, Wone C, Knefati Y, Kessler M and Peluso M (1990) *Staphylococcus aureus* nasal carriage and infection in patients on continuous, ambulatory peritoneal dialysis. N. Engl. J Med. 322: 505-9.

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

Marinella MA, Pierson C and Chenolieth C (1997) The stethoscope: a potential source of nosocomial infection? Arch Intern Med 157: 786-90.

Marrie TJ, Haldane D, Bezanson G and Peppard R (1992) Each water outlet is a unique ecological niche for *Legionella pneumophila*. Epidemiol Infect.108: 261-70.

Mayer JA, Dubbert PM, Miller M, Burkett PA and Chapman SW (1986) Increasing handwashing in an intensive care unit. Infect Control 7: 259–262.

Mayon-White RT (1988) An international survey of the prevalence of hospital-acquired infection.J Hosp Infect 11 (Suppl. A): 43–48.

Meert KL, Long M, Kaplan J and Sarnaik AP (1995) Alterations in immune function following head injury in children. Crit Care Med 23: 822-8.

National Nosocomial Infections Surveillance (NNIS) System Report (1999) Data summary from January 1990-May 1999. Am J Infect Control 27(6): 520-32.

Neely AN (2000) A survey of gram-negative bacteria survival on hospital fabrics and plastics. J Burn Care Rehabil. 21(6): 523-7.

Neely AN and Maley MP (2000) Survival of enterococci and staphylococci on hospital fabrics and plastic. J Clin Micro. 38(2): 724-26.

Neely AN and Orloff MM (2001) Survival of Some Medically Important Fungi on Hospital Fabrics and Plastics. J. Clin. Microbiol. 39: 3360-61.

Nicola P, Drapeau C, Nicastri E, Martini L, Ippolito G, Moro ML and ANIPIO (2008) Surgical site infections in Italian Hospitals: a prospective multicenter study. BMC Infect Dis. 8: 34.

Niederman MS, Craven DE, Fein AM and Schultz DE (1990) Pneumonia in the critically ill hospitalised patient. Chest 97: 170-81.

Nikodemski T (1999) Bacteriological and clinical analysis of nosocomial infections in patients from the intensive care unit. Ann Acad Med Stetin. 45: 211-26.

O'Grady NP, Alexander M, Dellinger EP, Gerberding JL, Heard SO, Maki DG, Masur H, McCormick RD, Mermel LA, Pearson ML, Raad II, Randolph A and Weinstein RA (2002) Guidelines for the prevention of intravascular catheter-related infections. CDC. MMWR Recomm Rep 51(RR-10): 1-29.

Onarheim H, Hoivik T, Harthug S, Digranes A, Mylvaganam H and Vindenes HA (2000) Outbreak of multiresistant *Acinetobacter baumannii* infection. Tidsskr Nor Laegeforen 120(9): 1028-33.

Orsi GB, Raponi M, Franchi C, Rocco M, Mancini C and Venditti M (2005) Surveillance and inection control in an intensive care unit. Infect Control Hosp Epidemiol 26(3): 321-5.

Ozinel MA, Bakir M and Cek M (2004) Uriner kateter infeksiyonlarinin onlenmesi klavuzu. Hastane Infeksiyonlari Dergisi 8(1):3-12.

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

Pelczar MJ Jr, Chan ECS and Kreig NR (1993) Microbiology, Fifth Edition. Tata McGraw Hill Publishing Company Limited, New Delhi, India: 769-70.

Pfaller MA, Jones RN, Doern GV, Kugler K and The SENTRY Participants Group (1998) Bacterial pathogens isolated from patients with blood stream infection: frequencies of occurrence and antimicrobial susceptibility patterns from the SENTRY Antimicrobial Surveillance Program (United States and Canada,1997). Antimicrob. Agents Chemother. 42 (7): 1762- 70.

Philippon A, Labia A and Jacoby GA (1989) Extended spectrum beta-lactamases. Antimicrob. Agents Chemother 33: 1131-5.

Pilonetto M, Rosa EAR, Brofman PRS, Baggio D, Calvário F, Schelp C, Nascimento A and Messias-Reason I (2004) Hospital gowns as a vehicle for bacterial dissemination in an intensive care unit. Braz J Infect Dis. 8(3).

Pina P, Guezenec P, Grosbuis S, Guyot L, Ghnassia JC and Allouch PY (1998) An *Acinetobacter baumannii* outbreak at the Versailies Hospital Center. Pathol Biol (Paris) 46: 385-94.

Pingleton SK, Fagon JY and Leeper KV Jr (1990) Patient selection for clinical investigation of ventilator-associated pneumonia: criteria for evaluating diagnostic techniques. Chest 97: 170-81.

Pittet D, Allegranzi B, Sax H, Bertinato L, Concia E, Cookson B, Fabry J, Richet H, Philip P, Spencer RC, Ganter BW and Lazzari S (2005) Considerations for a WHO European strategy on health-care-associated infection, surveillance, and control. Lancet Infect Dis 5(4): 242-50.

Pittet D, Dharan S, Touveneau S, Sauvan V and Perneger TV (1999) Bacterial Contamination of the Hands of Hospital Staff during Routine Patient Care. Arch Intern Med. 159: 821-6.

Pittet D, Tarara D and Wenzel RP (1994) Nosocomial bloodstream infection in critically ill patients. Excess length of stay, extra costs, and attributable mortality. JAMA 271(20): 1598-601.

Pokhrel BM, Rawal S and Joshi H (1993) Bacteriological study at TU Teaching Hospital, Kathmandu, Nepal. Journal of Institute of Medicine.

Pollack M (2000) *Pseudomonas aeruginosa.* In: Mandell GL, Bennett JE and Dolin R (eds.) Principles and practice of infectious diseases. 5th edition, Pa: Churchill Livingstone, Philadelphia: 2310-35.

Prass K, Meisel C, Hoflich C, Braun J, Halle E, Wolf T, Ruscher K, Victorov IV, Priller J, Dirnagl U, Volk HD and Meisel A (2003). Stroke-induced immunodeficiency promotes spontaneous bacterial infections and is mediated by sympathetic activation reversal by poststroke T helper cell type 1-like immunostimulation. J Exp Med 198: 725-36.

Prescott LM, Harley JP and Klein DA (2005) Microbiology, Sixth Edition. McGraw Hill Higher Education : 840-1.

Rangel-Frausto MS, Houston AK, Bale MJ, Fu C and Wenzel RP (1994) An experimental model for study of *Candida* survival and transmission in human volunteers. Eur J Clin Microbiol Infect Dis 13: 590-5.

Rello J and Torres A (1996) Microbial causes of ventilator-associated pneumonia. Semin Respir Infect 11: 24-31.

Rello J, ausina V, Ricart M, Castella J and Prats G (1993) Impact of previous antimicrobial therapy on the etiology and outcome of ventilator-associated pneumonia. Chest 104: 1230-5.

Reshedko G, Sukhorukova M, Ivanchik N, Ryabkova E and RosNet Group (2007) Antimicrobial resistance of gram-negative bacteria in Russian intensive care units (ICUS). http//www.antibiotic.ru.

Reuter S, Sigge A, Wiedeck H and Trautmann M (2002) Analysis of transmission pathways of *Pseudomonas aeruginosa* between patients and tap water outlets. Crit Care Med. 30 (10): 2222-8.

Reybrouk G (1986) Handwashing and hand disinfection. J Hosp Infec 8(1): 5-23.

Richards M, Thursky K and Buising K (2003) Epidemiology, prevalence and sites of infections in intensive care units. Semin. Respir. Crit. Care Med. 24: 3-22.

Richards MJ, Edwards JR, Culver DH and Gaynes RP (1999) Nosocomial infections in medical intensive care units in the United States: nosocomial infection surveillance system. Crit. Care Med. 27: 887-92.

Richards MJ, Edwards JR, Culver DH and Gaynes RP (2000) Nosocomial infections in combined medical-surgical intensive care units in the United States. Infect Control Hosp Epidemiol 21(8): 510-515.

Ronveaux O, Jans B, Suetens C and Carsauw H (1998) Epidemiology of nosocomial blood stream infections in Belgium, 1992-1996. Eur J Clin Microbiol Infect Dis 17(10): 695-700.

Rotimi VO, al-Sweih NA and Feteih J (1998) The prevalence and antibiotic susceptibility pattern of Gram-negative bacterial isolates in two ICUs in Saudi Arabia and Kuwait. Diagn Microbiol Infect Dis 30: 53-9.

Rutala WA *(*1996) APIC guideline for selection and use of disinfectants. Am J Infect Control 24 (4)*:* 313-42.

Safdar N, Dezfulian C, Collard HR and Saint S (2005) Clinical and economic consequences of ventilator-associated pneumonia; a systemic review. Crit Care Med. 33: 2184-93.

Sattar SA and Ijaz MK (1987) Spread of Viral infections by aerosols. Crit. Rev. Environ. Control 17: 89-131.

Savaş L, Duran N, Savaş N, Nlen Y and Ocak S (2005) The Prevalence and Resistance Patterns of *Pseudomonas aeruginosa* in Intensive Care Units in a University Hospital.Turk J Med Sci 35: 317-22.

Schaberg DR, Culver DH and Gaynes RP (1991) Major trends in the microbial ecology of nosocomial infections. Am J Med 91(3): 72-5.

Schwalbe RS, Stapleton JT and Gilligan PH (1987) Emergence of vancomycin resistance in coagulase-negative staphylococci. N. Engl. J. Med. 316: 927-28.

Scott E and Bloomfield SF (1990) The survival and transfer of microbial contamination via cloths, hands and utensils. J. Appl. Bacteriol. 68: 271-8.

Sengupta S, Sirkar A and Shivananda P (2000) Stethoscopes and Nosocomial Infection. Indian J Pediatr 67(3): 197-9.

Sexton t, Clarke P, O'Neill E, Dillane T and Humphreys H (2006) Environmental reservoirs of methicillin resistant *Staphylococcus aureus* in isolation rooms:

correlation with patient isolates and implications for hospital hygiene. J Hosp Infect 62(2): 187-94

Shaikh JM, Devrajani BR, Shah Ali SZ, Akhund T and Bibi I (2008) Frequency, Pattern and Etiology of Nosocomial Infection in Intensive Care Unit: An Experience at a Tertiary Care Hospital. J Ayub Med Coll Abbottabad 20(4).

Shannon SC (2005) Chronic clinical illness. In Jesse BH, Gregory AS, Lawrence DH, eds. Principles of Critical Care. 3rd edition, McGraw Hill: 207-15.

Sharma S (2006) Microbial flora among visitors and the hospital environment in ICU and SICU at TUTH. A dissertation submitted to the Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal.

Shehabi AA and Baadran I (1996) Microbial infection and antibiotic resistance patterns among Jordanian intensive care patients. East Mediterr Health J. 2 (3): 515-20.

Shlaes DM and Rice LB (1995) Emerging mechanisms of B-lactam resistance: an update. Infect Dis in Clin Prac 15: 35-85.

Simor AE, Lee M, Vearncombe M, Jones-Paul L, Barry C, Gomez M, Fish JS, Cartotto RC, Palmer R and Louie M (2002) An outbreak due to multiresistant *A. baumannii* in a burn unit: risk factors for acquisition and management. Infect Control Hosp Epidemiol 23: 261-7.

Singh Naz N and Sprangua BM (1996) Risk factors for nosocomial infections in critically ill children: A prospective cohort study. Crit Care Med, 24: 875-8

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

Smith MA, Mathewson JJ, Ulert A, Scerpella GE and Ericsson CD (1996) Contaminated stethoscopes revisited. Arch Intern Med 156: 82- 4.

Spencer RC (1994). Epidemiology of infection in ICUs. Intensive Care Med 20: 52-6.

Spencer RC (1996) Predominant pathogens found in European Prevalence of Infection in Intensive Care Study. Eur J Clin Microbiol Infect Dis 15(4): 281-85.

Talbot GH, Bradley J, Edwards JE Jr, Gilbert D, Scheld M and Bartlett JG (2006) Bad Bugs Need Drugs: An Update on the Development Pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clin Infect Dis 42: 657-68.

Tambekar DH and Gulhane PB (2003) Studies on pathogenic air flora of hospitals in Amravati. 44th Annual Conference of Association of Microbiologists of India, Dharwad. November 12-14.

Tambekar DH, Gulhane PB and Bhokare DD (2007) Studies on Environmental Monitoring of Microbial Air Flora in the Hospitals. J. Med. Sci 7(1): 67-73.

Tennant I, Harding H, Nelson M and Roye-Green K (2005) Microbial isolates from patients in an intensive care unit, and associated risk factors. West Indian Med. J 54: 225-31.

Thornsberry C (1995) Trends in antimicrobial resistance among today's bacterial pathogens. Pharmacotherapy, 15(1 pt 2): 3S–8S

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

Tortorano AM, Bernhardt H, Kibller CC, Peman J, Faure O, Biraghi E, Canton E, Zimmerman K and Seaton S (2004) Epidemiology of candidaemia in Europe:

results of 28-month European confederation of Medical Mycology (ECMM) hospital–based surveillance study. Eur J of Clin Microbiol Infect Dis 23(4): 317-22.

Tortorano AM, Kibller C, Peman J, Bernhardt H, Klingspor L and Grillot R (2006) Candidaemia in Europe: epidemiology and resistance. Int J Antimicrob Agents 27(5): 359-66.

Trautmann M, Michalsky T, Heidemaire W. Radosavljevic V and Ruhnke M (2001) Tap water colonization with *Pseudomonas aeruginosa* in a surgical intensive care unit (ICU) and relation to Pseudomonas infections of ICU patients. Infect Control Hosp Epidemiol 22: 49-52.

Trilla A (1994) Epidemiology of nosocomial infections in adult intensive care units. Intensive Care Med 20: 1-4.

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

Vincent JL (2000) Microbial resistance: lessons from the EPIC study European Prevalence of Infection. Intensive Care Med 26 (suppl 1): S3–S8

Vincent JL, Bihari DJ, Suter PM, Bruining HA,White J, Nicolas-Chanoin M, Wolff M, Spencer RC & Hemmer M (1995) The Prevalence of Nosocomial Infection in Intensive Care Units in Europe: Results of the European Prevalence of Infection In Intensive Care (EPIC) Study. JAMA 274(8): 639-44.

Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, Moreno R, Lipman J, Gomersall C, Sakr Y, Reinhart K, for the EPIC II Group of Investigators (2009) International Study of the Prevalence and Outcomes of Infection in Intensive Care Units. JAMA 302(21): 2323-29.

Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, Moreno R, Carlet J, Le Gall JR, Payen D (2006) Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 34: 344–53

Vosylius S, Sipylaite J and Ivaskevicius J (2003) Intensive care unit acquired infection: aprevalence and impact on morbidity and mortality. Acta Anaesthesiol Scand 47(9): 1132-1137.

Wagh H and Acharya D (2009) Ventilator Associated Pneumonia: an Overview. BJMP 2(2): 16-9.

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

Weber DJ, Raasch R and Rutala WA (1999) Nosocomial infection in the ICU. Chest 115: 34- 41.

Weinstein, RA (1991) Epidemiology and control of nosocomial infections in adult intensivecare units. Am. J. Med. 91(Suppl. 3B):179S-184S.

Weistein RA (1998) Nosocomial Infection Update. Emerging Infectious Diseases, 4(3):416-20

Wendt C and Herwaldt LA (1997) Epidemics: identification and management. In: Wenzel R, ed. Prevention and control of nosocomial infections. 3rd ed. Baltimore, MD; Williams andWilkins: 175–213.

Wenzel RP, CA Osterman, LG Donowitz, JW Hoyt, MA Sande, WJ Martone, JE Peacock Jr., JL Levine and GB Miller Jr (1981) Identification of procedure-related nosocomial infections in high-risk patients. Rev. Infect. Dis. 3:701-706.

WHO (1994):http://whqlibdoc.who.int/hq/1994/HBI_TER_WTW94.02.pdf

WHO (2002) Prevention of hospital-acquired infections: A Practical Guide, 2nd edition. pp 1-46.

WHO (2009) WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care is Safer Care: 12-29.

Wilks M, Wilson A, Warwick S, Price E, Kennedy D, Ely A and Millar MR (2006) Control of an Outbreak of Multidrug-Resistant *Acinetobacter baumannii-calcoaceticus* Colonization and Infection in an Intensive Care Unit (ICU) Without Closing the ICU or Placing Patients in Isolation. Infect Control Hosp Epidemiol 27(7): 654-8.

Woodford N, Tierno PM Jr, Young K, Tysall L, Palepou MFI, Ward E, Painter RE, Suber DF, Shungu D, Silver LL, Inglima K, Kornblum J and Livermore DM (2004) Outbreak of *Klebsiella pneumoniae* Producing a New Carbapenem-Hydrolyzing Class A β-Lactamase, KPC-3, in a New York Medical Center. Antimicrob Agents Chemother 48(12): 4793–9.

Woodward ED, Friedlander B, Lesher RJ, Font W, Kinsey R and Hearne FT (1988) Outbreak of hypersensitivity pneumonitis in an industrial setting. J. Am. Med. Assoc. 259: 1965-69.

Wright IMR, Orl H and Porter G (1995) Stethoscope contamination in the neonatal intensive care unit. J Hosp Infect 29: 65-8.

Ylipalosaari P (2007) Infections in Intensive Care; Epidemiology and Outcome. Academic dissertation to be presented, with the assent of the Faculty of Medicine of the University of Oulu, Acta Univ. Oul. D 925, Oulu, Finland.

Zer Y, Karaoglan I, Namiduru M, Balci I, Karagoz ID, Ozaslan M, Kilic HI and Suner A (2009) Investigation of nasal colonization of health care workers by methicillin-resistant *Staphylococcus aureus* with using new generation real-time PCR assay: Discussing of risks. Afr J Biotechnol 8 (20): 5542-6.

Zhanel GG, DeCorby M, Laing N, Weshnoweski B, Vashisht R, Tailor F, Nichol KA, Wierzbowski A, Baudry PJ, Karlowsky JA, Lagace-Wiens P, WalktyA, McCracken M, Mulvey MR, Johnson J, The Canadian Antimicrobial Resistance Alliance (CARA) and Hoban DJ (2008) Antimicrobial-Resistant Pathogens in Intensive Care Units in Canada: Results of the Canadian National Intensive Care Unit (CAN-ICU) Study, 2005-2006. Antimicrob. Agents Chemother. 52 (4): 1430–7

Zolldann D, Spitzer C, Häfner H, Waitschies B, Klein W, Sohr D, Block F, Lütticken R and Lemmen SW (2005) Surveillance of Nosocomial Infections in a Neurological Intensive Care Unit. Infect Control Hosp Epidemiol 26(8): 726-31.

Zuliani- Maluf ME, Maldonado AF, Bercial ME and Pedroso SA (2002) Stethoscope: a friend or an enemy? Sao Paulo Med J/Rev Paul Med 120 (1): 13-5.

APPENDICES

Appendix-I

Clinical and Microbiological profile of patient

Name of patient: Date:

Age: sex:

Specimen

Lab no.:

Clinical history of patient:

Microbiological investigation:

Day-1

Appearance of specimen

- color
- clear
- cloudy

Microscopic examination of specimen

- Bacteria
- White blood cell
- Yeast cell
- epithelial cell

Gram's staining

- Gram positive cocci / bacilli
- Gram negative cocci / bacilli

Inoculation of sample into BA, MA and CA

Day-2

Cultural characteristics

MA	***Colony characteristics***	***Catalase***	***Oxidase***	***Coagulase***	***Gram reaction***	***Morphology***	***Inference***
1.							
2.							
3.							
4.							
BA	***Colony characteristics***	***Haemolysis***	***Gram reaction***			***Morphology***	***Inference***
1.							
2.							
3.							
4.							
CA	***Colony characteristics***	***Haemolysis***	***Gram reaction***			***Morphology***	***Inference***
1.							
2.							
3.							
4.							

Culture on NB for 4 hours (from MA if gram negative, from BA if gram positive)

Day-3

Biochemical characteristics

S.No.	**MR/VP**	**Citrate utilization**	**SIM**	**TSI**	**Urea hydrolysis**	**Inference**
1.						
2.						
3.						
4.						
5.						

Organism identified as:

Antibiotic Sensitivity Testing: Kirby-Bauer method

S.No.	***Antibiotics used***	***Zone of inhibition (mm)***	***Interpretation***

Performed by.....

Appendix-II

Equipments and materials used during the study

Autoclave	
Hot air oven	Chitransh (India)
Incubator	Associated Scientific Technologist (India)
Refrigerator	LG (Korea)
Microscope	Olympus (Japan)
Weighing Machine	Tanita (Japan)
Centrifuge	Gemmy international corporation (Taiwan)
Microscope	Olympus (Japan)

Appendix - III

A. 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.

6. Mannitol Salt Agar (MSA)

Composition	gm/litre
Proteose Peptone	10.00
Beef Extract	1.000
Sodium Chloride	75.00
D-Mannitol	10.00
Phenol Red	0.025
Agar	15.00
Final pH (at 25°)	7.4 ± 0.2

111gm 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.

7. MacConkey Broth Purple (for MPN method)

Composition	gm/litre
Peptic digest of animal tissue	20.0
Lactose	10.0
Sodium Chloride	5.00
Sodium Taurocholate	5.00
Bromocresol Purple	0.01
Final pH (at 25°)	7.4 ± 0.2

40gm of the medium was dissolved in 1000ml of the distilled water and then boiled to dissolve completely. The medium was then distributed in test tubes with inverted Durham's tube and autoclaved at 121°C (151bs pressure) for 15 minutes. The sterilized medium was then cooled to room temperature.

B. 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.

C. 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%H_2O_2)

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.

4. 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. Then to 99.4 ml of this solution, 0.6 ml of 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, was added and mixed well. Then 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 - IV

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

B. Procedure for Antibiotic Sensitivity Testing (AST) by Disc Diffusion Method

In the treatment and control of infectious disease, antimicrobial susceptibility test is done to select effective antimicrobial drugs against suspected organisms. Disc diffusion method is a standard method for antimicrobial susceptibility test.

The following steps are involved in AST by Disc diffusion method:

1. An isolated colony of organism was suspended in the nutrient broth and incubated at 37^{o}C for 4 hours. The turbidity was matched with 0.5 McFarland standards.
2. A sterile cotton swab was introduced into the standardized tube and swabbed onto the MHA plate.
3. The pate was allowed to dry and the antibiotics discs were placed over the media in the plate and incubated at 37^{o}C. The result was noted after 18 hours.

Appendix-V

Zone size interpretative chart of antibiotics

Antibiotics used	Symbol	Disc potency (mcg)	Diameter of zone of inhibition in mm		
			Resistant	Intermediate	Sensitive
Ampicillin When testing Enterobacteriaceae When testing Staphylococci	A	10	 13 28	 14-16 _	 17 29
Amikacin	Ak	10	14	15-16	17
Cefotaxime	CE	30	14	15-22	23
Cefoperazone / Sulbactam	CC	75/30µg			
Cefepime / Tazobactam	CPT	30/10			
Cotrimoxazole (Trimethoprim+ Sulfamethoxazole)	C0	1.25+ 23.75	10	11-15	16
Chloramphenicol	C	30	12	13-17	18
Ciprofloxacin	CF	5	15	16-20	21
Cephalexin	CP	30	14	15-17	18
Cloxacillin	CX	5	11	12-13	14
Erythromycin	E	10	13	14-22	23
Gentamicin	G	10	12	13-14	15
Imipenem	I	10	13	14-15	16
Norfloxacin	NX	10	12	13-16	17
Nitrofurantoin	NF	100	14	15-16	17
Nalidixic acid	Na	30	13	14-18	19
Ofloxacin	OF	5	14	15-17	18
Piperacillin / Tazobactam When testing Enterobacteriaceae When testing Staphylococci & *P. aeruginosa*	PT	100/10	 17 17	 18-20 _	 21 18
Polymyxin B	PB	300 units	11	_	12
Vancomycin	VA	30	_	_	15

(Source: Product Information Guide, HiMedia Laboratories Pvt. Limited, Mumbai, India, Cheesebrough (2000) and Becton, Dickinson and Company)

Appendix-VI

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, H2S ⟶ Glucose, Lactose/ Sucrose fermenter, H2S producer.

b. Red (Alkali) / Yellow (Acid), No Gas, No H2S ⟶ Glucose fermenter, Lactose/Sucrose nonfermenter, Anaerogenic, H2S 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. Coagulase test

The Coagulase test is used to especially differentiate species within the genus Staphylococcus. *S. aureus* (usually positive) is differentiated from Staphylococcus saprophyticus and *S. epidermidis* (usually negative). A positive coagulase test is usually the final diagnostic criterion for the identification of Staphylococcus species.

The exact mechanism and chemical structure of coagulase is unknown. It is known, however, that this enzyme plays a thromboplastin like role in clotting by converting fibrinogen to fibrin. In vitro, coagulse increases the rate of plasma clotting , the end result of which is the formation of fibrin clot.

Two types coagulase are produced by most of the *S. aureus*:

A) Free coagulase, which converts fibrinogen to fibrin by activating a coagulase reacting factor present in plasma. It can be detected by the appearance of the fibrin clot in the tube coagulase test.

B) Bound coagulase, also known as clumping factor, converts fibrinogen directly to fibrin without requiring a coagulase reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide coagulase test.

Slide coagulase test

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

Tube coagulase test

For the organism showing positive test on slide coagulase test, tube coagulase test is performed. In the tube coagulase test, 0.5 ml of the diluted plasma (1:10 in physiological saline) was pipetted into 18-24 hours broth culture tube inoculated with test organisms. After mixing gently, the tube was incubated at 37^{o}C for 2-6 hours. The clotting is observed by gently tilting the tube for positive coagulase test.

j. 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-VII

Data analysis (χ^2 - Test)

Association of presence of *P. aeruginosa* in clinical and environmental (air) sample of ICU

	Presence of *P. aeruginosa*	Absence of *P. aeruginosa*	Total
Clinical specimens	248	156	404
Air sample	24	6	30
Total	272	162	434

Test statistic is χ^2

Ho : The occurrence of *P. aeruginosa* in clinical and air sample are not associated with each other.

H_1 : The occurrence of *P. aeruginosa* in clinical and air sample are associated with each other.

Calculation of χ^2 Value:

Expectation of 248= 404×272/434 = 253.20

So, the table of expected frequencies will be as follows:

Observed value (O)	Expected value (E)	$(O-E)^2$	$\chi^2_{cal}=\frac{(O-E)^2}{E}$
248	253.20	27.04	0.11
156	150.80	27.04	0.18
24	18.80	27.04	1.44
6	11.20	27.04	2.41
			$\Sigma\chi^2_{cal}=\frac{(O-E)^2}{E}$ = 4.14

Calculated value of χ^2 (χ^2_{cal}) = 4.14

Here, the degree of freedom (d.f.) = (r-1) × (c-1) = (2-1) × (2-1) = 1

According to the table of χ^2 (χ^2_{tab}) at 5% level of significance (P> 0.05) for 1 d.f.=3.84

Since the calculated value of χ^2 (χ^2_{cal}) is lesser than the corresponding tabulated value of χ^2 (χ^2_{tab}), the null hypothesis is rejected. This implies that the *P. aeruginosa* isolated from clinical and air sample of ICU are associated with each other. Similar type of analysis was performed for *K. pneumoniae*, *E. coli*, *Acinetobacter* spp. and *S. aureus.*

Appendix-VIII

Calculation of median value

Age groups	Frequency (f)	Class boundaries	Cumulative frequency (c.f)	Median age
1-10	3	0.5-10.5	3	
11-20	5	10.5-20.5	8	
21-30	10	20.5-30.5	18	
31-40	7	30.5-40.5	25	
41-50	5	40.5-50.5	30	**47.5**
51-60	14	50.5-60.5	44	
61-70	10	60.5-70.5	54	
71-80	3	70.5-80.5	57	
Total	**57**			

In the above table,

Median= size of n/2th value = 57/2=28.5th value

Here, 28.5th value lies in the interval 40.5-50.5. This interval is called median class.

Now, using the formula,

Median=L + h/f (n/2- c.f)

Where,

L = lower limit of the class containing the median value(s)
c.f = cumulative frequency of the class preceding the median class
f = frequency of the class containing the median value(s)
h= class-width of the median class

Then, L = 40.5, c.f =25, f = 5, h = 10. Substituting these values in the formula, we get

Median=40.5+10/5 (28.5-25)
=40.5+2(3.5)
=40.5+7
=47.5

Therefore, the median age group of patients was 47.5.

Appendix-IX

Methods of hand washing (WHO, 2009)

Photograph 1: Culture plate showing growth of microorganisms in the hand imprint samples of HCWs before hand wash

Photograph 2: Culture plate showing growth of microorganisms in the hand imprint samples of HCWs after hand wash

Photograph 3: Culture plate of *S. aureus* and CoNS in the MSA plate

Photograph 4: MPN bottles sets showing positive results for coliforms

Photograph 5: Antibiotic sensitivity testing of *Pseudomonas aeruginosa* on MHA CPT-Cefepime/ Tazobactam, CE-Cefotaxime, CF-Ciprofloxacin, G- Gentamicin, I-Imipenem, Ak - Amikacin

Photograph 6: Sample Processing on Microbiology Laboratory

Specimens from patients:

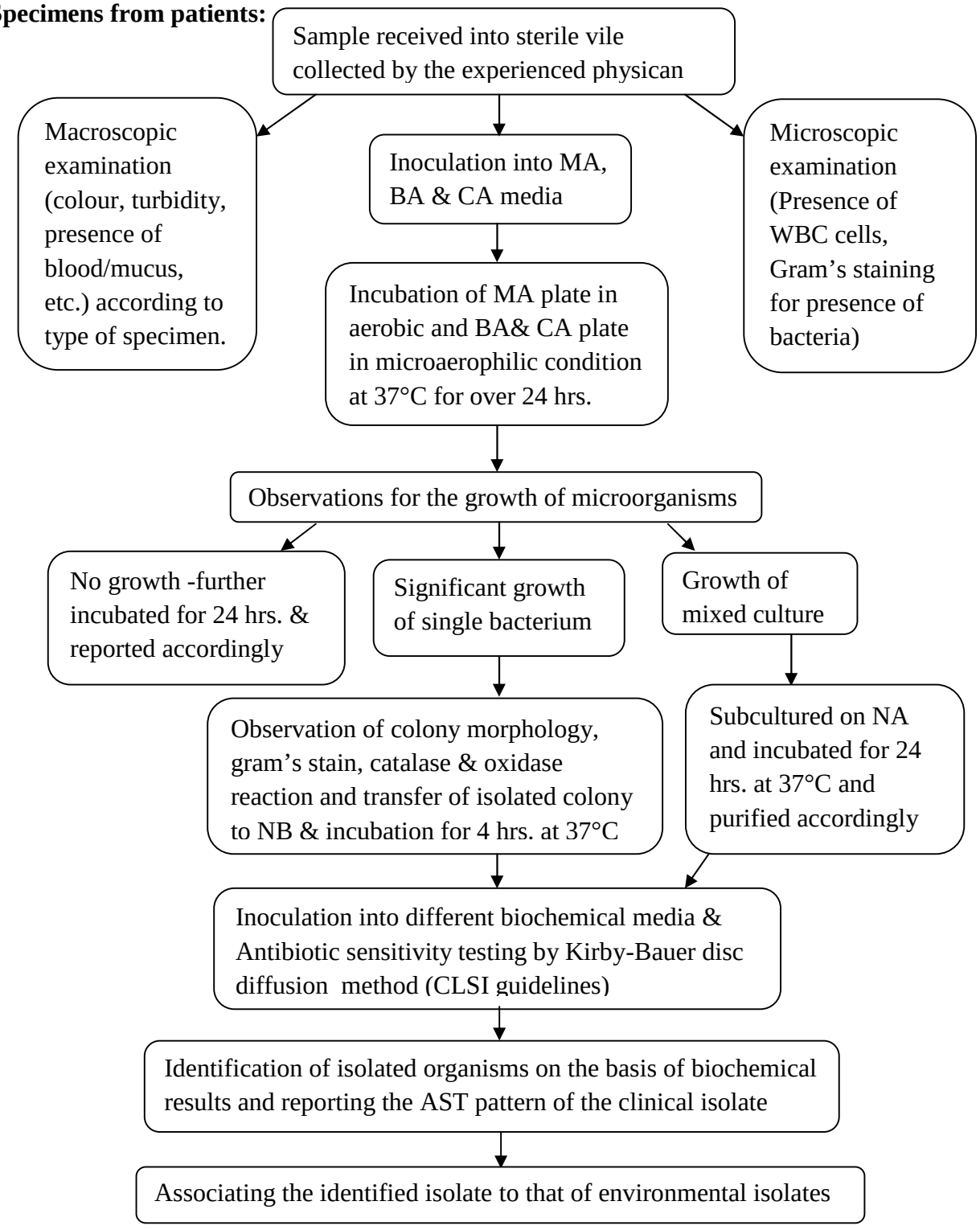


Figure 6: Flowchart of methodology showing collection and processing of clinical specimens of ICU patients

Sample from HCWs:

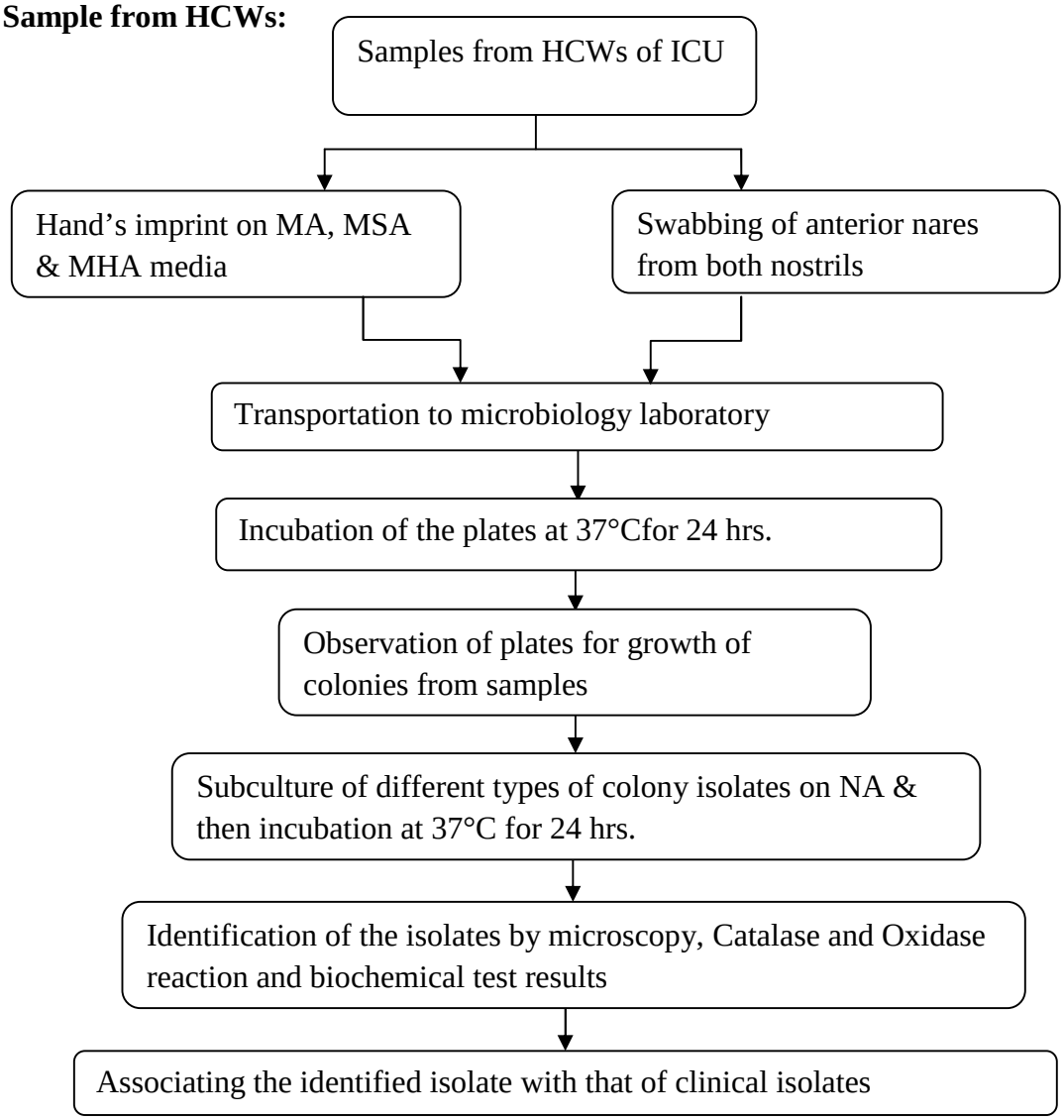


Figure 7: Flowchart of methodology showing collection and processing of hand imprints and nasal sample of HCWs

Fabrics/ clothes and inanimate surface samples:

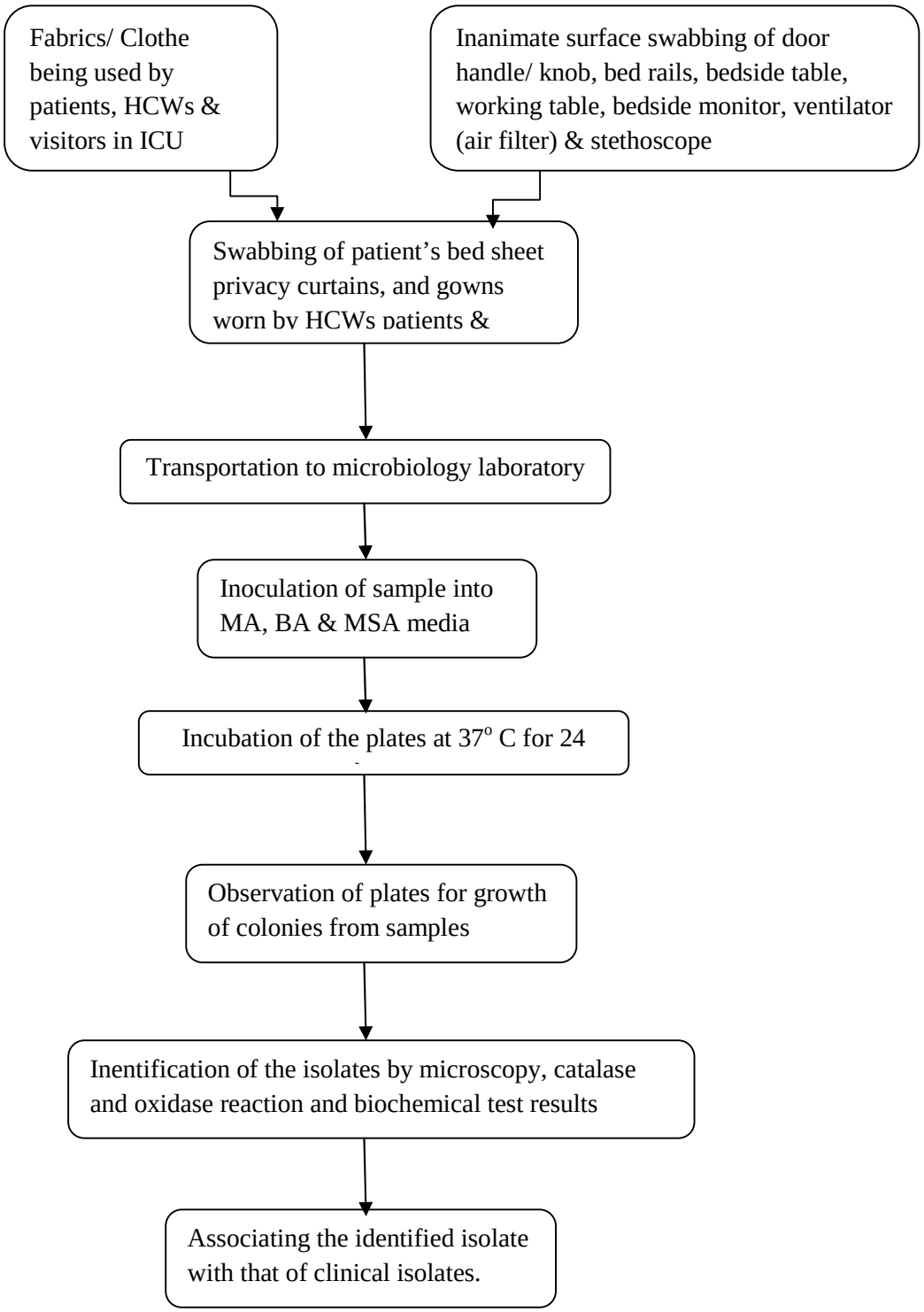


Figure 8: Flowchart of methodology showing collection and processing of inanimate surfaces around ICU & various fabrics/ clothes worn by patient, HCWs and visitors

Air and water samples from ICU:

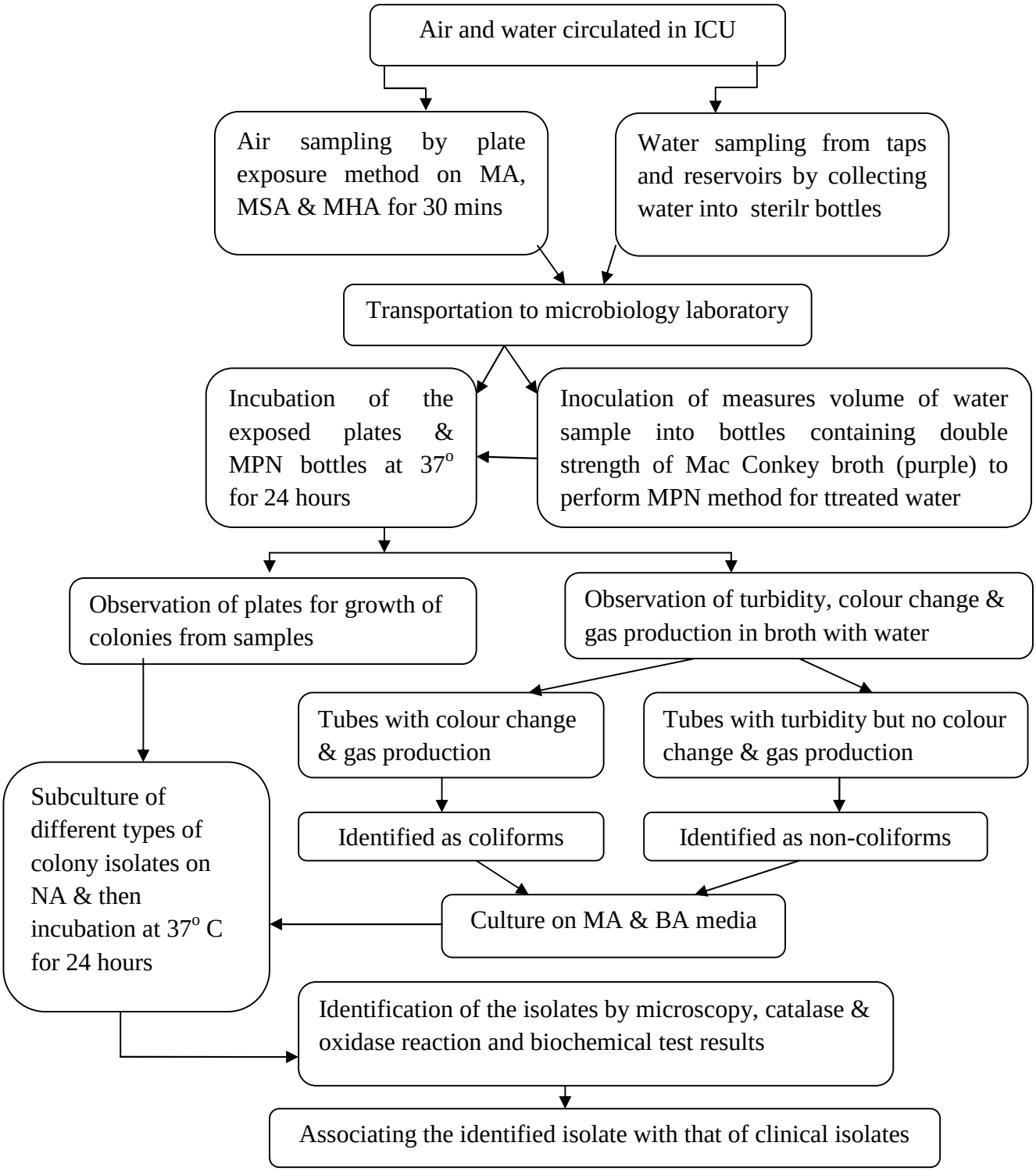


Figure 9: Flowchart of methodology showing collection and processing of air & water sample from inside the ICU room