NOSOCOMIAL INFECTION IN POST OPERATIVE CARE UNIT OF DIFFERENT HOSPITALS

PhD Thesis

DEPARTMENT OF MICROBIOLOGY
FACULTY OF BIOLOGICAL SCIENCES
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2010

SUBMITTED BY
MOHAMMAD MURSHED
REG. NO : 15
SESSION : 2005-2006

NOSOCOMIAL INFECTION IN POST OPERATIVE CARE UNIT OF DIFFERENT HOSPITALS

A THESIS SUBMITTED TO THE UNIVERSITY OF DHAKA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN MICROBIOLOGY

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DEDICATED TO

MY PARENTS

DECLARATION

I do hereby declare that the work submitted as a thesis entitled "**Nosocomial Infection in Post Operative Care Unit Of Different Hospitals**" to the Department of Microbiology, University of Dhaka for the degree of Doctor of Philosophy by the results of my own investigation and carried out under supervision of Professor Dr. Md. Abdul Malek, And Professor Dr. Md. Majibur Rahman, Department of Microbiology, University of Dhaka. The research work has not previously been submitted for any degree.

Mohammad Murshed
Department of Microbiology

CERTIFICATE

We Hereby certify that the research work embodying the results reported in this thesis entitled "**Nosocomial Infection in Post Operative Care Unit of Different Hospitals**" submitted by Mohammad Murshed bearing registration no:15, session:2005-2006 for submission in the form of thesis has been carried out under our guidance and supervision for the degree of Doctor of Philosophy in Microbiology, University of Dhaka.

Supervisor

Professor Dr. Md. Abdul Malek
Department of Microbiology
University of Dhaka

Joint Supervisor

Professor Dr. Md. Majibur Rahman
Department of Microbiology
University of Dhaka

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Mohammad Murshed
DHAKA

ABSTRACT

A comparative study of post operative care unit of a government managed hospital and private managed hospital was carried out to determine the nature of nosocomial infection with their sources and the prevention of transmission of the infection by identifying the risk factors. Among the ninety six infected patients studied, the most vulnerable for male were ranging from 30-60 years of age and for female was about 30 years. More than 80% of the infected patients in the private hospital were found to be suffered from diabetes and the habit of smoking was higher in government hospital (75%). Minor operations like cut injury presented with cleans wound and abscess wound was dirty type. The healing process of wounds took more than two weeks. The major pathogens of wound infection in both hospitals were *E. coli* and *S. aureus*. Microbial isolates especially *E. coli* was obtained from the hands of hospital staffs and attendance in both hospitals and hands of the attendances (60%) contained more bacteria than hospital staffs (40%). Anterior nares of hospital staffs and attendances contained *S. aureus*. Samples were tested from the patients surroundings of operative and post operative room of both hospitals contained *E.coli, S. aureus, Pseudomonas* and *Proteus*. All the strains of *E. coli* were found to be 100% resistance to ampicillin and the most potent drug against *E. coli* was found to be imepenem. All the strains of *S. aureus* were found to be resistant to penicillin and most potent drug against *S. aureus* was vancomycin. More than 75% strains of *S. aureus* were methicillin resistance and more than 60% of *E. coli* was extended spectrum β-lactamase. Sensitivity and specificity of latex agglutination test of *S. aureus* was 100% and methicillin resistant *S. aureus* isolates from both patients and carrier showed same coagulase type VI. Different plasmid patterns of *E. coli* strains varied in molecular weight ranging from 1 to 140 MDa and a correlation ascertained between plasmid pattern and drug resistance. DNA fingerprinting analyses using pulsed field gel electrophoresis of *Xba*I restriction-digested genomic DNA showed that clonal relatedness between the two clinical and environmental isolates of *E. coli* was 100%. 70% of *E. coli* isolates contained resistance gene marker *intl1*. After implementing the infection control program, the hand washing with antimicrobial soaps, wearing masks and gloves, sanitation with 70% ethyl alcohol was found to be very effective in reduction of microbial contaminates.

The Author

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ABBREVIATIONS

APHA	American Public Health association
bp	base pair
CDC	Centre for disease control and prevention
CoNS	Coagulase negative *Staphylococcus*
cfu	Colony forming unit
DMCH	Dhaka Medical College Hospital
DD	Double Disc
dNTP	Deoxy nucleotide triphosphate
EDTA	Ethylene diamine tetra acetic acid
ESBL	Extrended Sprectum β lactamase
HFRCMCH	Holy Family Red Crescent Medical College Hospital
Ig	Immunoglobulin
intI1	gene for class 1 integron
ICDDR,B	International centre for diarrhoeal disease research, Bangladesh
kb	kilobase
kda	kilodalton
LAT	Latex agglutination test
MRSA	Methicillin resistance *Staphylococcus aureus*
MSSA	Methicillin suseptible *Staphylococcus aureus*
MDa	Mega Dalton
PCR	Polymerase chain reaction
PBP2a	Penicillin binding protein 2a
PFGE	Pulsed field gel electrophoresis
SDS	Sodium dodecyl sulfate
WHO	World Health Organization

CHAPTER 1

CHAPTER 1
INTRODUCTION

Nosocomial infection, an infection that become manifest to hospitalized patients two days after admission, is one of the major causes of mortality and morbidity of hospital admitted patients (Garner *et al.*, 1988). The post operative nosocomial infections caused by different bacteria are given attention in recent years (Anaya and Dellinger, 2006). *Escherichia coli* and *Staphylococcus aureus* are the two major pathogens of post operative surgical site infection and the Methicillin resistance *Staphylococcus aureus* (MRSA) and extended spectrum β-lactamases (ESBL) are the two major antibiotic resistance patterns to deal with.

Different molecular and serological techniques are applied to detect the nosocomial pathogens and to make a correlation between clinical and environmental samples (Singh *et al.*, 2006). The Methicillin resistance *Staphylococcus aureus* (MRSA) is detected by latex agglutination test and correlation of environmental and clinical isolates of *Staphylococcus aureus* are determined by coagulase typing. The prevalence of *Eschericia coli* and the extended spectrum β-lactamases (ESBL) in both clinical and environmental samples are established by plasmid profile analysis, pulse field gel electrophoresis (PFGE) and polymerase chain reaction (PCR).

There are various sources and risk factors which mediate the transmission of post operative surgical site infection. The relationship of these risk factors depends on bacterial characteristics like types and colonization of microorganisms in body surface, surgical site characteristics like pre-operative, operative and post-operative risk factors and patient related factors like diabetes, smoking, immunosupression, etc. The control of transmission of post operative nosocomial infection also depends on the management system of different hospitals. The proper control of nosocomial infection also can be achieved through a sound infection control program by implementing contact precaution, droplet precaution, air borne precaution and as a whole standard precaution (Siegel *et al.*, 2007). It is necessary to study the nature and risk factors and the control or care for preventing the post operative nosocomial infection.

1.1 Nosocomial Infection

The term "nosocomial" derived from two Greek words- "nomos" means "disease" and 'komeion" means "to take care of". The nosocomial infection is a disease contacted by a patient while under medical care. Generally, a patient who has been in the hospital for less then 48 hours and develops an infection is considered to have been incubating the infection before hospital admission. Therefore, infections that become manifest after 48 hours are considered to be "nosocomial" (Garner *et al.*, 1988; Jarvis, 2001).

Through nosocomial infections have existed as long as there have been hospitals, but attention was not directed until the middle of the nineteenth century. During this time, Florence Nightingale improved hospital design and higher standards of nursing care, Ignaz Semmelweiz furnished the methodology to control the hospital epidemics of puerperal sepsis by hand washing (Rotter, 1998).

During World War I and II, nosocomial infections were usually caused by *Streptococcus* spp. and *Staphylococcus* spp. After the discovery of penicillin, streptococcal infections were dramatically reduced but it has only temporary effect on staphylococcal infections. The frequency of infections caused by Gram-negative bacteria and fungi ware increasing since 1960s (Eickhoff, 1982). Methicillin resistance *Staphyloccus aureus* (MRSA) is still one of the important causal agent of nosocomial infcetion and Gram-negative bacilli being the predominant organisms (Kapur *et al.*, 1985).

1.2 Impact of Nosocomial infection

Every year, at least 5% of the patients admitted to acute care hospitals may acquire an infection that was neither present nor in the incubation stage when they entered the hospital (Haley *et al.*, 1985). In the USA, it has been estimated that about 10% of the hospital patients acquire a nosocomil infection and a huge expenditure was required for additional treatment. Surgical wound infections also result in an excess length of hospital stay (LOS) of over 6 days and an increase in hospital costs of over $3000 per infection (Kirkland,1999).

1.3 Infecting Microorganisms and Sites of Nosocomial Infection

Nosocomial infections are usually caused by bacterial, viral and fungal pathogens. National Nosoconial Infection Surveillance (NNIS) system surveyed from January 1992 to May 1999 and found that Gram-negative bacilli dominate the list of nosocomial pathogen though *Staphylococcus aureus* remains the most important pathogen of all sites except the urinary tract according to Centre for Disease Control, USA (CDC, 1999).

The most common nosocomial infections are urinary tract infections (33%), followed by surgical site infections (15%), ventilator associated pneumonia (15%) and blood stream infections (13%) , etc. (Forbes *et al.*, 2002).

1.4 Sources of Microorganisms

Human sources of the infecting microorganisms in hospitals may be the patients, the health care workers or the visitors. Human source may include persons with disease, persons in the incubation period of a disease and persons who are colonized by an infectious agent but have no apparent disease or persons who are choric carrier of an infectious agent. Other sources of infecting microorganisms can be the patients own endogenous flora, which is difficult to control and inanimate environmental objects that have become contaminated (Jilani *et al.*, 2007).

1.5 Mode of Transmission of Nosocomial Infections

Transmission of infection within a hospital requires three elements: a source of infecting microorganisms in hospitals, a susceptible host and a means of transmission for microorganisms. The infections in the hospital could spread from one person to another through direct or indirect contact or droplet transmission or with the help of vehicles such as food, water etc, and vectors like arthropods (Haley, 1985).

1.6 Classification of the Post Operative Wounds

Post operative wound is usually synonyms as surgical site wound and vice versa. The CDC's National Nosocomial Infection Surveillance (NNIS) system has developed and

standardized the criteria for defining surgical site infection. By these criteria surgical site infection (SSI) are classified as being incisional or organ/ space (Figure 1.1). Incisional SSI are further divided into those involving only skin and subcutaneous tissues (superficial incisional SSI) and those involving deeper soft tissues of incision (deep incisional SSI). Organ/space SSIs involve any part of the organ or space other than incised body wall layers that was opened or manipulated during an operation (Alicia *et al.*,1999).

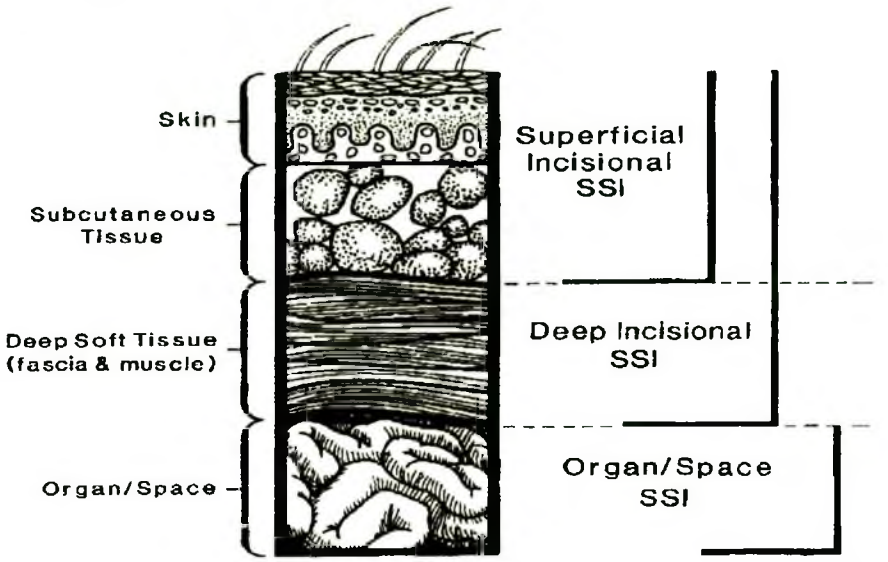


Figure 1.1 Cross section of abdominal wall depicting classification of surgical site infection (Alicia *et al.*, 1992).

1.7 Types of surgical site wounds

On the basis of degree of microbial contamination surgical site infections can be classified into four major groups (Anaya and Dellinger, 2006).

Clean site wounds (C): Clean wound with no inflammation. No penetrating (blunt) trauma after operation.

Clean contaminated wounds (CC): Clean wound without unusual contamination. No evidence of infection or major break in sterile technique is encountered.

Contaminated wounds (CO): Fresh accidental wound or operations with major break in sterile technique and acute non purulent inflammation.

Dirty or infected wounds (D): Old traumatic wound with foreign body or fecal contamination and pus is encountered.

The rate of wound infection was determined by the following degree of contamination: 1% to 5% for clean wounds; 3% to 11% for clean-contaminated wounds; 10% to 17% for contaminated wounds and more than 27% for dirty wounds (Cruse and Foord, 1980).

1.8 The Risk Factors and Pathogenesis of Post Operative Infection

Three major factors determine the likelihood that a given patient will acquire a post operative nosocomial infection (Figure 1.2).

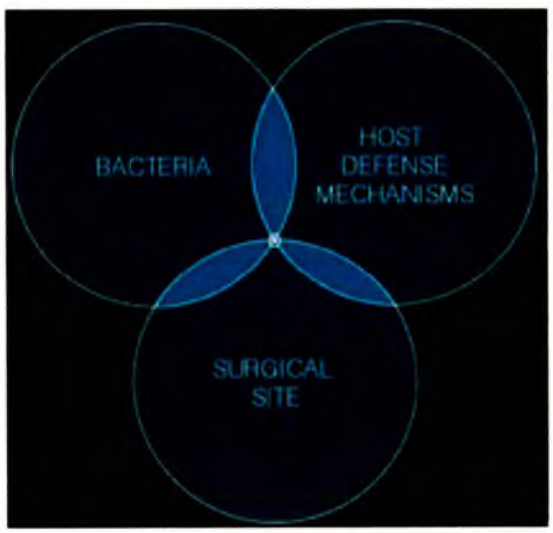


*Figure 1.2 The relationship between the three risk factors of post operative wound infection (*Culver *et al.*,1991).

Firstly, the virulence of the infecting organisms (Microorganisms especially bacterial factor) like preoperative colonization of bacteria in body surface. Second factor is the susceptibility of the patient to the infection (host or patient factor) like immunosuppressant, smoking, diabetes, obesity, etc. The third factor is the surgical factor or local factor like preoperative shaving, surgical drains, suture, etc. On the basis of number of positive risk factors chance or risk of developing post operative nosocmial infection is determined (Culver *et al.*, 1991).

1.8.1 Pathogenesis of infection following surgical operation

Post- operative infection constitutes clinical diseased states in which the multiplication of microbes in wounds provokes local and systemic reactions. It has been reported that 3-11% of surgical incision become infected (Nicholas, 1995). Wound pathogens entered during the surgical procedure are the normal flora of the viscous. The risk of infection after surgery depends on different factors including the degree of microbial contamination at the operation site, the length of the surgical procedure, the age, underlying conditions and previous history of the patients, the skill of the surgeon and type and timing of pre-operative antibiotic prophylaxis (Nichols, 1998).

1.8.2 Epidemiology of wound infection

The most common underlying event for all wounds is trauma. Trauma my be accidental or intentionally induced. The latter category includes hospital acquired wounds, which can be grouped according to how they are acquired, such as surgically and by use of intravenous medical devises. Hospital acquired wounds can be caused by pressure sores of local ischemia. They are also referred as decubitus ulcers and when such wounds become infected they are often colonized by multiple bacterial species (Janda *et al.*,1997).

A wide range of bacteria are responsible for surgical site wound infection. Wariso and Nwachukwu (2003) from Nigeria investigated a total of 2458 wound swab samples. In descending order of frequency, the organisms include *Staphylococcus aureus* (31.60%) *Escherichia coli* (25.97%), *Pseudomonas aeruginosa* (21.21%) and *Klebsiella* spp. (10.82%), *Proteus* spp. (8.23%), non haemolytic *Streptococcus* (1.29%). Berceanu and co-workers (2003) in Romania studied 119 bacterial strains isolated from post operative nosocomial wounds. Regarding their frequency, the following strains were isolated: *Escherichia coli* (57%), *Staphylococcus aureus* (31%) *Pseudomonas aeruginosa* (8%) and *Proteus* spp (4%).

In the case for post operative nosocomial infection, *Escherichia coli* is the most prevalent among Gram-negative organisms and *Staphylococcus aureus* from Gram-positive organisms.

1.9 The *Escherichia spp.* in Nosocomial Infection

Escherichia coli are Gram-negative bacilli and one of the members of the family, Enterbacteriaceae. Most *E. coli* ferment lactose, reduce nitrates and are methyl-red-positive. Approximately 10% of the species are late lactose fermenters; some of them are non-lactose fermenters (Thomas, 1988). *E. coli* produce indole, gives a negative Voges-Proskauer reaction and do not utilize citrate as the sole carbon source. Isolates of these genera usually do not produce extra cellular DNase, H_2S, Phenylalanine deaminase, urease, and do not grow in inositol and KCN containing media. Typical gas production from glucose, indole, lysine, arabinose, mannitol, ortho-nitrophenyl galactoside (ONPG), trehalose, and xylose is found in these genera (Farmer *et al.*, 1985).

E. coli represents a wide cluster of biotypes. For many years, *E. coli* was considered to be the only species in the genus *Escherichia,* but four additional species are now recognized (Farmer *et al.*, 1985). The other three species *E. fergusonii, E. vulneris, E. hermannii* were isolated from human sources. However, these new *Escherichia* species are uncommon, and the species, *E. coli* accounts for more than 99% of *Escherichia* isolates (Schaechter and Neidhardt, 1987).

1.9.1 The *E. coli* genome

The *E. coli* chromosome is very simple in structure consisting of a single covalently bonded circular molecule of DNA with a size of about 3800 kilo base pairs. Absence of any surrounding membrane allows most of the chromosomal DNA to remain in contact with the cytoplasm. The *E. coli* chromosome was previously thought to be simply a naked molecule of DNA, but now it is known that there are associated proteins which form complex with the DNA and maintain the chromosome in a highly condensed or compact state. Four specific nucleoid-associated proteins have been identified and it is likely that these proteins maintain the nucleoid structure in the same way as specific histone and non-histone proteins are responsible for the structural organization of eukaryotic chromosome (Johnson *et al.*,2007).

The length of *E. coli* chromosome is enough to harbor nearly 2500 genes. So far, fourteen hundred and three gene loci have been detected (Bachmann, 1990). All these genes code mainly for the products involved in metabolic pathways, structural components, and various type of resistances. The unrevealed genes may control integrated cell physiology, e.g., responses to environmental changes, survival under stress, and cell division However, all the genes in *E. coli* may not be located in the chromosomal DNA; some of them may be located on plasmids and transposons (Gyles,1994).

A restriction map of the entire *E. coli* chromosome is now available and hence chromosomal deletions can be performed in a way that indicates whether a region of the chromosome contains essential genes or not. It has been found that most of the chromosomally located genes are essential and are stably maintained; whereas plasmid borne genes give extra survival advantage e.g. survival under antibiotic stress. Plasmid-borne genes are usually not as stable as chromosomally located genes. They are prone to both vertical and horizontal propagation, whereas the chromosomal DNA propagate only vertically (i.e. parent cell to daughter cell). Plasmids are often responsible for virulence of bacteria which cause diseases in man, animals, fish and plants. Some coliphage DNA may also exist in the *E. coli* cell and may be maintained within the chromosome DNA through lysogenic cycle. These DNA contain a number of genes, e.g. the phage 933j carry the Shiga-like toxin I (SLTI) gene and induce its production in enterohaemorrhagic *E. coli* strains by phage conversion (O'Brien *et al.*, 1984).

1.9.2 *E. coli* as a model organism

In the 1950s, scientists who were interested in studying basic processes of biology decided that the best strategy was to choose a simple free-living microorganism as a model system and study that one organism intensively. The model microorganism chosen was, of course, *E. coli.* In ensuing years, *E. coli* was used as a model system for just about everything imaginable; metabolic pathways, genetic regulation, signal transduction, cell wall structure, and even sex (in the form of bacterial conjugation). Oddly enough, although *E. coli* is an important cause of human and animal diseases world wide, few

laboratories turned to the study of *E. coli* is a pathogen until fairly recently. Despite this, a considerable body of information has now been gathered about the virulence factors of *E. coli*, and it is possible to begin to understand why different strains of *E. coli* cause different types of disease (Honda *et al.*,1984).

1.9.3 *E. coli* as a highly adaptive organism

Strains of *E. coli* are found habitually in the large intestine of vertebrates, usually as minority members of the normal flora. They can cause diseases in other body sites, and they spread between individual hosts, often after a relatively short extracoporeal residence. Almost never do they find themselves in a contrast, unchanging environment. Rather, they feed only intermittently in the large intestine and undergo partial desiccation when voided from their host. If deposited in a body of water they face the problems of nutrition at low concentrations of foodstuffs. Between hosts they undergo shifts in temperature, some subtle, some drastic. These organisms sometimes face these circumstances plus some new ones, when they are grown in laboratory media and intermittently saved in Bijou bottles, dunked in glycerol, and placed in a freezer, or lyophilized. Given such a varied existence, it can be expected that *E. coli* is highly adaptive. This species and many of its relatives can synthesize the preponderance of their constituents, often from a single organic compound and a few minerals. Their repertoire of nutritional abilities is quite impressive. They respond to changes in temperature and available nutrients by making rapid adjustments in the synthesis of regulatory molecules. As a consequence, they can vary considerably in size and chemical composition (Osek, 2001).

E. coli is also highly efficient in the way they scavenge their energy resources. When presented with different kinds of nutrients, they grow at different rates. The richer the array of nutrients provided, the faster they grow, sparing themselves the task of synthesizing many of the compounds provided exogenously. Under conditions of active growth they only synthesize the quantities of small and large molecules that they require. Only under conditions of starvation or other stresses do they accumulate constituents that they cannot use immediately. Under such conditions, a certain non-growing cells contain a small but significant number of ribosome's that are not engaged

in presence at the time of re-feeding allows the cells to resume growth faster (Hartl *et al.*, 1984).

The grand strategy that allows these organisms to be both highly adaptive and very efficient is difficult to perceive. Unlike cells of metazoans, bacteria and other normally unicellular organisms have evolved an extraordinarily sensitive and complex set of controlling mechanisms that sense both the external and the internal environment. A common characteristic of these sensing and controlling mechanisms is that they operate very fast. They have evolved a series of "alarm reactions" which permit them to repair damaged DNA, shut off the synthesis of unwanted RNA or protect their membranes. Most of these responses lead to the selective shut-off and turn-on of specific macromolecules. Although recombination of chromosomal genes apparently is infrequent in natural populations of *E. coil*, it may, of course, have important long-term evolutionary consequences through the creation of novel, adaptive genotypes The potential importance of transduction in the exchange of genetic information in *E. coli* in nature is under-scored by the demonstration that 70% of several hundred isolates is derived from a wide-host-range bacteriophage (Hartl *et al.*, 1984).

1.9.4 Response of *E. coli* to physical environment

The response of *E. coli* to its physical environment is in no respect exceptional among bacteria; the enteric organism classified as mesophiles with respect to growth temperature and as neutrophiles with respect to pH range permitting growth. *E. coli* grow over the mid range of temperatures, pH values, water activities, and pressure in which bacterial growth occurs (Ingraham, 1978).

Temperature: The growth rates of most wild-type strains of *E. coli* respond similarly to changes in temperature. The normal temperature range extends from 21 to 37°C, with a value of 13,000 to 14,000 cal/mol. The maximum temperature at which bacterial growth can be sustained is approximately 49°C. In wild type strains, the extent of the normal temperature range and the value of temperature characteristic within it are unaffected by nutrition. The values of these parameters are the same for cultures growing in a complex medium and in a mineral salts medium with glucose; in the latter, the rate of growth is

correspondingly lower at all temperatures. Nor does the richness of the medium affect the minimum temperature of growth, although this growth parameter in a number of other bacteria is affected by nutrition (Ingraham, 1978).

Extracellular pH: *E. coli* is a representative of the large neutrophilic class (that grow over the mid range, from about p^H 5.0 to 9.0);it grows optimally between p^H 6.0 and 8.0 and can grow more slowly at p^H values of a unit or so beyond these limits.

Intracellular pH: *E. coli* as well as many other neutrophiles (Padan *et al*, 1981), has evolved remarkably effective mechanisms of maintaining homeostasis of intracellular pH. Homeostasis of pHi (internal pH) is essential to growth of *E. coli.* When, as a consequence of environmental stress, pHi drops to 7.2, growth rate is decreased by a factor of 2; growth ceases completely when pHi drops to 6.6 to 6.8. *E. coli* is highly intolerant to perturbations that cause pHi to become more alkaline (Booth, 1985).

1.9.5 *E. coli* as an opportunistic pathogen

While most strains of *E. coli* live as benign commensals, many perhaps all, are opportunistic pathogens of humans and animals. They are the prime agents in surgical and other nosocomial (hospital-acquired) infections (wounds, secondary pneumonia, peritonitis) in compromised patients. In addition, *E. coli* is the major cause of neonatal septicemia, neonatal meningitis, and urinary tract infections in humans and of a variety of invasive diseases in mammals and birds, including mastitis in cattle and sepsis in chickens. *E. coli* is the infecting organism in more than 80% of cases of urinary tract infections, which include asymptomatic bacterimia, cystitis, and pyelonephritis. It is also a leading cause of diarrheal diseases in humans and other mammals (Levine, 1984).

1.9.6 Extended spectrum β-lactamases

Majority of ESBL producing strains are *E. coli*, *K.pneumoniae* and *K.oxytoca.* Other organisms producing ESBLs include *Enterobacter* spp. *Salmonella* spp, *Proteus* spp, *Serratia* marcesens, *Shigella* dysentery, *Pseudomonas aeruginosa*, *Burkholderia ecpacia* and *Capnocytophage ochracea* (CDC,1999). Organisms previously known to produce β-

lactamase occasionally such as *E .coli* are now frequent and on the other hand those originally beta-lectamas negative, such as *Haemophilus influenzae* and *Neisseria gonorrhoea* are now β-lactamase producers. Previous study showed that *E. coli* and *K. pneumoniae* were identified as predominant pathogens associated with UTI and wound infection. ESBLs producers have become widespread through the world and ESBLs are now found in other Enterobacteriaceae (Breadford, 2001).

Bacterial antibiotic resistance has become a major clinical concern world wide including Bangladesh. Recently, the use of second and third generation cephalosporins had led to the selection of Gram-negative organisms resistance to β-lactamase stable cephalosporins. This resistance is attributed to the production of Extended sprectum β lactamases. Extended spectrum β-lactamases are enzymes that mediate resistance to extended spectrum (third generation) cephalosporins i.e. ceftazidime, ceftriazone, cefotaxime and monobactams, i.e, aztreonam but do not affect cephamycine i.e. cefoxitin and of carbepenems i.e. meropenem or imepenem (Rahman *et al.*, 2004).

1.9.7 Types of ESBL

Most ESBLs are derived of TEM or SHV enzymes. There are now more than 90 TEM type β lactamases and more than 25 SHV type enzymes (for amino acid sequences for TEM, SHV and OXA). With both of these groups of enzymes a few point mutations at selected loci within the gene give rise to the extended spectrum phenotype.TEM and SHV type ESBLs are most often found in *E. coli.* They have also found in and other genera of Enterobacteriaceae. All of these enzymes are closely incorporated with *intl-1* gene (Bush *et al.*, 1995).

1.10 *Staphylococcus aureus* in Nosocomial Infection

Staphylococcus is the only genus of medical importance in the family Micrococcaceae. The name Staphylococcus, derived from the Greek noun staphyle (a bunch of grapes) and coccus (a grain or berry), was introduced by early investigators to describe the organism seen in pus from surgical infections. The genus staphylococcus has at least 30 species.

The main species of clinical important are *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. Some are members of the normal flora of the skin and mucous membranes of human; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia (Duguid,1989).

Staphylococcus aureus is a facultative anaerobe but growth is more abundant under aerobic environment. Staphylococcus aureus can grow on usual bacteriological media at optimum pH between 7.4 to 7.6 at 37^0C optimum temperature. On nutrient agar the colonies are circular and given golden yellow or may be white/lemon yellow. In blood agar media, the colonies produce zone of hemolysis. Media incorporating 10-15% sodium chloride acts as selective media. They are non motile, non-spore forming. It produces enzymes catalase and coagulase. Catalase converts Hydrogen peroxide into water & oxygen. Coagulase production indicates pathogenic capacity. It probably protects the organism by producing a fibrin barrier and prevents phagocytosis and destruction by phagocyte (Choudhury, 1999).

1.10.1 Methicillin resistance *Staphylococcus aureus* (MRSA)

Staphylococcus aureus has developed resistance to many common antibiotics. Strains that are oxacillin and methicillin resistant, historically termed as methicillin–resistant *S. aureus* (MRSA), are resistant to all beta-lactam agents, including cephalosporins and carbapenams. In 1963, the first major nosocomial epidemic of a methicillin-resistant strain of *S. aureus* was described. The strains were first isolated from an infant who had been treated with penicillin. Eventually, the strains were isolated from one nurse and 37 children in eight wards. One of these children admitted into surgical ward, having infections in his wounds with the MRSA strains; treated with methicillin and streptomycin and died ultimately. (Brooks *et al.*,2004).

Methicillin-resistant *Staphylococcus aureus* are significant pathogens that have emerged over the past 30 years and now cause both nosocomial and community-acquired infections. In the United States and in some European countries, MRSA accounts for 10 to 40% of all *S aureus* isolates. The organism can be difficult to control because its early

symptoms are benign and because frequently delay seeking medical intervention until the organism has begun to spread. This bacterium is usually confined to hospitals and in particular to vulnerable or debilitated patients. A proportion of the patients become infected particularly if they have been put at greater risk, such as following an operation, or have a malignancy, or the presence of a bladder catheter, intravenous infusion or surgical drain. MRSA infection on surgical wards is becoming increasingly common, especially in critically ill patients who have spent prolonged periods on the intensive care unit (Louic *et al.*, 2000).

Resistance to methicillin in *staphylococci* is characterized by presence of *mecA* genes. Staphylococci resistance to oxacillin /methicillin occurs when an isolate carries an altered penicillin-binding protein, PBP2a; which is encoded by the *mecA* gene. The alteration of the penicillin-binding protein does not allow the drug to bind well to the bacterial cell, causing resistance to *beta*-lactum producing antimicrobial agents (Willet, 1992).

In Bangladesh, a few survey was carried out for detection of MRSA. Khan and co-workers (1991) in the out door department of Dhaka Shishu hospital found that, a total of 214 patients showed positive culture for *S. aureus* out of 250 patients. Of them 134 were MRSA. In another study by Jahan and co-workers (2004) in National institute of Traumatology and Orthopedics rehabilitation (Nitor) found that among post operative wound infection cases, out of 6 isolates of *S. aureus* ; MRSA was 83.33% and only 16.67% was methicillin sensitive *S. aureus* (MSSA). The rate of MRSA was found to be very high in that study may be due to very small sample size.

1.10.2. Danger of MRSA

There are increasing numbers reports of community- acquired *S. aureus* being methicillin resistant. In the 1950s, penicillinase producing strains of *S. aureus* were so common that penicillin was becoming useless against staphylococcal infections (Herold *et al.*, 1998).

Heterogeneous strains can be considered to be composed two phenotypes of bacteria: relatively susceptible and highly resistant. Homogenous strains on the hand are composed of single phenotype of the bacteria. All of which may be highly resistant to the antibiotics. Numerous medical centers, first in Europe in the 1960s and then in the United States in the1970s, have reported outbreaks of nosocomial infections cause by methicillin resistant *S. aureus.* Methicillin-resistant strains have also become established outside the hospital, particularly among intravenous drug abusers (Haley *et al.*,1985).

Most patients from whom MRSA is isolated are colonized with this organism rather than infected. Colonization means the presence of the organisms on the skin, or in the back of throat but without any illness. A proportion of the patients become infected particularly if they have been put at greater risk, such as following an operation, or have a malignancy, or the presence of a bladder catheter, intravenous infusion or surgical drain. The patients may then develop illness similar to those caused by methicillin sensitive *S. aureus* (MSSA) such as wound and skin infection, urinary tract infections, pneumonia and bacteraemia or blood poisoning (Herold *et al.*, 1998).

1.10.3 Mechanism of resistance in MRSA

The most common and best understood mechanism of methicilin resistance in *S. aureus* is production of an unique low affinity binding protein referred to as PBP2a or 2'. The normal penicillin binding proteins of *Staphylococci* include protein that function as transpeptidase, endopeptiadses or carboxypeptidase in the cross linkage of macromolecules in the cell wall during cell division. Intrinsic methicllin resistance results from production of a unique penicillin binding protein (PBP) of slightly different molecular size that has very low affinity for binding various β-lactum antibiotics. PBP2A is encoded by a chromosomal genome of approximately 2.1 kilobases contained within a region referred to as *mec* (Jorgensen, 1991).

Methicillin resistance is genetically and biochemically complex. Numerous other factors are undoubtedly present. These other factors may include components for the regulation

of PBP2a expression, a transposase gene for integration of *mec* into the chromosome and genes whose products provide resistance to other compounds such as mercury, tetracycline or tobramycin etc. This can account for many of the properties typical to methicillin resistance Staphylococci except for PBP2a production; the elements involved in the heterogenous or homogenous expression of resistance remain to be defined (Sieradzki *et al.*, 1999).

1.10.4 Molecular basis of resistant of MRSA

Approximately 30 to 50 kb of additional chromosomal DNA, *mac,* not found in susceptible staphylococci is present in methicillin-resistant strains. The *mac* contains *macA,* the structural gene for penicillin binding protein 2a (PBP2a); *mecl* and *mecRl,* regulatory elements controlling *mecA* transcription; and 20 to 45 kb of *mac-* associated DNA (Hiramatsu, 2001).

The mecA gene

The mecA encodes PBP 2a (also termed PBP2), an inducible 76-kDa PBP that determines methicillin resistance. There is no *macA* homolog in susceptible strains. Both susceptible and resistant strains of *Staphylococcus aureus* produce four major PBPS1, 2, 3, and 4, with approximate molecular mass of 85, 81, 75 and 45 KDa. Penicillin binding proteins (PBPs) are membrane bound DD-peptidase that has evolved from proteases, and their biochemical activity is mechanically similar to that of the serine proteases (York *et al.*, 1996). These enzymes catalyze the transpeptidation reaction that cross-links the peptidoglycan of the bacterial cell wall. β-lactam antibiotics are substrate analogs that covalently bind to the PBP active-site serine, inactivating concentration of enzymes. In methicillin-resistant cells, PBP 2a, with its low affinity for binding β- lactam antibiotics can substitute for the essential functions of high affinity PBPs at concentration of antibiotic that are otherwise lethal. How *mecA* was acquired by methicillin-resistant staphylococci is not known, but transposition is a plausible mechanism. The *mecA* is highly conserved among staphylococcal species. The *B-lactam*ase plasmid may provide a temporary insertion site for the *mec-* containing transposon (Chambers,1997).

Mec1* and *macR1

The *mec1* and *mecR1* are divergently transcribed regulatory genes located immediately upstream from the *mecA* promoter. They are similar in molecular organization, structure, function and mechanism of regulation to staphylococcal β-lactamase regulatory elements, *bla1* and *blaR1*.The *bla1* is a DNA–binding protein that represses *B-lactam*ase gene transcription. The *blaR1* is a signal-transduction PBP that in the presence of β-lactam antibiotic leads to *B-lactam*ase gene transcription (Chambers,1997).

The *mec*-associated DNA

The *mecA*, *mecR1*, and *mec1* are encoded by approximately 5 kb of DNA that is itself located within 25 to 50 kb of additional DNA that may contain up to 100 open reading frames. Transposons (Tn554, located 5′ of mecA) and insertion sequence (IS431, is located 3′ of mecA) are present. The IS431 elements has the ability to trap and cluster resistance determinates with IS elements explains the multiple drug resistance phenotype that is the characteristics of methicillin-resistance staphylococci (Chambers,1997).

1.10.5 MRSA carriage

The major reservoirs of MRSA in hospitals are infected or colonized patients. Transient hand carriage on the hands of health care workers is the predominant mode for patent to patient transmission. Colonization on environmental surfaces also serves as a reservoir for MRSA. The anterior nares, axillae, perineal, perianal and inguinal regions are common site of MRSA carriage. An important feature MRSA is their propensity to spread and colonize debilitated patients. Since these strains tend to be multiple antibiotic resistances, they pose a major difficulty in treating systemic infections (Sachdev *et al.*,2003).

The largest reservoir for spread of MRSA has been asymptomatic carrier. MRSA usually spreads through direct contact with the contaminated hands of healthcare workers, but nasal carriage among hospital staff has accounted for nosocomial MRSA outbreaks. Three patterns of carriage can be distinguished. First, approximately 20 percent of healthy people almost always carry a MRSA strain. Second, a large proportion of the

population (approximately 60 percent) harbors *S. aureus* intermittently and third only a minority of people (approximately 20 percent) almost never carries *S. aureus*. The most frequent risk factors in those with nasal carriage of MRSA. Others are previous hospitalization, antibiotic use in past 6 months, referral to a hospital clinic in the past year and close contact with hospital staff (Cesur and Cokca, 2004).

1.11 The Pattern of Antimicrobial Resistance

Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic. Antibiotic resistance bacteria can live and multiply in the presence of therapeutic level of antibiotics. They can be resistance in their natural state or they can acquire resistance genes by mutation or from other bacteria. There is a large reservoir of resistance genes in bacterial genomes and in extra chromosomal piece of DNA that encode different mechanism of resistance (Soulsby, 2005).

1.11.1 Causes of microbial resistance to antibiotics

In the last 50 years bacteria have demonstrated a remarkable ability to develop and share resistance to every antibiotic often by quite unexpected mechanisms. Antibiotic resistance is a consequence of evolution via natural selection or programmed evolution. The antibiotic action is an environmental pressure; those bacteria which have a mutation allowing them to survive will live on to reproduce. They will then pass this trait to their offspring, which will be fully resistance (Spratt, 1994).

Pattern of antibiotic usage greatly affect the number of resistance organisms that develop. Overuse of broad spectrum antibiotics, greatly hastens the development of resistance, even in organisms that have never been exposed to the selective pressure of these antibiotics. Other factors contributing towards resistance include incorrect diagnosis, unnecessary perceptions, improper use of antibiotics by patients and the use of antibiotics as livestock feed additives for growth promotion (Bouza, 2002).

1.11.2 Transfer of drug resistance genes among organisms

During times of stress, commensals and pathogenic bacteria demonstrate the ability to become hyper-mutable and they duplicate and share survival information. Such as

resistance genes that can be encoded on plasmids, transposons and integrons. Spontaneous mutations that produce new resistance or strengthen existing resistance occur readily in bacteria. Bacteria may require resistance genes through a number of rotes. They may inherit the genes from their resistance forerunners. Alternatively they may acquire resistance through transfer via a virus or plasmid vector or by free DNA uptake (Shapiro,1999).

1.11.3 Antibiotic resistance mechanism

The four main mechanisms by which microorganisms exhibit resistance to antimicrobials.

a) Drug in activation or modification: enzymatic deactivation of penicillin G in some penicillin resistance bacteria through the production of β-lactamases.

b) Alteration of target site: alteration in topoisomerase genes (gyrA, gyrB and parC) which are associated with quinolones and/or floroquinolones resistanxce, b) alteration of PBP- the binding site of penicillins- in MRSA(mechicillin resistance Staphylococcus aureus) and other penicillin resstance bacteria.

c) Alteration of metabolic pathway: some sulfonamides resistance bacteria do not require para amino benzoic acid (PABA); an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides. instead, like mammalian cells, they turn to utilizing performed folic acid

d) Reduced drug accumulation: by decreasing drug permeability and/or increasing active efflux.

1.12 The Antibiotic Resistance Marker Gene *intl-I*

The spread of antibiotic resistance among bacteria has been recognized as an increasing problem in the veterinary and medical fields, and mobile DNA elements, including plasmids, transposons, and integrons, facilitate the proliferation of resistance genes in bacteria (Liebert *et al.*, 1999 and Speer *et al.*, 1992). The class 1 integron has been

identified as the most prevalent of the five classes of integrons in clinical isolates and has been associated with multidrug resistance (MDR) in pathogenic bacteria (Hall *et al.*, 1993 and Recchia *et al.*, 1995). The class 1 integron acquires resistance genes in the form of gene cassettes via site-specific recombination. Among the same gene cassettes, some nucleotide changes tend to be present, and some of these are shared by other bacteria. Certain of those nucleotide changes can be classified as singlenucleotide polymorphisms (SNPs), and these have proven useful in the fine differentiation of bacterial isolates, as well as in studies of the molecular evolution of class 1 integrons (Sokurenko *et al.*, 1999).

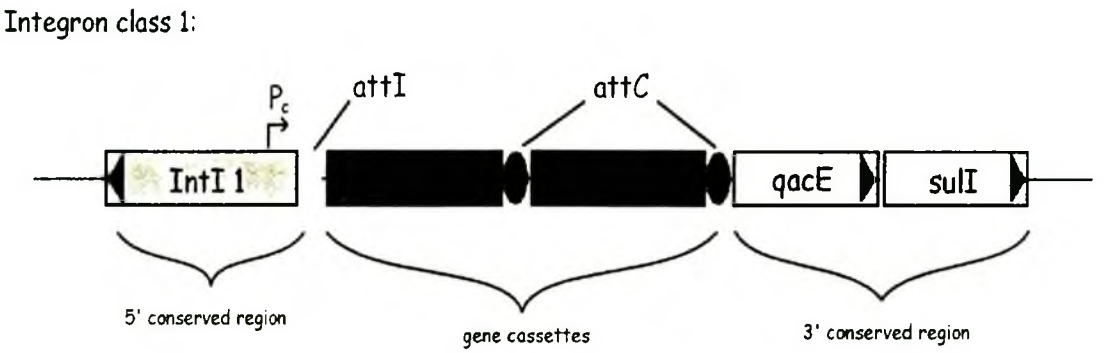


Figure1.3 The integron class 1 gene cassettes site specific recombination system (Boucher et al, 2007).

Class 1 integrons are most commonly found in nosocomial and community environments followed by class 2 integrons, other integrons classes being scarcely reported to date. ESBL located on integrons like structures being increasingly reported worldwide (Canton *et al.*,2003; Bonnet , 2004).

1.13 The Plasmid

Extrachromosomal resistance plasmids have been recovered from bacteria isolated and stored in pre-antibiotic era. The term plasmid was proposed by Lederberg (1952) for all extrachromosomal genetic structures that can replicate autonomously. Plasmid are characterized either by size as measured in kilo bases (kb) or molecular weight, as measured in millions of Daltons or mega Daltons (MDa). Most strains of

Enterobacteriaceae have a molecular weight of between $2x10^3$kilo base (kb) pairs. Plasmids are varying in size between 1.5 and 300kb pairs (for conversion 1 MDa is equivalant to 1.51 kb). There are not indispensable except certain circumstances. For example, growth of bacteria in presence of ampicillin, due to resistance plasmid encoded β-lactamase, it follows that the plasmid is indispensable in presence of the drug. Most plasmids whether from Gram-positive or Gram-negative bacteria are circular element, but linear plasmid have been reported in *Streptococcus* spp. (Hinnebush and Tilly,1993).

1.14 Polymerase Chain Reaction (PCR)

The PCR technique did not excite general interest until the mid 1980, when Mullis and co-workers (1986) developed PCR into a technique that could be used to generate large amounts of single copy genes from genetic DNA.

PCR is an *in vitro* method for system of nucleic acid in which a particular segment of DNA can be specifically amplified. The polymerase chain reaction uses multiple cycles of template denaturation, primer annealing and primer elongation to amplify DNA sequences. The primers hybridize to the opposite strands of the target sequence and are oriented so that DNA synthesis by DNA polymerase proceeds across the region between the primers. Since the extension product themselves are also complementary to and capable of binding primers in successive cycles of amplification essentially double the amount of the target DNA synthesized in the previous cycles. The result is a exponential accumulation of specific target DNA fragment approximately up to 2n, when n is the numbers the cycles of amplification performed (Saiki *e. al.*,1988).

The template must be sufficient to be able to visualize PCR products using ethidium bromide. Usually 100ng of genomic DNA is sufficient to defect a PCR product from a single copy mammalian gene. A member of contaminants found in DNA preparation can decrease the efficiency of PCR. These include urea, the detergent SDS, sodium acetate and sometimes components carried over in purifying DNA from agarose gels. The most frequently used thermostable polymersae is Taq DNA polymerase. Taq DNA polymerase permitted the use of higher temperatures for annealing and extension which improved the

stringency of primer template hybridization and this specificity of the products. A very important property of Taq DNA polymerase is its error rate, which was initially estimated at $2x10^{-4}$ nucleotides/cycle (Mullis *et al.*, 1986).

1.15 Pulsed Field Gel Electrophoresis (PFGE)

PFGE has been shown to be a valuable typing method for epidemiological investigations of several bacterial pathogen and clonality studies. In this finger printing method, chromosomal DNA is digested with a restriction endonuclease that generates large fragments. The restriction fragments are resolved in a pattern of discrete bands. The DNA restriction patterns of the isolates are then compared with one another to determine their relatedness. For the analysis of chromosomal DNA restriction patterns produced by PFGE, Tenover and co-workers (1995) have suggested that macro restriction profiles of an isolate differing by more than six fragments positions can be considered to represent different strains. Choice of restriction enzyme is an important factor to obtain a reproducible and well discriminatory banding pattern in PFGE. A number previous study suggested that *NotI* gave the best discriminatory banding pattern for *Vibrio* spp. since it has a long ranged DNA cutting site and cut the DNA infrequently (Safa *et al.*, 2005). *Xba*I endonuclease is used for typing of all isolates in the present study

1.16 MRSA Latex Agglutination Test

Nakatomi and Sugiyama (1998) described a simple rapid slide latex agglutination assay that was developed to directly detect penicillin-binding protein 2a (PBP2a) from isolates of staphylococci. PBP2 present in MSSA where as PBP2a present in the members of MRSA. MRSA latex agglutination test is based on the reaction of latex particles sensitized with monoclonal antibodies against PBP 2a. To extract PBP 2a from the tested colonies, a loopful of cells is supended in 4 drops of extraction reagent 1. This suspension is placed in heating block (>95°C) for 3 minutes. After allowing the suspension to cool to room temperature, 1 drop of extraction reagent 2 is added and the mixture is vortexes throughly. The suspension is then centrifuged at 1500xg for 5 minutes. The latex

agglutination test is performed with the supernatant. 50 µl of supernatant is mixed with 1 drop of sterilized latex. For negative control 50 µl of supernatant is mixed with 1drop of negative control latex. Mixing for 3 minutes is performed in shaker. When agglutination occurred within 3 minutes it was visually quantified as a score between 1 and 3.

Presence of PBP2a means presence of *mecA* gene and So, MRSA is detected (Figure 1.4).

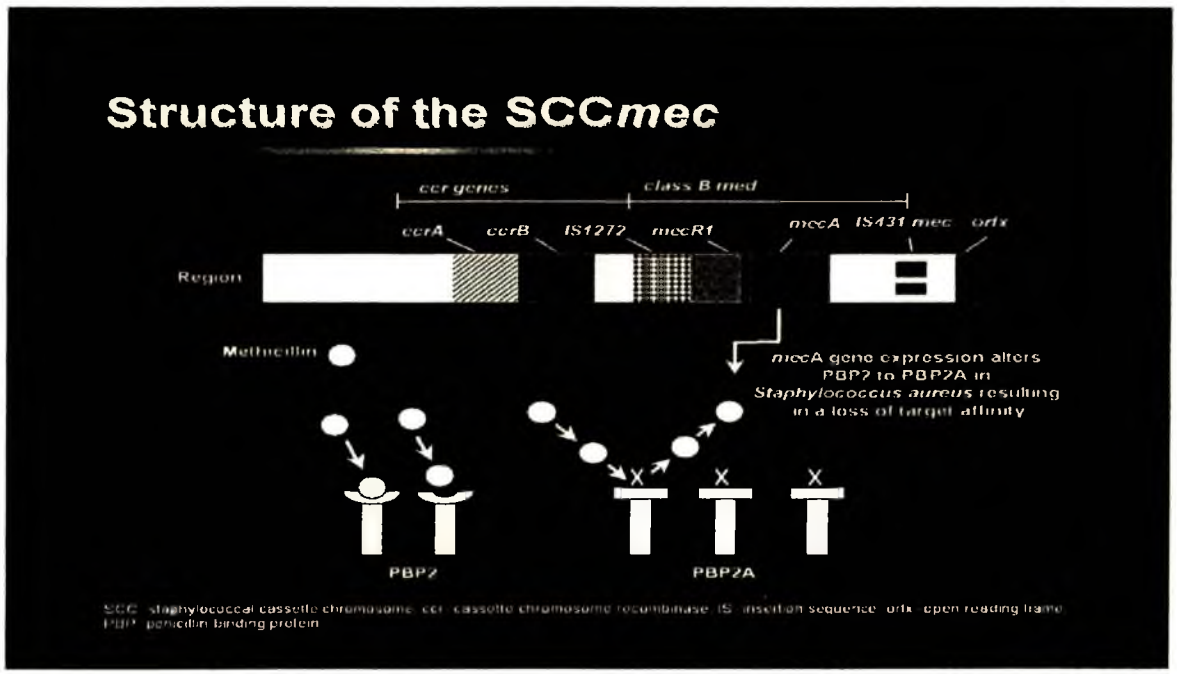


Figure1.4 The mecA gene expression alters PBP2 to PBP2a in Staphylococcus aureus (Rybak and Laplante, 2005).

1.17 Infection Control in Post Operative Care Unit

Infection control in post operative room is based on following principles.

(WHO/CDS/CSR/EPH. 2002.12)

1. Isolation policy 2. Surveillance 3. Antibiotic policy 4. Waste management

5. Use of antiseptics and disinfectants

1.17.1 Isolation police

Isolation policy is developed on the basis of CDC guideline for isolation precaution. (Siegel *et al.*, 2007). The major components of the guidelines are:

A. Standard precautions- applied in all patients regardless of infection status

B. Transmission-based precautions- applied for known or probable infected patients. Three types of transmission based precautions:1. Airborne precaution.2.Droplet precaution. 3. Contact precaution.

Standard precautions

Standard precaution is to be applied to any person regardless of his or her infection status.

Key Components of Standard Precaution

a) Hand hygiene. b) Barrier precautions /use of Personal protective equipment (PPE) 1. Gloves 2. Gowns 3. Shoe covers 4. Head covers 5. Mask 6. Goggles 7. Face shield c) Disinfection of equipments/ floor/ surface and management of spillage. d) Sharps precautions .e) Safe disposal of hospital waste. f) Care of skin and mucous membrane. g) Management of injuries.

Contact precautions

It is meant to prevent transmission of organisms through contact. There are two types of contact transmissions: Direct and indirect. In direct contact transmission transfer of infection occurs directly by skin to skin contact during examining patient and procedures and in indirect contact transmission, transfer occurs indirectly through linen, utensil, stethoscope, BP machine and contaminated gloves that are not changed between patients.

Droplet precautions

When a person coughs or sneezes, very small mucous droplet particles are produced which can go as far as 3 feet. Thereafter the particles fall down to the floor due to gravity. If a patient carries respiratory infections, droplet precautions are needed whenever a HCW goes within the critical distance of 3 feet.

Air borne precautions

Some organisms can remain suspended in the air and be spread farther than 3 feet, the critical distance designated for droplet precaution. They need more rigorous precaution in the form of airborne precaution.

1.18. Nosocomial Infection Surveliance

The nosocomial infection rate in patients in a facility is an indicator of quality and safety of care. The development of a surveillance process to monitor this rate is an essential first step to identify local problems and priorities and evaluate the effectiveness of infection control activity. Surveillance, by itself, is an effective process to decrease the frequency of hospital acquired infections (Pottinger *et al.*, 1997).

1.19. Antibiotic Policy

Antibiotic use is widely accepted as being responsible for the selection and maintenance of antibiotic resistance. It is less obvious, however that it is also responsible for increasing transmissibility and pathogenicity of many multi resistance bacteria and may actually be increased the number of post operative nosocomial infection. With the dearth of new antibiotics coming to the marketplace and advance of multi drug resistance bacteria like MRSA and ESBL, it is not difficult to see untreatable life threatening bacteria infection becoming common. Antibioigram preparation, MRSA policy are some components of antibiotic policy. (Gould, 2008).

1.20 Waste Management

Waste management is one of the major components for prevention of transmission of nosocomial infection (Blenkharn, 2005). Waste management is divided into four compartments:

Sorting → Handling → Storage → Final disposal

There are 4 types of colored bins are used where different grades of waste products are deposited. Any sharp items are placed inside **red** bins. Infectious items or any soaked with body fluids are placed inside **yellow** bins. Non infectious items such as plastic begs, tubes, unbroken vials, liquids are placed inside **blue** bins. Domestic wastes were placed inside **gray** bins

1.21 Proper Use of Disinfectants and Antiseptics

The following antiseptics and disinfection has been used for hospital equipments and environment. This chart has been made based on The Association for Professionals in Infection Control and Epidemiology (APIC) guideline for infection control practice (Ratula,1996).

Table 1.1 Use of disinfectant and antiseptics in cleaning environment.

Type	Trade Name	used for	comments
Quantenary Ammonium Compounds	*Zephiran* *Bactyl*	Cleaning surface of equipment, surface of environment e.g., furniture, sink, floor, B/P Apparatus, bed, patient's file, etc.	Dilute Zephiran 1:200 Dilute Bactyl 1:100 both bacteristatic abd bactericidal speed of action is slow.
Hypochloride	Chlorox Virkon	Cleaning surface contaminated with blood or secretion - Cleaning contaminated environmental surfaces	Dilute Chlorox 1:10 and Virkon 1:20 dilute 0.25% and 0.5% high level disinfectant. Corrosive to skin.

Table 1.2 Use of disinfectant and antiseptics in cleaning equipments.

Type	Trade Name	used for	comments
Alcohol 70%	Alcohol	Clean equipment used to contact with membrane of skin, e.g., Stethoscope and rectal thermometer	Intermediate level disinfectant useful against gram positive and negative bacteria and speed of action is fast
Chlorhexidine Cetrimide Alcohol	Savlon in Alcohol	Respiratory tubes	same as alcohol but mode of action is slow
Sodium Hypochloride	Alka	To be used in medical equipment cleaner	Intermediate level disinfectant. irritant to skin
Glutaraldehyde	Cidex Adecyde	Medical equipment, e.g, Endoscope	high level disinfectant good against all kind of microbes

1.22 The Scope and Objectives

Nosocomial post operative infection remains as an endemic and silent problem encountered in hospitalized patients throughout the world including Bangladesh. In the past, hospitals were notorious for infections and even in the contemporary history a little has been achieved in rendering hospitals a safe place. On the other hand, nosocomial infections in post operative rooms are preventable, perhaps between 30 to 50 percent are primarily caused by problems in patient care practices, such as the use of and care of urinary catheters, as well as hand washing practices and surgical skills. It remains one of the major causes of morbidity and mortality leading to increase in cost of hospitals and to the emergence of new health hazards of the community (*Garner et al., 1996*).

Nosocomial infection in post operative room may occur through endogenous and exogenous rout. The main nature of infection of surgical sites are respiratory infection, device related infection, urinary tract infection, blood and blood product infection and major surgery procedures related infection, etc. Among the microorganisms *Staphylococcus aureus* is the hospital pathogen of major concern. *E. coli* and Enterobacteriaceae become synonymous with nosocomial infection Different drug resistant strains like Methicillin resistant *S. aureus* (MRSA) and Extended spectrum β lactamases (ESBL) are emerging very rapidly in Bangladesh and it is becoming a real concern for critically ill patients.

At present In Bangladesh, a few studies have been conducted to predict the infection rate of nosocomial infection. There was no substantial evidence on the sources that can be responsible for transmitting these nosocomal pathogens. There is also no integrative effort to identify and fight against the problem. It is necessary to understand the common causes, major risk factors and proper management of the problem.

The objectives of the present study

1) *To investigate the nature of nosocomial infection at post operative care unit.*
2) *To isolate and identify the causative organisms and their antimicrobial resistance.*
3) *To determine the sources and risk factors influencing the spread of infections.*
4) *To set up a guideline for prevention of post operative nosocomial infections.*

CHAPTER 2

Chapter 2

MATERIALS AND METHODS

The investigation was carried out in the laboratory of Department of Microbiology, University of Dhaka; Department of Microbiology of Holy Family Red Crescent Medical College Hospital Dhaka and in the laboratory of Clinical Microbiology, ICDDR'B, Dhaka. Most of the samples were collected from Dhaka Medical College Hospital and Holy Family Red Crescent Medical College Hospital during the period of January 2008 to July 2009.

2.1 General Procedures and Equipment

2.1.1 Sterilization

All equipment and glassware which had to be sterilized were autoclaved (HA Model 240M Japan) at 15 p.s.i for 20 minutes. Autoclaved materials were dried overnight at 95^0C and glassware was sterilized at 170^0C for two hours in a hot air oven (Heraeus, model 0042, Germany) prior to use. All media and solutions were sterilized under the same conditions of autoclaving. Agar plates were prepared, dried at room temperature and all inoculations were performed under aseptic condition in a laminar flow cabinet (HA Model 48, Italy).

2.1.2 Growth of microorganisms

For growth of the microbial strains in liquid media in shake flasks, a lateral shaker (Gallen Kamp, England) was used. Inoculated culture media were incubated in an EN 500 incubator at 37-45^0C and in Gallen Kamp cool incubator at 25^0C. A colony counter (Gallenkamp P1460, England) was used to count the microbial colonies on solid agar plates. A phase contrast light microscope (ZEISS Model460701-9903, Germany) was used for microscopic study of bacterial strains.

2.1.3 Preparation of solutions

Accurate weights of the components of various solutions and reagents were measured using a Mettler balance (Model B5, Switzerland) and for approximate weight a top pan

balance (Mettler PC 440, Switezerland) was used. Measurement of pH values of buffer solutions, culture supernatant, etc., was performed by a Seiko pH meter (Model Delta-320, UK).

2.1.4 Maintenance of cultures, reagents and solutions

Preservation and maintenance of different materials including microbial strains, heat labile chemicals or reagents, buffers, enzymes, substrates, etc. were carried out in refrigerator at 4^0C or in a freezer at 78^0 C. The pure bacterial cultures were routinely maintained on Nutrient Agar slants and stored at 4^0C until required. Subcultures were prepared every two-month on fresh agar slants. Prior to use, the bacterial cultures was checked on a nutrient agar plate. The plate was then considered as stock plate from which the inoculum was prepared for experimental works. For long term preservation, the pure bacterial culture was kept in an ampoule and kept at 78^0C.

2.2 Media Used for Cultivation of Microbial Isolates

The dried complex medium such as Nutrient Agar (Oxoid), McConkey Agar (Difco), Mannitol Salt Agar (Oxoid), Sabouraud Dextrose Agar (Oxoid), Muller Hilton Agar (Oxoid), KIA medium (Oxoid),T_1N_1 Broth (Difco), Loffler's serum (Difco), etc. were used for growth and characterization of the bacterial isolates. The compositions of some important media are illustrated below:

2.2.1 Blood Agar

Blood agar media contained 14g tryptone, 4.5g peptone, 4.5g yeast extract, 5g NaCl, 12.5g Agar per liter distilled water and pH was adjusted to 7.3. Blood Agar media was specially used for enrichment and identification of *Streptococcus* Spp. and *Staphylococcus* Spp.

2.2.2 MacConkey Agar

MacConkey agar media was selective for Enterobactericeae. MacConkey Agar media was used for identification of *Eschericia coli*, *Salmonela* Spp. *Pseudomonas* Spp. *Proteus*

spp. MacConkey Agar media acts both as a selective and indicator media. MacConkey agar media contained 13.5g agar, 17.8g peptone, 5.8g NaCl, 15g bile salt, 3g neutral red in 1000 ml distilled water.

2.2.3 Mannitol Salt Agar

Mannitol salt agar Medium is a useful selective medium for recovering *Staphylococcus aureus*. Mannitol Salt Agar contained 10g agar, .025gm phenol red, 75g NaCl, 10g D-Mannitol, 10g Beef extract, 10g proteose peptone in a liter of distilled water and pH was adjusted to 7.2. Mannitol salt agar is also be used to screen for nasal carries. *S. aureus* ferments mannitol and is able to grow on agar containing 70-100 g/l sodium chloride. Mannitol salt agar containing 75 g/l sodium chloride is recommended, particularly for isolating MRSA strain.

2.2.4 Sabouraud Dextrose Agar

Sabouraud Dextrose Agar is composed of 17g agar, 20g dextrose and 10g neopeptone per liter, is adequate for the isolation of most Candida species. *Candida albicans* grows well on Sabouraud Agar and produces cream colored pasty colonies at 35-37^{0}C after 24-48 hours incubation. The colonies have a distinctive yeast smell. On agar to which corn meal extract has been added, *C.albicans* can be identified by the formation of pseudohyphae and chlamydospores. Chloramphenical (90.05 to 0.02 g/liter) and Gentamycine (0.02g/litre) are added to inhibit bacteria. pH is adjusted to 4.5 to 5.5.

2.3 Selection Criteria of Patients and Carriers

Patients were selected on the basis of their wound characteristics with some associated features. The features were revealed by recording history and examination of the sign and symptoms of the diseases.

2.3.1 Patients

Inclusion criteria

Clinically diagnosed patients of all age group and of both sexes having wound infection, with pus or discharge draining from the infection site or wounds having any signs of

infection (pain or tenderness, localized swelling, redness or heat) or the wound has not healed within 3 days after the injury were included in the study.

Exclusion criteria

Patients who received antibiotic within 24 hours or having wound dressed with any antiseptic solution were excluded from the study.

2.3.2 Carriers

Inclusion criteria

Anterior nasal swabs from hospital staffs having no history of disease within 6 months, working in Surgery and/or Gynaecology Department of Dhaka Medical College Hospital (DMCH) and Holy Family Red Crescent Medical College Hospital (HFRCMCH) were included in the study.

Exclusion criteria

Medical staffs with history of hospitalization, undergoing surgery or treatment of any kind and intake of antibiotics in the past 6 months were excluded from the study.

2.3.3 History of the patients

History of the patients including time of surgery, type of surgery, implantation history, temperature of the body, use of any antibiotics and antibiotic prophylaxis, wound class, type of surgical site infection were recorded according to The American Society of Anesthesiologists (ASA) physical status classification (Cruse and Foord, 1980).

The onset of fever and an elevated white blood cell count more than $10,000/mm^3$ after an operation was recorded as a signal of an infectious process. A careful examination of the patient, guided by history was also taken. Laboratory tests and x-rays were complementary for the detection of infected cases.

Inspections of site of surgery along with different classes of wounds were also performed. The wounds classes divided into four major groups: Clean, Contaminated, Clean-

contaminated and Dirty. History of site of surgery was recorded according to condition of incision: Superficial, deep or organ/space incisional surgical site infection History of the use of empirical antibiotics or the prolonged administration of preoperative prophylactic antibiotics in the absence of a specific diagnosis was noted.

2.3.4 The ASA classification of operated patients

The guidelines of the American Society of Anesthesiologists (ASA) physical status classification were followed for the prediction and assessment of the surgical risks.

Class 1: Healthy patient, no medical problems.

Class 2: Mild systemic disease.

Class 3: Severe systemic disease, but not incapacitating.

Class 4: Severe system disease that is a constant threat to life.

Class 5: Moribund, not expected to live 24 hours irrespective of operation.

2.4 Sample Collection and Cultivation

Samples were collected from two different models of hospitals.

A) Government hospital (DMCH).

B) Private hospital (HFRCMCH).

2.4.1 Collection of sample

Samples were collected aseptically from different sites. A screw capped test tubes were cleaned and sterilized in the autoclave at 121^0C. The samples were collected by using sterile cotton swabs that were immediately transferred into the sterile screw capped test tube. The tubes were then properly capped, labeled and transferred to the laboratory at the earliest convenience.

2.4.2 Wound swabs

From each operated patient duplicate wound swabs were collected; one for preparation of smear for microscopy and other for seeding of culture Sterile cotton tipped swab was

used for collecting the sample by using zig-zag motion to swab wound surface and rotating the swab during collection. Special attention was given to avoid contact surrounding skin. Specimen was immediately transferred to the sterile test tube. After collection, the tubes were capped properly, labeled and transferred to the microbiology laboratory promptly (Jahan *et al.*,2004).

2.4.3 Nasal swabs

Right and left sides of anterior nares were sampled by rubbing up to one and half cm of nostrils and making five rotations in each nostril by the sterile cotton wool swabs moistened with sterile normal saline (0.89% NaCL solution). All swabs were inoculated within one hour of collection on appropriate culture media and incubated at 37^0C (Ashiq,1989).

2.4.4 Air sampling

Settling plate technique was performed for tapping of microbes in a Nutrient agar medium of known surface area are for a measured period of time (Pasquarella *et al.*,2000). Blood Agar and MacConkey Agar plates were also exposed for fifteen min for each sampling. The plates ware incubated at 37^0C for 24 hours. After incubation the bacterial colony forming units were counted from plates through necked eye observation.

2.4.5 Water Sampling

Tap water was collected from the post operative room and operation theatre (OT) in a 500 ml flask aseptically and sent immediately to the microbiology lab. T water was filtered through standard membrane filter technique (APHA, 1992).

The Total Coliform Count(TCC) by the membrane filtration technique

MFC (Gidco laboratories USA) agar media was used for detection of coliform organisms. At first the sterile millipore filter (Miillipore corporation, Bedford) was placed over the plate of filtration apparatus. Then the collected samples were sucked down through filter

and a small amount of R·nger's solution was added for uniform dispersion of organisms on the membrane. After unlocking, filter was removed with sterile forceps and placed on MFC agar plate and inoculated at 37^0C for 24 hours. Pink to red color with metallic sheen colonies were considered as the total coliform (According to APHA standard, 1992 for the examination of water and waste water). The total coliform density was counted by nacked eye with the colony range and finally the number of organisms was calculated per 100 ml.

The Feacal Coliform Counts (FCC) by membrane filter technique

Filter was placed on the MFC agar media with the same procedure described in total coliform count. The Petri dish was incubated at 45^0C for 24 hrs. Blue color colonies on membrane filter with faint blue to yellowish cream background considered as feacal coliform. Then the count was done in the same manner of total coliform count.

2.4.6 Sampling of bacteria from hands

Samples were collected from both hands (10 nail folds, 8 inter digital spaces and both palms) of surgeons and OT nurses, patient's attendants with no skin problem by five finger impression technique (Rotter, 2001). The microorganisms were also collected from hand washing water to determine the total and faecal coliform count by membrane filtration technique described in collection of water sample.

2.4.7 Sampling from OT equipments, dressing materials, and bed sheets

Samples were collected monthly from bed sheets of surgical ward and OT, different OT equipments, dressing materials and others in operation theatre during operation and dressing materials during dressing, by eight times swabbing (15X15 cm area) with sterile swab stick which remoistened with saline water and immediately inoculated in relevant culture medium. Liquids samples were agited frequently and sterile swab stick was impregnated aseptically. The swab stick was rubbed on to Blood and MacConkey agar plate on the sampling site. All plates were then incubated at 37^0C for 24 hours.

2.4.8 Sampling from the surface of the floor

Samples were collected randomly from the floor of OT, surgical ward and other related toilets during operation by eight times swabbing (15x15 cm area) with sterile swab sticks which were remoistened with saline water and streaked sampling side on to Blood agar and MacConkey agar plate and incubated at 37^0C for 24 hours. The plates were incubated at 37^0C for 24 hours (Dharan *et al.*,1999).

2.5 Characterization and Identification of the Isolates

2.5.1 Study of culture characteristics

Microbial colonies grown on Nutrient agar for 24-48 hours were morphologically studied for size, shape, edge, elevation, opacity, color and luster, etc. Other selective solid media, e.g. McConkey Agar (MCA), Mannitol Salt Agar (MSA), Sabouraud Dextrose Agar (SDA), Trypticase soy Agar (TSA), etc. were used for identification of the isolates.

2.5.2 Microscopic examination

Conventional procedures were followed for the study of microscopic characteristics of the potential isolates. The size, shape, cell arrangement, spore formation, motility, etc. of the strains were observed under light microscope.

2.5.3 Study of physiological and biochemical Properties

The biochemical tests that were performed in the study were Indole Citrate Utilization (ICU), Methyl Red (MR) test, Voge's Proskauer (VP) test, Kligler's iron agar (KIA) test, Nitrate Reduction (NR) test, motility, sucrose, nitrate, etc. Description of some of the important biochemical tests given below:

Catalase Test (Colle and Miles, 1990)

One or two colonies were picked up from primary culture plates with a sterile took pick and were placed into a small test tube containing hydrogen peroxide solution. Immediate gas bubbles production indicated positive reaction *Staphylococcus aureus* was used as positive control and *Streptococcus pyogens* as negative control.

Oxidase Test (Cheesbrough,1993)

A strip of filter paper was soaked with freshly prepared oxidase reagent (1% solution of Tetramethyl-p-phenylene diamine dihydrochloride). Immediately after that, a portion from a colony from the culture was picked up with a sterile tooth pick and rubbed on socked filter paper. A positive reaction was indicated by development of a deep purple color within 5-10 seconds. A negative reaction was considered by absence of coloration or development of color later than 10 seconds. Pseudomonas was used as positive control and salmonella as negative control.

Coagulation Test (Cheesbrough,1993)

The test is used to identify *S.aureus* which produces the enzyme coagulase

Slide test method

A drop of distilled water is placed on two separate slides. A colony of the test organism was emulsifying in each of the drops to make two thick suspensions. A loopful of plasma was added to one of the suspension. Look for clumping of the organisms within 10n seconds. No plasma was added to the second suspension. Clumping within 10 seconds is positive reaction and no clumping indicates negative reaction.

Tube test method

Three small test tubes was taken and labeled as 'T' for test organism (18-24 h broth culture); 'Pos' for positive control(18-24 h *S.aureus* broth culture); and 'Neg' for negative control (Sterile broth). 0.2ml of plasma was added in each tube. 0.8 ml of the test broth was added to tube 'T' then 0.8 ml of *S.aureus* was added to the tube labelled 'Pos' and 0.8 of the sterile broth to the tube labelled 'Neg'. After mixing, incubated the three tubes at 35-37^{0}C.and examine for clotting after one hour .If no clot was occurred the tubes were examined after 3 hours. Clotting of tubes indicates positive reaction and no clotting indicates negative reaction.

Methyl red reaction (Colle and Miles, 1990)

Sterile glucose phosphate peptone water was inoculated with few colonies of the test organism from plate and incubated at 37^{0}C for 24 hours. Then 5 drops of methyl red

reagent were added in the inoculated medium. Positive test was indicated by bright red color and negative by yellow.

Voges –Proskaur test (Colle and Miles, 1990)

Glucose phosphate peptone water was inoculated with the test organism and incubated at 37^0C for 48 hours. Then 3 ml of 5% α-napthol solution in absolute alcohol was added to each tube followed by 1 ml of 40% KOH in aqueous solution. Positive reaction was indicated by development of pink color in 20 minutes, which turned to crimson-red within 30 minutes. Negative reaction was indicated by development of yellow color.

Nitrate reduction test (Colle and Miles, 1990)

A heavy inoculum of the test organism was inoculated in 0.5ml of sterile nitrate reduction test medium and incubated at 37^0C for 24 hours. Then 1 drop of sulphanilic acid reagent and 1 drop of α-napthylamine reagent was added to the test culture and shaken. A red color developed within a few minutes indicating positive reaction. No color indicates negative reaction.

Kingler's Iron Agar (KIA) test (Colle and Miles, 1990)

KIA test was performed by using KIA medium (Appendix). A sterile straight wire was touched on an isolated colony, stabbed into the soft agar butt along the centre of the tube (without touching the wall) upon the bottom and streaked gently over the surface of the slant. The tube was incubated at 37^0C. The result was recorded after 24 hrs. yellow colorization of both slant and butt indicated that the growth was a lactose fermentar. Yellow colorization of butt, pink-red color in the slant indicated the growth was a non-lactose fermenter. Black colorization along the stabbing line indicated H_2S production and cracking of the media indicated gas production.

Motility, Indole and Urease (MIU) test (Colle and Miles, 1990)

MIU test was carried out in motility, indole, and urea semisolid medium (Appendix). An isolated colony was picked up with a sterile wire and stabbed into the agar very carefully down the tube at once without touching the bottom of the tube. A paper strip impregnated

with indole reagent (Appendix) was inserted between the cotton plug and the agar surface of the tube and incubated at 37^0C for 24 hrs. Spreading turbidity of the medium along the line of inoculation indicated motility of the organisms. Pink colorization of the indole paper strip and agar was regarded as positive test for indole and urease production respectfully.

Test for motility by hanging drop preparation (Colle and Miles, 1990)

Motility of the bacterial isolates determine by examining a drop of bacterial suspension, grown in nutrient broth hanging from a cover glass, supported by a rim of vaseline on a glass slide. The slide was examined under the microscope by using the 40x objective. The condenser iris was sufficiently closed to give maximum contrast. True bacterial motility was progressive, whereby the organisms moved in different directions and changed their positions in the field. It was distinguished from (1) passive drifting of organisms in same direction in a conventional current in the fluid and (2) from Brownian movement, which was an oscillatory movement about a nearly fixed point, possessed by small bodies suspended in fluid and due to irregularities in their bombardment by molecules of water

Carbohydrate utilization test (Colle and Miles, 1990)

A number of different carbohydrates included :Monosaccharides: i) Pentose-Arabinose ii) Hexose-glucose, Mannose, and Disaccharides: i) Sucrose ii) Maltose Polyhydric alcohols i) Mannitol ii) Inositol Glycosides i) Salicin ii) Esculin were used as carbon substrates for determine their ability of utilization of the substrates.

It was assayed in 1% peptone water containing 1.25 ml of 2% (w/v) solution of bromothymol blue indicator per 100ml, to which each of the carbohydrates was added in (5% w/v). A suspected colony was grown in Mulller-Hilton broth (Appendix) for 4 hours. With a sterile pasteure pipette, 2 drops of the bacterial suspension was added to tubes containing 2 ml of carbohydrates broth supplemented with the specific substrates. The positive tests were indicated by the change of color from blue to yellow after incubation

at 37^{0}C for 24 hours. In the tube containing glucose, a durham tube was introduced for collection of gas found during the growth of the isolates.

2.5.4 Specific detection of *Candida albicans*

Candida albicans was detected in unstained wet preparation or Gram stained preparations of skin and mucosal surface collected from surgical site lesion. They were small, oval measuring 2-4 μm in diameter. It stained smears, the yeasts was attached to pseudohyphae. *Candida albicans* was identified by a simple germ tube test (Cheesbrough,1993).

The germ tube formation by *Candida albicans* was detected in serum. Colonies of the isolates from a SDA media were picked with a wire loop. The loop was placed in a test tube containing 0.3-0.5 ml of serum (human or preferably bovine or rabbit) and the material was incubated at 37^0 C for 3 hours. A drop of the serum yeast culture was transferred to a glass slide and covered with a cover glass. The preparation was observed under a microscope and detects the yeast cells that were a tube like outgrowths from the cells known as "germ tubes".

2.6 Assay of the Pattern of Antimicrobial Resistance

Antimicrobial susceptibility was determined by Disc diffusion method using Muller-Hilton agar and commercially available antimicrobial discs (Oxoid Ltd, UK). The concentration of some important antimicrobials in the discs are given in the appendix-C-3.2.

Staphylococcus spp. and *Streptococcuus* spp. were tested against Penicillin (P), Ampicillin (AP), Ciprofloxacxin (CIP), Gentamycin (CN), Vancomycin(Va), Tetracycline, Cloxacillin (OB), Cephalexin (CL), Ceftriazone (CRO). *E.coli* and *Klebsiella* and for other gram- negative bacteria Imipenem (IP) and all the above discs except Penicillin (P), Cloxacillin (OB), Erythromycin (E) were used.

Susceptibility test was performed by disc diffusion method described by Barry and co-workers (1991).

2.6.1 Antibiotics sensitivity test

One loopful of the test organism was inoculated in 5ml amount of nutrient broth and incubated at 37^0C for 4-6 hours. turbidity due to the growth of the organism in the medium was the adjusted by sterile broth to a turbidity equivalent to that of one half of MacFarland tube No. 1, which approximately corresponds to $1.5x10^8$ organisms per ml. Mueller-Hinton medium (appendix A-1.1), was used for sensitivity test. A sterile cotton swab was dipped into the bacterial suspension. Excess fluid was removed by rotating and pressing the stick firmly against the inner wall of the tube above the fluid level. The swab was then streaked on the dried surface of the Mueller-Hinton agar plate in three different planes (by rotating the plate 60^0 angle each time) to get a uniform distribution of the inoculum. Then the antibiotic discs were placed firmly to ensure complete contact with the agar and allow the antibiotics to diffuse for 30 minutes. The discs were placed 15 mm away from the edge of the Petri dish and 24 mm away from the adjacent discs. The plates were then incubated at 37^0C for 24 hours.

2.6.2 Reading of the results of sensitivity tests

Each plate was examined after the incubation period was over and the diameter of complete zone of inhibition were measured with the help of a scale placed on the under surface of the petridish. Zone of inhibition was measured in two directions at right angels to each other through the center of the disc and the averages of the two readings were recorded and expressed in mm. Inhibition zones produced by each drug was translated into 3 susceptibility categories, namely Sensitive(S), Intermediate(I) and Resistance(R) with the help of (Appendix C- 3.2)

2.6.3 Detection of methicillin resistance *Staphylococcus aureus* (MRSA) by oxacillin disc diffusion test

Methicillin resistance in Staphylococci was detected by using 1μg disc containing oxacillin and incubating at 35^0C for 24 hours. A zone of inhibition less than 10 mm or any discernible growth within zone of inhibition was indicative of methicillin resistance

Oxacillin in preferred in place of methicillin to detect MRSA for its stability and more reliable detection of Methicillin resistance *Staphylococcus aureus* (Chamber,1997).

2.6.4 Detection of extended strectum β -Lactamases by double disc synergy test

Synergy between a disc of augmentin (amoxycillin and xlavulanic acid) and 3rd generation cephalosporins was detected by double disc synergy test. The clavulanate in augmentin disc diffuses through the agar and inhibits the β –Lactamase surrounding 3rd generation cepholosporin disc (Jarlier *et al.*, 1988). Muller Hilton Agar plates were prepared and inoculated with standardized inoculum (corresponding to 0.5 McFarland tube) with sterile cotton swab. Augmentin (20μg amoxycillin and 10μg of clavulanis acid) disc was placed in the center of the plate. The third Generation cephalosporin-ceftazidime, ceftriazone, cefotaxime and aztreonam disc was placed 20-30 mm distance from augmentin disc. The plate was incubated overnight at 35^0C. ESBL production was found to be positive when the inhibition zone around the tested antibiotic disc increasd towards the augmentin disc or neither disc was inhibitory alone but tehbacterial growth was inhibited where two antibiotics were diffuse together. (Appendix C-3.1)

2.7. Serological Determination and Typing of the Methicillin Resistant *Staphylococcus aureus* (MRSA)

Staphylococcus aures were tested with MRSA latex agglutination test for their serological confirmation and coagulation typing was performed for epidemiological characterization.

2.7.1 MRSA latex agglutination test

Latex particles against sensitized with a monoclonal antibody against penicillin binding protein 2a (PBP2a) will specifically react with methicillin resistance *Staphylococci* to cause agglutination visible to the unaided eye. Extracts are prepared by boiling a suspension of *S. aureus* cells under alkaline conditions followed by neutralization and centrifugation. The supernatant is subsequently mixed with the latex reagent on a test card and visible clumping or agglutination was seen within three minutes with no change in the control indicates the presumptive presence of MRSA (Nakea *et al.*,1990).

The commercial MRSA Latex agglutination kit was used to detect the monoclonal antibody against the penicillin binding protein. Some of the important contents of the kit mentioned below:

a) Sensitized latex (Latex particles are sensitized with a monoclonal antibody prepared against PBP2a). b) Control Latex (Latex particles are sensitized with a monoclonal antibody of the same IgG subclass but against a human protein showing no reactivity with proteins of *Staphylococcus aureus*). c) Extraction reagent (1:0.1mol/L NaoH).d) Extraction reagent (2 : 0.5 mol/ L KH_2PO_4). e) Test cards f) Mixing sticks and g) Package insert were used in the MRSA Latex Agglutination test.

2.7.2 PBP2a extraction procedure

Extraction reagent 1 (200µL) was added to a centrifuge tube. Sufficient freshly grown bacterial was taken and suspended in the tube approximately $1.5x10^9$ cells/ tube. The tube was capped , placed into boiling bath and heated for 3 minutes. The Tube was removed and allowed to cool at room temperature about 30^0C. Extraction reagent 2 (50 µL) was added to the tube and mixed it well. The mixture was centrifuged at 3000rpm for 5 minutes to separate the cell from supernatant. The supernatant was used as test specimen.

2.7.3 Latex agglutination procedure

For each specimen, two circles were labeled on the test card (one as the test and other as the control). 50 µL of specimen was placed on the test and control circles. Then one drop (25 µL) of sensitized latex was added to the test circle and one drop (25 µL) of control latex was added to the control and mix thoroughly. The test card was rotated by hand for three minutes and placed in bench and resulting agglutination pattern was read by naked eye (Cavassini *et al.*, 1999). The interpretation of results was:

Strong agglutination against a clear background	**3+**
Agglutination against a slightly turbid background	**2+**
Slight agglutination against a turbid background	**1+**
Homogenous white suspension with no visible agglutination	-

2.7.4 Coagulase typing of MRSA

Coagulase typing can classify Staphylococcus spp. by its antigenic property into eight different types (Coagulase type I-VIII). Animal plasma coagulated when it is treated with coagulase, but this is inhibited if coagulase has been treated with antiserum containing homologus type antibodies. Neutralizing rabbit antisera specific to eight coagulase types (I to VIII) and normal rabbit serum (as a control) is added to supernatant obtained from an overnight culture of test isolates. After incubation rabbit plasma is added. Inhibition of coagulation after further incubation indicates the coagulase type (Jorgensen,1991) .

One colony of *S.aureus* was inoculated in Brain heart Infusion broth (37g dehydrated brain heart infusion broth media per liter distilled water) to produce coagulase and incubated at 37^0C overnight. the culture fluid was centrifuged 3000 rpm for 30 minutes and supernatant was used as the test specimen of "Coagulase antigen solution". Coagulase typing kit contained : 1.Coagulase typing immune sera (type I –type VIII).2..Normal rabit serum. 3. Dried normal rabbit plasma 4.Polypeptone (2.0% w/v) 5.Sodium citrate (1.0% w/v) 6. Sodium azide (0.1% w/v).

Nine test tubes were prepared. An amount of 50 µL of coagulase antigen solution was added into each of the tubes. Into the first tube, 50 µL of anti-type immune serum was added. Similarly 50 µL of anti-type II-VIII immune sera were put into the second to eight tubes. In the final ninth tube 50 µL of diluted rabbit sera (negative control) was added. The test tubes were stirred with mixer and then incubated at 37^0C for one hour. An amount of 100 µL of diluted normal rabbit plasma was added into all the tubes. The tubes were again stirred well with mixer at 37^0C. After one hour, judgment of coagulation was done and in undetermined case, judgment was done 2, 4, 24, or 48 hours after addition of the rabbit plasma. Test tubes were declined and clotting was observed in each test tube including the negative control (the 9^{th} tube containing normal rabbit serum) (Khan *et al.*, 2007).

2.8 Determination of the Plasmid profile of *E.coli*

Plasmid DNA was prepared according to the simplified alkaline lysis method of Kado and Liu (1981)

2.8.1 Isolation of plasmid DNA

An isolated colony of each strain was inoculated into 1.5 ml of TSB broth with 0.3% yeast extract (YE) and incubated overnight at 37°C on a water bath shaker. Cells were collected in a polypropylene micro centrifuge tube, by centrifuging the broth culture in an Eppendorf centrifuge at 14,000 rpm for 3 minutes. Supernatant was removed and the pellet was suspended in 100 μl of solution I (Appendix D) by pipetting, Then 200 μl of solution II (Appendix D) was added and was mixed gently by rapid inversion of the tube and was incubated at 55^0C for 45 minutes to 1 hr. in a water bath. After incubation, the tubes were taken out and an equal volume of solution III (30 μl) (Appendix D) was added and mixed well by slowly inverting the tubes until a milky white suspension was formed. Then the tubes were centrifuged at room temperature for 7 minutes at 12,000 rpm. It formed three layers, the upper layer was the plasmid solution, middle layer consisted of cell debris together with other pretentious fractions, and the lower layer was the phenol. The bottom layer consisted of phenol were reduced as much as possible using micropipette. Then centrifuged again for 8 minutes at 12,000 rpm. Finally by using a cut tip the plasmid solution (upper layer) was taken a new carefully in a new Expender tube. At this stage plasmid can be stored at 4^0C for about 2 weeks.

2.8.2 Separation of plasmid DNA by Agarose Gel Electrophoresis (AGE)

Plasmid DNA was separated by horizontal electrophoresis in 0.8% agarose slab gels in a Tris-burate EDTA buffer at room temperature at 100 volt (50 mA) for 3h. 30 μl of plasmid DNA solution was mixed with 3 μl of tracking dye and was loaded into the individual well of the gel. The gel was stained with 0.5 μg/ml of ethidium bromide for 30 min at room temperature. DNA bands were visualized and photograph was taken using Gel Documentation with UV Tran illuminator. The molecular weight of the; unknown plasmid DNA was determined on the basis of its mobility through agarose gel and was compared with the mobility of the known molecular weight plasmid present in strains E. *coli* PDK- 9 (140, 105, 2.7 and 2.1 MDa).

2.9 Polymerase chain reaction (PCR)

A single colony from MacConkey agar plate was inoculated to 3ml of LB broth. Incubated at 37°c with agitation (120 rpm).Then 1.5 ml of sample was taken in an Eppendorf tube. The sample was centrifuged at 13000 rpm for 10 min .The supernatant was discarded. The pellet was dissolved in the sterile normal saline. The sample was centrifuged at 13000 rpm for 10 min. The supernatant was discarded. The pellet was dissolved in 200µl of normal saline. The sample was boiled for 10 min. The sample was cooled in ice for 30 min. The sample was centrifuged at 13000 rpm for 10 min. The supernatant was transferred to a fresh Eppendorf tube and store at -20°c for use template DNA in PCR. 25µl of reaction mixer containing 2.5 µl of PCR buffer(10X) 1.5 µl of 50Mm $MgCl_2$) 2.0 µl Mm dNTPs, 1 µl of primer (forward and reverse) together with 0.1 unit of Taq DNA polymerase was mix gently by pippeting. Then 22 µl of reaction was taken in each PCR tube and then also took 3 µl of DNA templates from sample .Spin for few seconds and added one drop of PCR oil in each tube. PCR assays were performed in a DNA thermal cycle(model 480, Perkin-elmer Cetus, Emerville, USA). Each PCR tubes used the same basic setup: denaturation at 94^0C for 1min, annealing at 60^0C for 1min 15 seconds & Extension at 72^0C for1min 45 seconds. After putting all the samples in the machine, the temperature was increased at 94^0C. Finally aliquots of PCR products were analyzed by agarose (1.8%, wt/vol) gel electrophoresis in 0.5X Tris-borate-EDTA buffer, stained in ethidium bromide, and visualized with a Fluoro-S MultiImager (Bio-Rad, Inc.).

2.9.1 Primer used in the study

Target gene	Sequence (5'-3')	Amp (size)	Reference
intl1	in F: GGC ATC CAA GCA GCA AGC in B: AAG CAG ACT TGA CCT GAT	600bp	Dalsgaard *et al.*, 2000

2.9.2 Purification of PCR product

The PCR product was cut from the gel and purified by using commercially available Microcon Centrifugal Filter device, USA according to the following way:

The desired band was cut from the gel and purified by the Freeze 'N Squeeze DNA Gel Extraction Spin Columns. Then partial purified sample reservoir was inserted into screw cap vial. The entire solution (PCR product) was pipetted into sample reservoir (0.5 ml maximum volume), without touching the membrane with the pipette tip. The attached cap of screw cap vial was then sealed. The assembly (screw cap vial with sample reservoir) was placed in a centrifuge machine. When placing the assembled device into the centrifuge rotor, the cap strap was aligned toward the centre of the rotor. The assembly was spin at 5X1000 rcf (rotation centrifugal force) for 12.5min. The assembly was then removed from the machine and the vial is separated from the sample reservoir. The sample reservoir was placed upside down in a new vial, and then spins at 10X1000 rcf for 3.5 min to transfer the concentrate to vial. The sample reservoir was then separated from vial and discarded. Tris EDTA (TE) buffer (7µl) was added to vial containing the purified PCR product. Concentration of PCR product was measured and diluted to 10ng/µl with PCR water.

2.9.3 Cycle sequencing

The primer was diluted to 3.2p moles using sterilized water. The reagents added to a separate tube for each reaction are : a) 4µl of Terminator ready reaction mix. b) 1 µl of Template, 10 ng/µl c) 1 µl of Primer, 3.2pmoles d) 14 µl Distilled water – total 20 µl.

The contents of the tubes were mixed well and spin briefly. The tubes were placed in the thermal cycler (Applied Biosystem 9700, USA) and the following cycle sequencing program employed is summarized in the Table 2.1. The sample was then stored at 4^0C and hold at this temperature until ready to purify.

Table 2.1 Cycle Sequencing Program.

Step	Temp(OC)	Time	No. of cycle
Initial denaturation	96	5 min	1
Denaturation	96	10 sec	25
Annealing	50	5 sec	25
Extention	60	4 min	25
Final Extention	60	1 min	1

2.9.4 Purification of cycle product

Two micro liter of 3M sodium acetate (pH 4.6) and 50 µl of 95% ethanol were mixed in a 1.5 ml micro centrifuge tube. The entire contents of each reaction was pipetted into a tube of sodium acetate and ethanol mixture and mixed thoroughly. The tubes were then incubated at room temperature at least 18 min to precipitate the extension products. The tubes were then incubated at 13,000 rpm for 20 min. The supernatant was discarded carefully using a micropipette. The pellet was washed with 250 µl of 70% ethanol by washing the walls of the tubes. The tubes were spin at 14,000 rpm for 10 min. The supernatant was discarded very carefully using a micropipette and tips were used to remove the supernatant from the bottom of the tubes.

2.9.5 Detection of the nucleotides by ABI- Prism 310

The purified cycle sequence products were analyzed by electropnoresis in the genetic analyzer (Prism 310, USA). DNA is separated and detected by the laser beam. When the nucleotides reach a detector window in capillary electrophoresis, the fluorescent labeled fragments were excited by the laser beam of the machine. The laser excites the fluorescent dye labels and emitted fluorescence was collected by CCD camera. The fluorescence intensity data is interpreted into sequence data by specific bioinformatics software. The sequences were supplied as chromatographic file in floppy discs.

2.9.6 Sequence analysis

The sequence were then edited by bioinformatics software Chromas and subjected to BLAST search. After editing, the sequences were aligned and analyzed using bioinformatics software Bioedit, DNAstar, Clastal X and Genedoc.

2.10 Molecular Typing of *E.coli* by Pulsed Field Gel Electrophoresis (PFGE)

Pulsed field Gel Electrophoresis (PFGE) was performed to determine the clonal relationship of different strains of *E. coli* collected from clinical and environmental samples.

2.10.1 Preparation of PFGE agarose plugs from cell suspensions

Cell suspensions were made in 2 ml Cell Suspension Buffer from 14-16 h Gelatinase agar (GA) plates. The concentration of the cell suspensions were adjusted with the Dade Micro scan Turbidity Meter as described in the following steps:

The digital output on the instrument was tested by inserting CSB "blank" tube in the left position (position 1) of the turbidity meter and one of the other tubes of CSB in sample position (right) (position 2) of the turbidity meter. The reading should be 0.00±0.01. A cotton swab was used to remove growth from the agar plates into the appropriate Falcon tube that contains 2 ml of CSB. The cells were suspended by rubbing against the wall the tube gently so that the cells are evenly dispensed and formation of aerosol is minimized. The tubes were inserted into the sample position to check the reading (desired range is 0.48-0.52). If the reading was greater than 0.60, additional CSB was mixed and the reading was checked again until it is within the desired range. The cell suspensions were kept in ice-bath. 98 µl of each cell suspensions were transferred to labeled micro centrifuge tubes using 100 µl pipette and tip. The micro centrifuge tubes were placed in floating rack and incubated at 37°C water bath for 5 minutes. The cell suspensions were removed in 1.5 ml tubes from water bath and 4 µl Proteinase K was added to each 98 µl cell suspension and mixed by closing tubes and tapping side of tubes. 98 µl melted 1% Seakem Gold agarose which was kept in 56°C water bath was added to one of the 98 µl

cell suspensions and mixed gently by pipetting. Immediately part of mixture was dispensed into previously labeled appropriate wells in plug molds. The plugs were allowed to solidify for 10-15 min at room temperature.

2.10.2 Lysis of cells in agarose plugs

25µl Proteinase K Stock solution (20mg/ml) was added per 5 ml of cell lysis buffer (50mM Tris: 50mM EDTA, pH 8.0 + 1% Sarcosine) just before use. 5 ml of cell lysis buffer was dispensed to each of the 50 ml tubes. The plugs were disposed in the cell lysis buffer. The tubes were placed in a rack in 54°C shaking water bath and incubated for 1 hour with constant agitation.

2.10.3 Washing of agarose plugs after cell lysis

Tubes were removed from shaking water bath and the lysis buffer was poured off. 15 ml pre-warmed (50°C) sterile Type 1 water was added to each tube and returned to shaking water (50°C). Tubes were shaken for 15 minutes. Water was poured off and these wash steps were repeated. After that, the water was poured off and 15 ml pre-warmed (50°C) sterile TE was added and the tubes were shaken in shaking water bath (50°C) for 15 minutes. This steps were repeated for four times and TE was poured off each time and finally 1 ml TE was added and the plugs were stored at 4°C for subsequent step(s) in 1.5 ml tubes.

2.10.4 Restriction digestion of DNA in agarose plugs with *Xba*I

Plugs were removed from tubes containing TE with wide end of spatula and were placed in a sterile disposable petri dish and were cut at a 2 mm wide slice from test samples and transferred to the labeled 1.5 ml micro centrifuge tubes containing 200 µl diluted H buffer (1:10 dilution). The rest of plugs were replaced in original tubes that contained 1 ml TE Buffer. Two 2 mm wide slices of *Salmonella* ser. *Braenderup* standard plugs were cut and transferred to tubes of diluted H buffer. The tubes were incubated in room temperature for 10-15 min. After incubation of plug slices H buffer were removed. Then each of the plugs was immersed in 200 µl of reaction mixer containing 20 µl of *Xba* I restriction buffer, 2 µl *Xba* I restriction enzyme, 2 µl of BSA (20U/ml) along with 176 µl

filtered deionized water. Then the samples and the control tubes were incubated at 37°C water bath for at least 18 hrs.

2.10.5 Casting agarose gel and loading restriction plug slices on the Comb

1 g of Seakem Gold (SKG) Agarose (Bio-Rad) was added to 100 ml of 0.5X TBE buffer in a 500 ml Erlenmeyer flask. The slurry was heated in the microwave oven until the agarose was dissolved completely. The temperature of the slurry was equilibrated to 54-58^0 C in a water bath. The casting apparatus of the PFGE was assembled according to the instruction manual (Bio-Rad). The comb was put on bench top and the plugs were loaded on the bottom of the comb teeth; The *Salmonella* ser. *Braenderup* standard plug slices were put on teeth, 1, and 15 and the samples were loaded on the remaining teeth. Using a Pasteur pipette, the edges of the casting platform were sealed with a small quantity of the agarose solution and allowed to set. Then the remainder of the warm agarose solution was poured into the casting stand for a thickness of approximately 5-6 mm. The gel was allowed to solidify for 30 minutes at room temperature.

2.10.6 Electrophoresis

PFGE was performed with the Contour Clamped Homogenous Electric Field (CHEF-DRII) apparatus from the Bio-Rad laboratories (Richmond, CA, USA). The electrophoresis chamber was filled with approximately 2.5 liters of running buffer (0.5X TBE). Preparations of the electrophoresis apparatus were performed according to the instruction manual (Bio-Rad). The gel and the platform assembly were placed into the frame. It was insured that the gel was covered by about 2 mm of buffer. The temperature of the running buffer was adjusted to 14^{0}C and the flow rate of the buffer through the electrophoresis cell was maintained approximately at 0.75 liter per minute. Electrophoresis was done at 6 volts for 18 h (initial switch time 2.2 s; final switch time 63.8 s). After the end of run, the gel was stained with ethidium bromide (0.5 μg/ml) solution for 30 minutes at room temperature and then de-stained in sufficient distilled water for 1 h. The gel was visualized on the UV transilluminator and photographs were taken as described previously. The DNA size standards used was the *Salmonella* serotype *Braenderup* (H9812) ranging from 20.5 to 1135 kb.

2.11 Procedure Used for Control of Post Operative Nosocomial Infection

Disinfectants used in the study

A. Disinfectant used for soiled floor, trolleys, tables, bedside lockers, beds:

Routine cleaning is done by using ordinary detergents (mostly quaternary compounds)

Disinfection is done when spillage of body fluid occurs. Hypochlorite [bleaching liquid (chlorox) 1/10 dilution in water] is used.

B. Disinfectant used for semi critical equipments like endotracheal tubes, endoscopes, laryngoscopes etc: Gluteraldehyde (Cidex) is used.

C. Unsoiled trolley, ECG machine, Echo machine, stethoscope, thermometer: 70% ethyl alcohol.

Hand washing materials were used

Soap, antimicrobial soap and providone iodine (provicep) and alcohol based hand rub.

Sterilizes used in the study

All surgical metalic and febric items: Autoclave

All platic irtems: Ethylene oxide (EO)

2.12. Flow Diagram of the Methods of the Study to Detect the Post Operative Nosocomial Infection

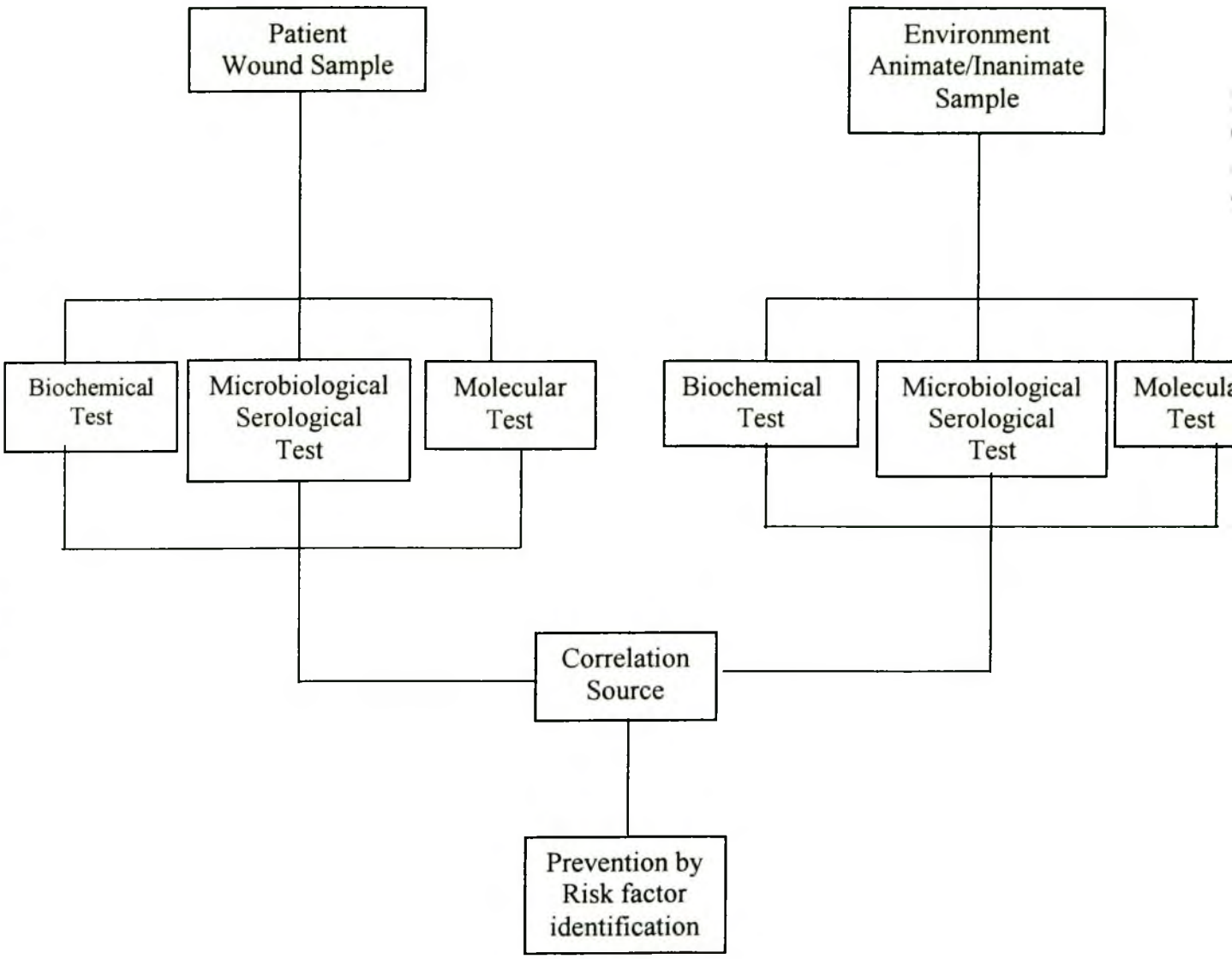


Figure 2.1 Flow Diagram showing the methods used to detect the post operative nosocomial infection.

CHAPTER 3

CHAPTER 3

NATURE OF THE NOSOCOMIAL INFECTION IN POST OPERATIVE CARE UNIT

The nature of nosocomial infections after surgery depends on many factors. These factors are categorized into various important wound assessment scales. In addition, the patient's preoperative, operative and post-operative history was also considered to assess the nature of the wound infections. A comparative study of the nature of infection in post operative room of government and private hospitals was evaluated from January 2008 to July 2009. Dhaka Medical College Hospital (DMCH) as a representative of government or public hospital and Holy Family Red Crescent Medical College Hospital (HFRCMCH) as a private hospital were selected for the present study.

3.1. History of the Patient's Infection

The patients' demographic characteristics were included to determine the effect of age and sex on the wound infection after surgery. A total of 96 infected patients characteristics were examined; out of which 48 infected patients were taken from DMCH and the rest of 48 infected patients were included from HFRCMCH. In public managed hospital (DMCH), 63% patients were male and 37% patients were female and in private hospital (HFRCMCH) 56% were male and 34% were female. In both hospitals, the most vulnerable age were for male ranging from 30-75 years and for female was about 30 years. The rate of infection in the different age groups is shown in Table 3.1.

Table3.1 Rate of infection among the different ages of patients in private and govt. hospitals.

Age	Male (%)		Female (%)	
	private	public	private	public
≤30	04 (15)	03 (10)	12 (57)	08 (44)
30-65	14 (52)	16 (53)	07 (33)	05 (28)
66-75	07 (26)	10 (33)	02 (10)	03 (17)
>75	02 (07)	01 (04)	0 (00)	02 (11)
Total	27(100%)	30 (100)	21 (100%)	18 (100)

From the clinical point of view, almost all infected patients were complained off fever and tenderness. The complication of discharge and bleeding from the wound site were higher in the public managed hospital (DMCH) than the private managed hospital (HFRCMCH). The sign and symptoms of the infected patients are shown in Figure 3.1.

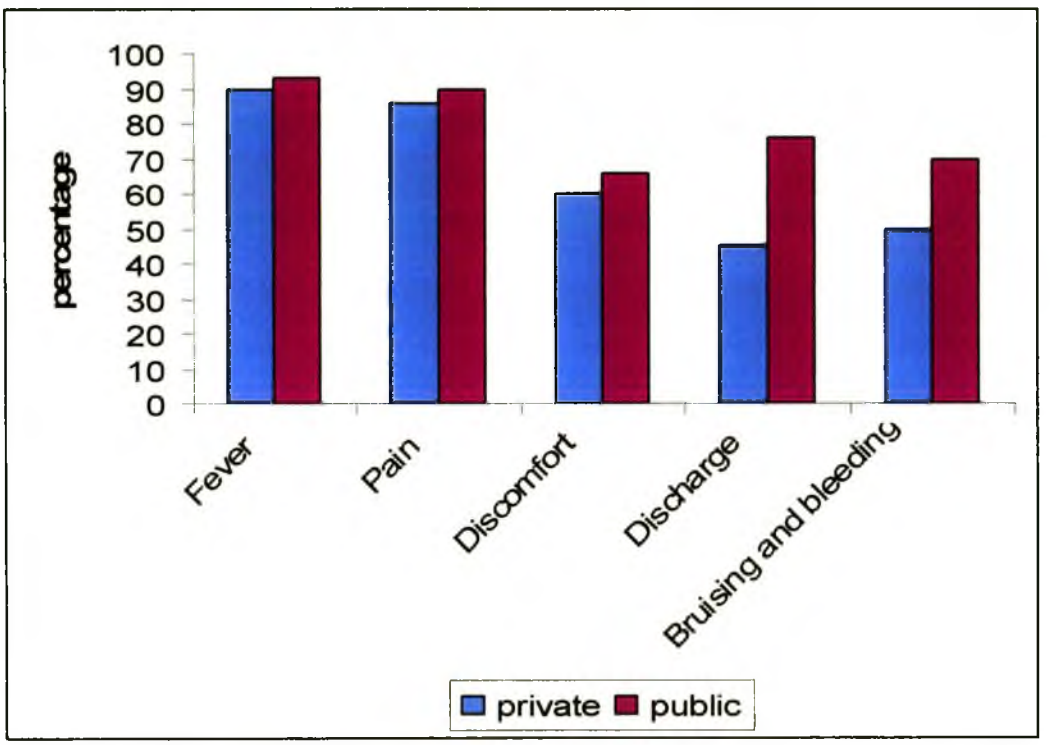


Figure 3.1 Sign and symptoms of the infected patients in private and public hospital.

The medical histories of 96 patients were recorded according to their associated diseases and habits which can influence the post operative surgical site infections. The history of diabetes, smoking, sepsis, steroid use, obesity and weight loss were asked to all 96 patients in both public (DMCH) and private (HFRCMCH) managed hospital. In public managed hospital, the history of the habit of smoking (75%) was significantly higher than that of private managed hospital (45%). More than 80% patients in the private hospital were suffering from diabetes and about 70% patients were obsessed. All these medical histories of the infected patients are presented in Figure 3.2.

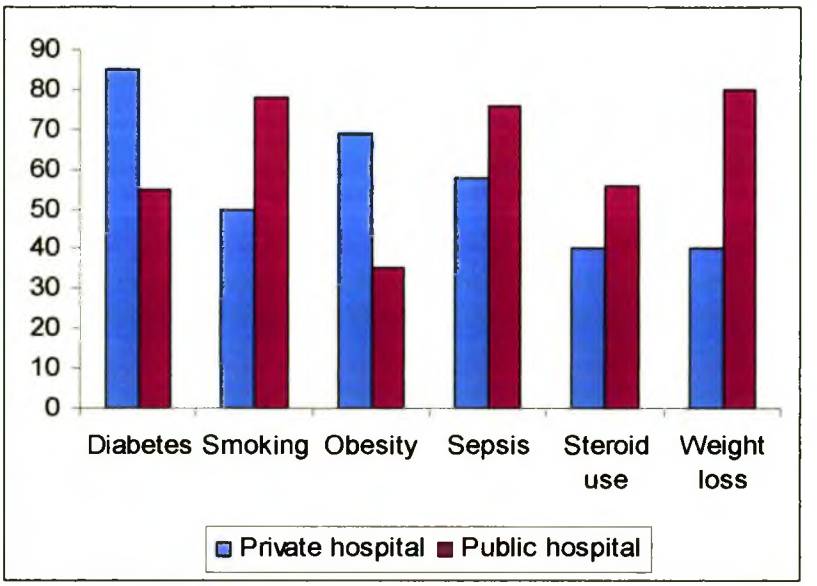


Figure 3.2 Medical history of the infected patients in private and public hospital.

Before surgery, the pre operative histories of the all the infected 96 patients were classified according to the American Society of Anesthesiologist (ASA). In both public and private managed hospital, the most of the surgical patients were in ASA class II. The prevalence of healthy patents before surgery in HFRCMCH (31%) was higher than that of DMCH (10%).The ASA score of the infected 96 patients are given in Table 3.2.

Table 3.2 Pre operative history of the infected patients in private and public hospital.

Class	Pre operative history of the infected 96 patients	No of patients	
		private	public
I	Healthy patient, no medical problems	15 (31)	05 (10)
II	Mild systemic disease	17 (36)	20 (42)
III	Severe systemic disease, but not incapacitating	10 (21)	15 (31)
IV	Severe systemic disease that is a constant threat to life	05 (10)	05 (10)
V	Moribund, not expected to live 24 hours	01 (02)	03 (07)

Patient's status during operation was recorded. Most of the patients (58%) in public hospital underwent urgent surgery and in private hospital the surgical patients were operated electively (73%) mostly. More than 50% of the operated patients in HFRCMCH need to stay for a week; whereas in DMCH most of the surgical patients were discharged in 2nd week. The operative histories of the all infected patient are shown in Table 3.3.

Table 3.3 Operative history of the infected patients in private and public hospital.

Operative history	Category	Number of patients (%)	
		public	private
Type of procedure	Elective	20 (42)	35 (73)
	Urgent	28 (58)	13 (27)
Type of anesthesia	Regional	37 (77)	30 (63)
	General	11 (23)	28 (37)
Duration of operation	≤ 60 min	18 (38)	15 (31)
	61-120	14 (29)	20 (42)
	121-180	10 (21)	06 (13)
	> 180	06 (12)	07 (14)
Length of hospital stay	≤7 days	11 (23)	30 (63)
	7-14 days	25 (52)	13 (27)
	> 14 days	12 (25)	05 (10)

3.2. Characterization of the Wound Infection

All the 96 infected patients were categorized by CDC guideline for prevention of surgical site infection (CDC,1999) as clean, clean-contaminated, contaminated and dirty wound. Clean wound presented with no inflammation with closed drainage following non penetrating trauma (Figure 3.3.a) found in minor operations. Unusual contamination in alimentary, genital and urinary tract operations with major break in technique were presented in clean-contaminated wound (Figure 3.3.b). Contaminated wounds presented with major breaks in sterile technique with non purulent inflammation (Figure 3.3.c). Dirty wound was found in major operations with pus, perforated viscous and fecal contaminations (Figure 3.3.d).

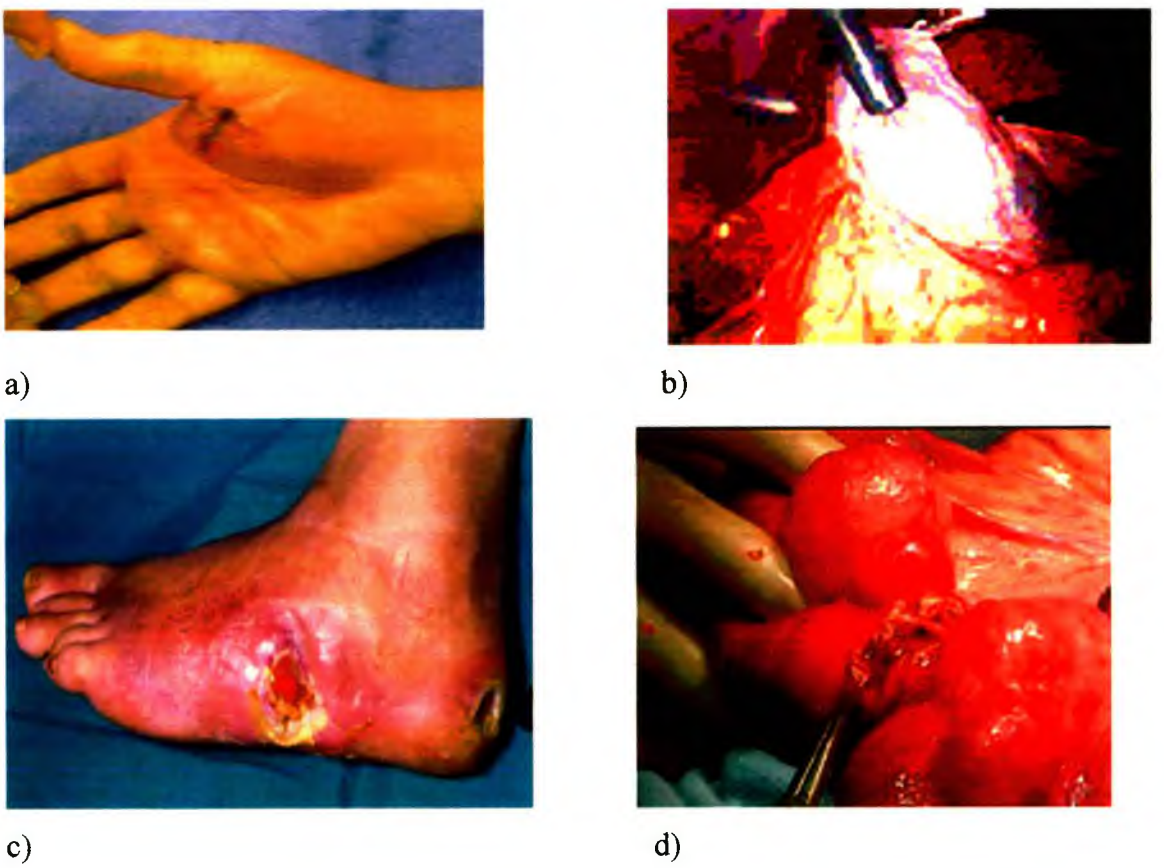
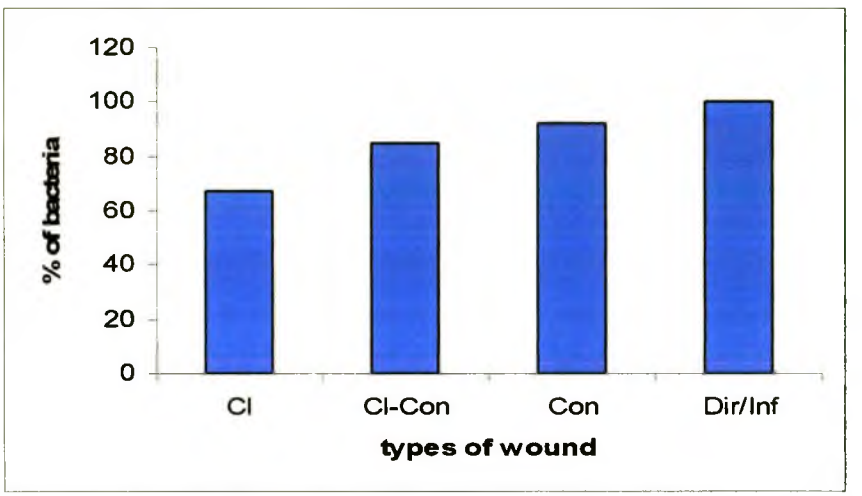


Figure 3.3 Photography of different types of wound infection after surgical operation.
a) Clean wound b) Clean-contaminated wound c) Contaminated wound d) Dirty wound.

Dirty or infected wounds contained abundant of bacteria (100%) followed by contaminated (90%), clean contaminated (85%) and clean wound (65%). The comparative bacterial contaminations in different types of wounds are shown in the Figure 3.4.



Note: Cl=Clean. Cl-Con=Clean- contaminated. Con=contaminated Dir/inf=Dirty/infected

Fig 3.4 Percentage of isolation of bacteria from different types of wound.

In Table 3.4, prevalance of different types of wound in some selected operations are presented. Throughout the study period, it was observed that, minor operations like cut injury or laparoscopic surgery presented with clean type of wounds. Appendicectomy and intestinal obstruction repair was very common operative procedures contained mostly clean- contaminated wound. The contaminated wound containing mixed types of bacteria was found in abscess drainage. The worst type of wound infection is dirty wound which was observed in this study in gangrene amputation. The wound types in different operations were characteristically similar both in Private managed hospital (HFRCMCH) and public managed hospital (DMCH).

Table 3.4 The relationship between different types of operations and wound infections.

Name of the operation	Type of wound	No of samples(n)
Laparotomy	Clean	23
Intestinal obstruction	Clean-contaminated	5
Herniotomy	Clean	7
Abscess	Contaminated	35
Gangrene	Contaminated / Dirty	10
Cyst	Clean	21
Cut injury of hand	Clean	2
Appendicitis	Clean-contaminated	13
Fibrosarcoma	Clean	5

3.3. Features of an Incisional Surgical Site Infection

After evaluation of the total 97 infected patients in DMCH and HFRCMCH it was revealed that infection after an operation in an incision was a major indicator for post operative surgical site infection. There were five cases of superficial incisional surgical site infection; three cases of deep incisional surgical site infection and only one organ/space incisional surgical site infection were detected. Out of these 9 incisional surgical site infections, seven of them were collected from DMCH post operative surgical unit and rest of the two was investigated in HFRCMCH. In case of five superficial surgical site infections; three were collected from abscess, two from circumcision site and one was from burn wound. These wounds contained purulent discharge and localized redness, swelling and heat developed nearly 4 weeks after operation. Deep incisional surgical site infections (3 cases) were developed when superficial incisional infection reached the deep soft tissues and one organ/space wound infection was found in a part of anatomy organ or space other then incision which was opened or manipulated during an operation.

A typical incisional wound after a surgery was shown in Figure 3.5. It was revealed that there was apposition of skin conneting the suture containing purulent discharge.

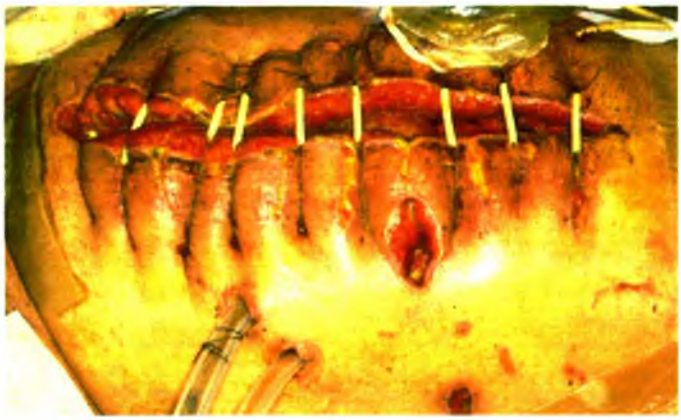


Figure 3.5 Photograph of an incisional wound of a surgical site infection (SSI)

3.4 Healing of the Wounds

All the infected wound cases were assessed according to their healing process. Diagrammatic presentation of healing process of a specific wound is given in Figure 3.6. In all 96 cases of wound healing cases, inflammation is the key for healing. Both active and passive inflammations were seen; which were characterized by redness, heat, tenderness around and in the centre of infection site. At the end, scar tissue was formed. Different wounds took different times to heal. The time distribution of all 96 wounds are presented in Table 3.5 and it was reveled that most of the wounds (60%) took a week to heal in both private (HFRCMCH) and public hospital (DMCH). The healing processes of the wounds were minutely followed and it was observed that only 6% wound took a month to heal.

Table 3.5 The healing process of 96 wounds in days.

Days	Characters	Number of wounds (n=96)
Day 1	Primary intension Secondary intension	03(03)
Days 2-3	Primary intension Secondary intension	12 (12)
Days 4-5	Primary intension Secondary intension	18 (19)
Days 7	Primary intension Secondary intension	57(60)
Days 30	Primary intension Secondary intension	06 (06)

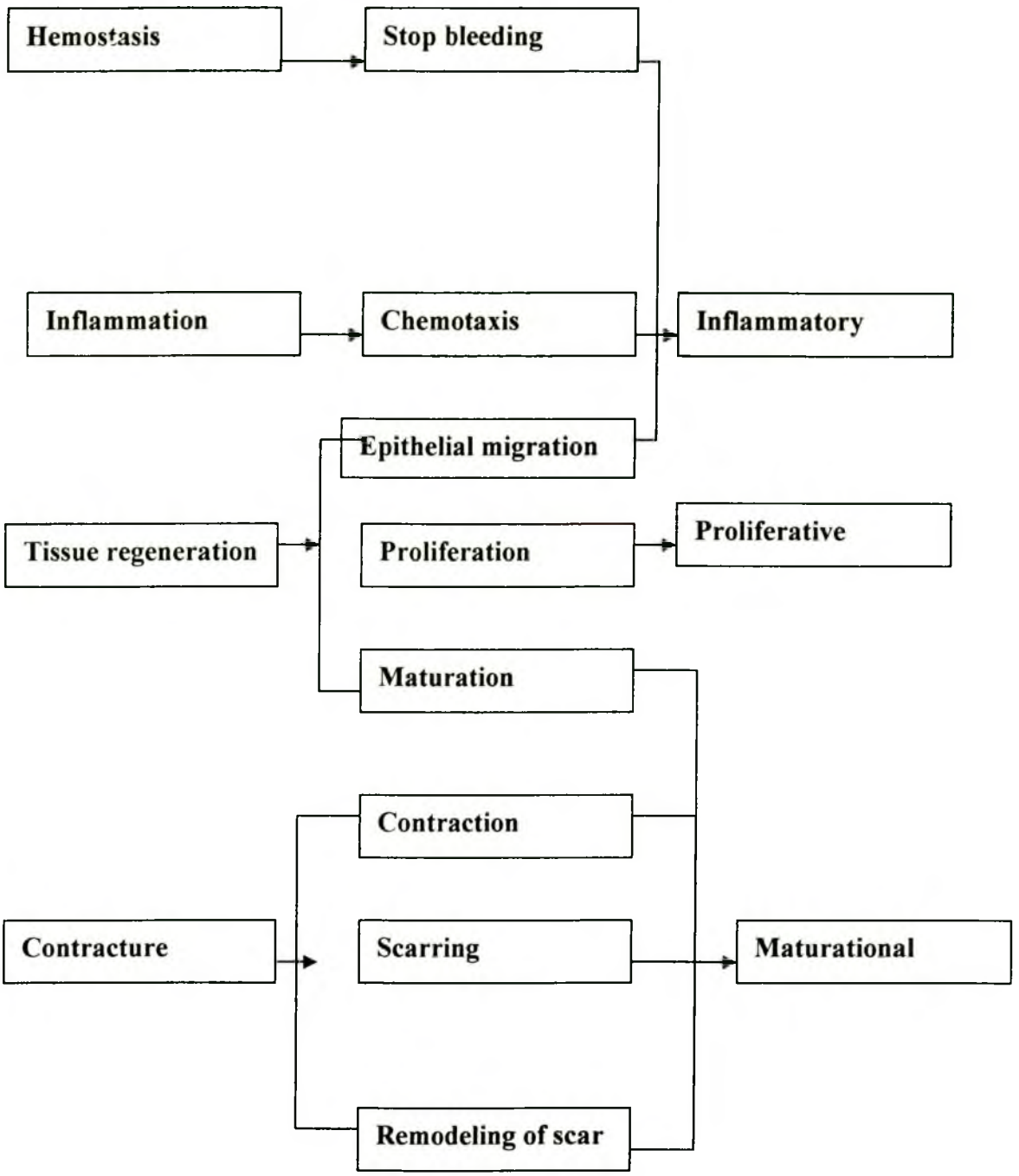
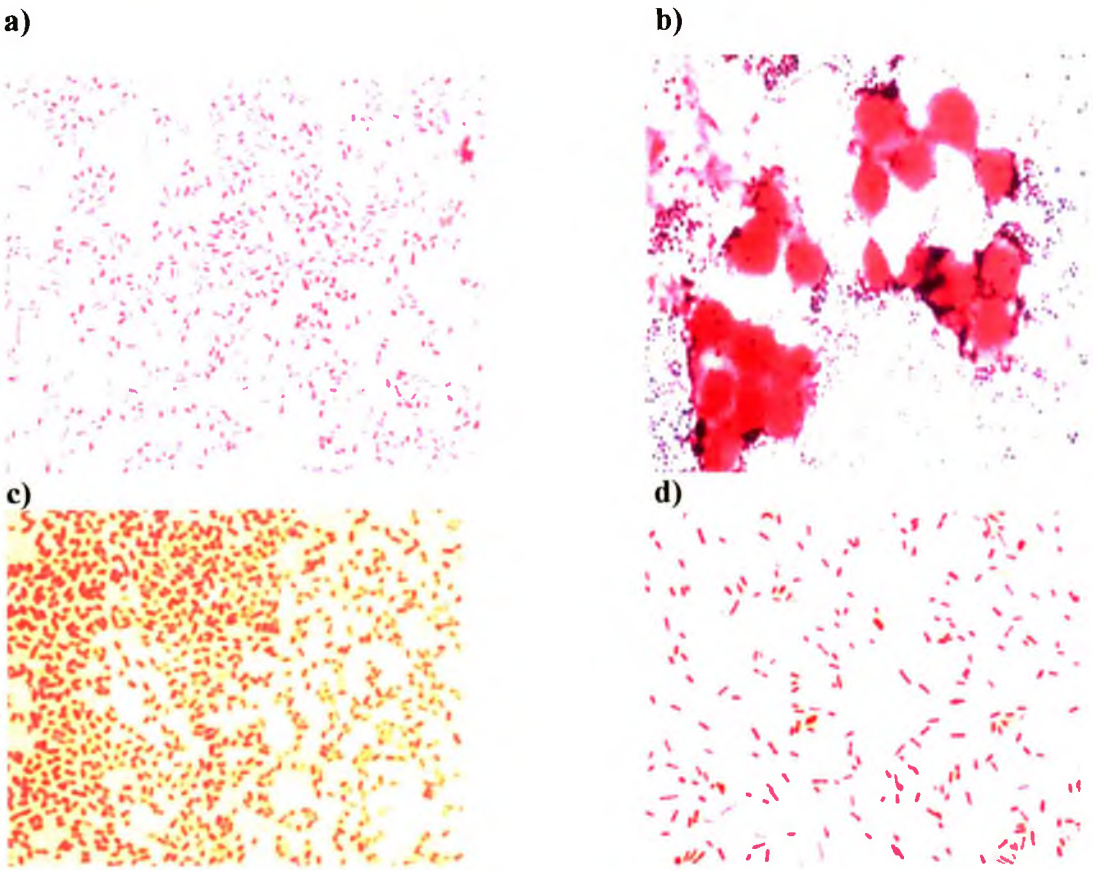


Fig 3.6 Diagrammatic features of a wound healing process.

3.5 Isolation and Identification of Microorganisms in Wound Infection

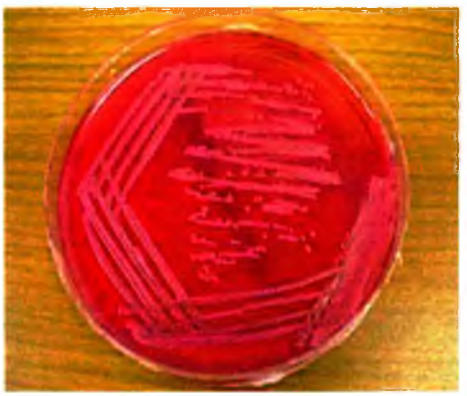
Samples collected from different wounds infections were investigated for determining the specific microorganisms involved in the infections after surgical operations. Each of these organisms was grown in different media for identification, based mainly on culture characteristics, microscopic studies and physiological and biochemical analyses, according to the Society of American Bacteriologists and Bergey's Manual of Systemic Bacteriology. A total of 48 samples, collected from different types of wounds were selected for isolation. The causative organisms, isolated from the samples, were characterized and identified as the member of genera of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* and *Proteus*. The photomicrographs of some important microorganisms involved in the wound infections are presented in Figure 3.7.



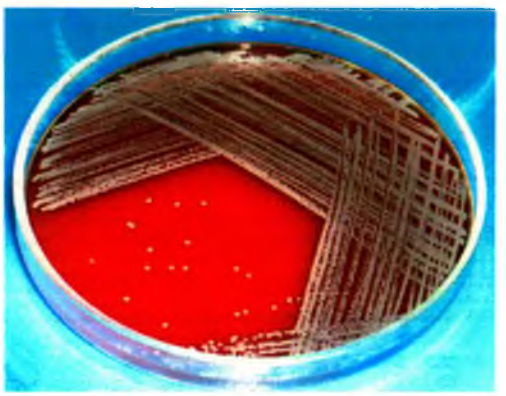
Photomicrograph scale :—= 5µm Microscopy: X40
a) *Escherichia coli* b) *Staphylococcus aureus* c)*Pseudomonas* d)Proteus

Figure 3.7 Photomicrographs of bacterial isolates involved in post operative infections.

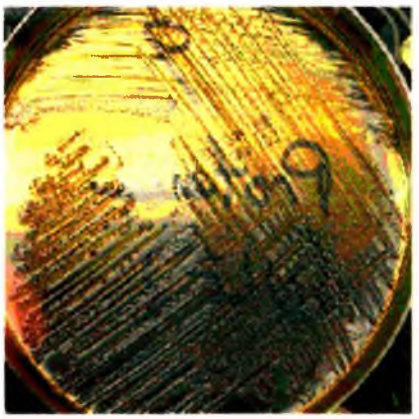
The results of the culture characteristics of the isolates are shown in Figure 3.8.. The selective media used for *Escherichia coli* was MacConkey Agar, which produced pink colonies by fermenting lactose (Figure 3.8.a). *S .aureus* produced white creamy colonies in blood agar media (Figure3.8.b) and *Proteus* produced swarming growth in MaCconkey agar medium after overnight incubation (Figure 3.8.c). The Pseudomonas showed large, round, mucoid, central smooth margin, flat, shiny and whitish colonies in MaCconkey Agar media (Figure 3.8.d).



a) *E. coli* on MacConkey Agar



b) *S. aureus* on Blood Agar



c) *Proteus* on MaCconkey agar

d) *Pseudomonas* on Nutrient agar

Figure 3.8 The photograph of colonial characteristics of the three most important microorganisms of post operative nosocomial infection on different culture media.

For identification a series of biochemical tests specific for *E. coli*, *S. aureus*, *Pseudomonas*, *Proteus* were carried out after initial identification by culture and microscopy. Isolates giving the following pattern of biochemical reactions were regarded as *E. coli, S. aureus*, *Pseudomonas and Proteus* (Table 3.6).

Table 3.6 Physiological and biochemical characteristics of the isolated organisms.

Organisms	Lactose*	Dextrose*	Sucrose*	H_2S Production	NO_3 Reaction	Indole Production	MR Reaction	VP Reaction	CitrateUse	Urease Activity	Catalase Activity	Oxidase Activity	Gelatin Liquefication	Starch Hydrolysis	lipid Hydrolysis
E.coli	ag	ag	a±	–	+	+	+	–	–	–	+	–	–	–	–
S.aureus	a	a	a	–	+	–	+	±	–	–	+	–	+	–	+
Pseudo monas	-	-	-	-	+	-	-	-	+	-	+	+	+	-	+
Proteus	-	ag	ag	+	+	+	+	-	±	+	+	-	+	-	-

*= Fermentation; ag= Acid and Gas; ± = Variable reaction

Twenty strains, S-1 to S-20 showed the characteristics to *Staphylococcus. Stapylococcus* were gram positive cocci characteristically produced white colonies in blood agar media (Figure 3.8b) and in biochemical reactions coagulase, mannitol, DNase positive (Table 3.6); suggestive of species *Staphylococcus aureus.* Another twenty strains E-1 to E-20, as an aerobe and facultative anaerobe cultured in MaCconkey agar media producing pink colonies (Figure 3.8a) with indole positive and lysine decarboxylase positive characterized as *E.coli.*

Three strains Ps-1 Ps-2 and Ps-3 produced pigments in blood agar media and in KIA medium produced pink red slope and butt and H_2S was produced and oxidase positive suggestive of *Pseudomonas spp.* Two strains P-1 and P-2 produced swarming growth with fishy odor in MaCconkey agar media and non lactose fomenter known as *Proteus.* All the bacterial isolates were characterized according to Bergey's Manual of Systemic Bacteriology (Volume 2; 1984). The classes of identified bacteria are shown in Table 3.7.

Table 3.7 Classification of identified bacteria.

Isolates	Probable genus	Family	Name given
S-1 to S-20	*Staphylococcus*	*Staphylococcaceae*	*Staphylococcus S-1 to S-20*
E-1 to E-20	*Escherichia*	*Enterobactericeae*	*Escherichia* E-1 to E-20
Ps-1, Ps-2 ,Ps-3	*Pseudomonas*	*Pseudomonadaceae*	*Pseudomonas* Ps-1,Ps-2 ,Ps-3
P-1 and P-2	*Proteus*	*Enterobactericeae*	*Proteus* P-1 and P-2

3.6: Prevalence of Different Pathogens in Wound Infection

The 48 samples ware collected from different types of post operative wounds in public managed hospital (DMCH) and private managed hospital (HFRCMCH) were investigated *E. coli* (48%) was the predominant organisms in HFRCMCH and *S. aureus* (31.3%) was the major pathogens for post operative wound infection in DMCH. The prevalence of *Pseudomonas* in DMCH was 20% and in HFRCMCH was 16%. The presence of *Proteus, Klebsiella, Streptococcus pyogens* were almost similar in both places. The prevalence of the pathogens obtained from wound infections are shown in Figure 3.9.

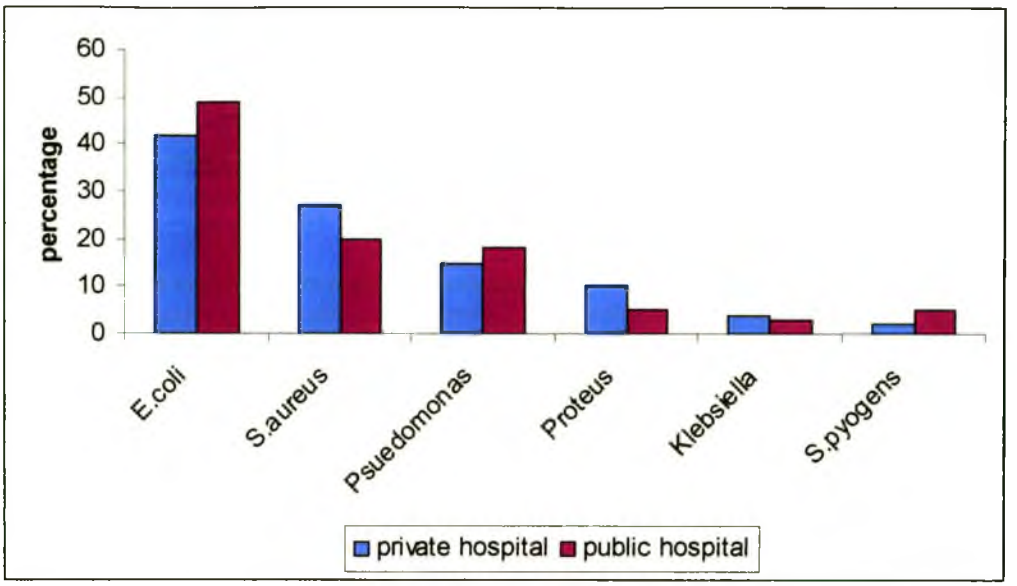


Figure 3.9 Prevalence of pathogens in various wound infection in public and private hospital.

The methicillin resistance *Staphylococcus aureus* was determined by oxacillin disc diffusion test. The average presence of MRSA in wound infection of public and private hospitals were almost similar. It was observed that the prevalence of Methicillin resistance *Staphylococcus aureus* was 76% and Methicillin sensitivity *Staphylococcus aureus* was 24%. Figure 3.10 demonstrates the percentage of prevalence of MRSA.

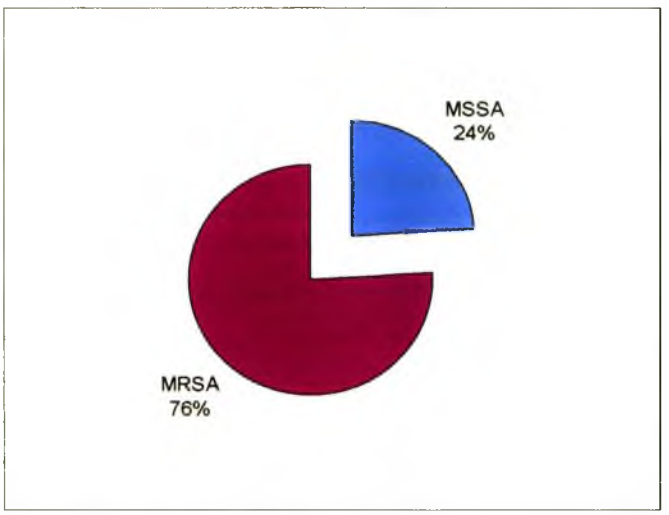


Figure 3.10 Percentage of distribution of MRSA and MSSA in S. aureus isolated from wounds of patients.

3.7 Discussion

Host susceptibility to infection can be estimated according to older age, severity of disease, physical-status classification, prolonged preoperative hospitalization, obesity, malnutrition, immunosuppressive therapy, smoking, preoperative colonization with *S. aureus*, and coexistent infection at a remote body site.

For assessing the patient's present status the clinical history is very important. In the study it was revealed that fever, pain, discharge, bleeding, bruising are the common phenomenon experienced by almost all patients. Significant differences in infection rates have been shown in patients with different illnesses. In one prospective study, the severity of underlying disease (rated as fatal, ultimately fatal, or nonfatal) was shown to have predictive value for endemic nosocomial infections: the nosocomial infection rate in patients with fatal diseases was 23.6%, compared with 2.1% in patients with nonfatal diseases (Giacometti *et al.*, 2000).

In private hospital most of the patients were suffering from diabetes. Recent preliminary findings from a study of patients who underwent coronary artery bypass graft showed a significant relationship between increasing levels of HgA1c and Surgical infection rates. Also, increased glucose levels (>200 mg/dL) in the immediate postoperative period (48 hours) were associated with increased SSI risk (Miskiri *et al.*, 1990).

In public hospital most of the patients presented with history of smoking for long years. The Nicotine use delays primary wound healing and may increase the risk of infection. In a large prospective study, current cigarette smoking was an independent risk factor for sternal and/or mediastinal SSI following cardiac surgery (Nagachinta *et al.*, 1987).

Theoretical arguments can be made for a belief that severe preoperative malnutrition should increase the risk of both incisional and organ/space SSI. Multivariate logistic regression modeling has shown that preoperative protein- calorie malnutrition is not an independent predictor of mediastinitis after cardiac bypass operations (Ziv *et al.*,

1996).The thesis reveled that most of the wound patients in public hospital were suffering from malnutrition.

Preoperative and operative status of the patients has been determined to evaluate the fitness of a patient for surgery. Preoperative fitness was asses by ASA score (Wilson, 1995). In both public and private hospital, the most of the surgical patients were in ASA class II. The prevalence of healthy patients in HFRCMCH is higher then DMCH.

The duration of operation has an inherent effect on acquiring wound infection. In this study it was observed that operation took more than three hours are at more risk of getting nosocomial infection than operation took one hour or less then one hour. In public hospital post operative stay of hospital is higher than private hospital. This might be due to the fact that, public hospital maintained proper infection control hygiene than public hospital. The preoperative characteristics based on ASA score and operative status of the present study showed similar result with post operative infection studied by Noel and co-workers (1997) and Yalcin and co-workers (1995).

The characterization of post operative wound was performed according to the CDC guidelines (CDC, 1999). Different types of operations contained different types of wound. Abscess and gangrene was found to be contaminated and dirty wound. Cut injury and laparoscopic surgery presented with clean wound. Multiple types of bacteria were observed mostly in infected and contaminated surgical wounds and single type of bacteria was abundantly found in clean surgical wound. Similarly according to the classes of wound the type of operation is classified by Simmons (1982).

In ninety six infected cases, there were some cases of incisional complication which were categorized as superficial, deep and organ/space incisional type of wound described in Giacometti and coworkers (2000). They were presented with purulent discharge. Most of the wounds was healed by two weeks. Some wounds in DMCH took one month to heel. Here also nutritional status, immune suppression influenced the healing process of the wound.

Considering the strains of bacteria, *E .coli* was the most frequently isolated organism from wound infection. Almost similar rate was reported by Saini et al (2004) from India. Higher prevalence of *E. coli* in our study might be due to its frequent presence in hospital environment from where study cases were selected and in most post operative wounds *E. coli* is usually the predominant organism (Zafar,1999).

This study shows an alarmingly high incidence of MRSA infection in both hospitals of Bangladesh, which is much higher than the reports of the researchers in different other countries (Anupubra *et al*, 2003, Majumder *et al*, 2000). Such a high rate of MRSA in the study may be due to several factors; non judicious use of antibiotics, lack of MRSA control measure, failure of proper detection of MRSA for which carriers remains undetected, overcrowding, poor hygiene etc.

A lower rate of *Pseudomonas* has been reported by Giacometti from Italy (23%) and Mahmood from Pakistan (13%). But Shasuzzaman and co-workers (2003) from Bangladesh has reported higher rate of Pseudomonas 37.2%. In this study the rate of Pseudomonas in wound infection is 10.5%. Other organisms in order of frequency of isolation included *Proteus* (8.1%), *Klebsiella* (4.2%) and *CoNS* (2.7%). Almost similar result has been reported by Shittu and co workers (2000).

The rate of infection by *Strep. pyogens* in this study has been observed to be quite low, similar result was reported by Mahmood (2000) from Pakistan. and Jinnah and co workers (1998) from Bangladesh (3.1%). This static and low arte of infection by Strep. pyogens in different studies may be due to the fact that penicillin has always been used successfully for combating infection by *Strep.pyogens.*

The nature of the nosocomial infection seems to be very diverse and it needs to be properly addressed to identify the risk factors and on the basis of the sources a proper management system has to be developed.

CHAPTER 4

CHAPTER 4

SOURCES OF MICROORGANISMS AND THEIR RELATIONSHIP WITH POST OPERATIVE WOUND INFECTIONS

Microorganisms in post operative care unit of public managed hospital (DMCH) and private managed hospital were isolated from various sources. Samples were collected form inanimate and animate objects of these two hospitals monthly at random basis form January 2008 to July 2009. Inanimate objects were air, water floor, bed sheets, surgical instruments, dressing materials etc. Animate objects were nasal swab of hospital staffs (surgeons and OT nurses) and attendants; nail fold, inter-digital space and palm of hospital staffs (surgeons and OT nurses) and attendants. The microorganisms were characterized with the help of molecular and serological techniques along with their drug resistance patterns. A relationship was also examined between the microorganism from these sources and microorganisms from the wound infections.

4.1 Microbial Isolates Obtained from Hands of the Hospital Staffs and Attendants

Twenty Samples were taken monthly from palms, inter-digital spaces of both hands of surgeons, post operative nurses and attendances for quantitative and qualitative analysis in private managed hospital. It was observed that the attendance hands contained more bacteria than that of Hospital staffs and *E. coli* was the predominant organism. The percentage of prevalence of *E. coli*, *S. aureus* and *Pseudomonas* were more in attendants' hands than in the hands of health care workers. (Figure 4.1)

Microorganisms were also isolated from the hands of hospital staffs and attendances in public managed hospital (Figure 4.2). The percentage of prevalence of *E. coli* was more in the hands of attendances (60%) than hospital staffs (40%) but the presence of *S. aureus* and *Pseudomonas* were less in attendances hands than hospital staffs hands. The other organisms that were present in hands of hospital staffs and attendances were *Proteus, S. epidermidis, Klebsiella* and *α-hemolytic Streptococcus*.

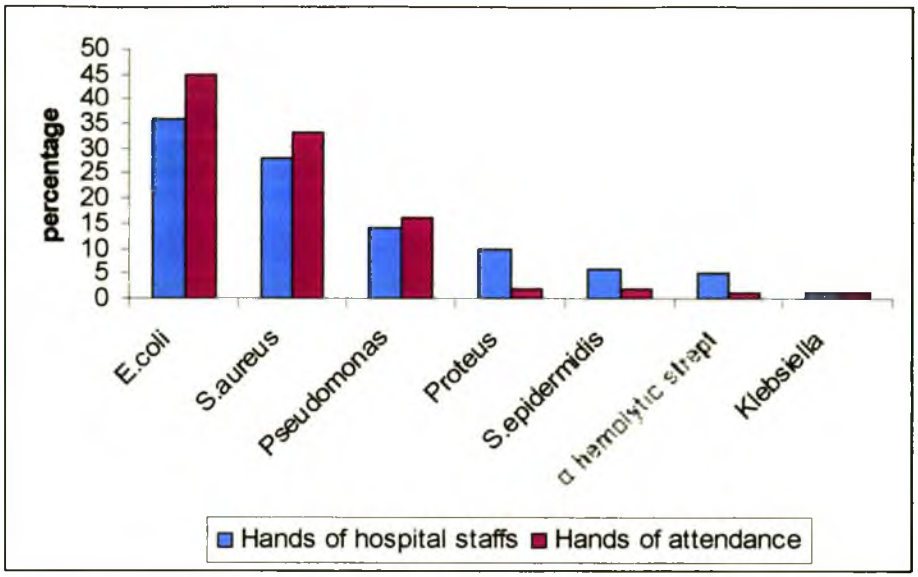


Figure 4.1 Microorganisms isolated from hands of hospital staffs and attendances in private managed hospital (HFRCMCH).

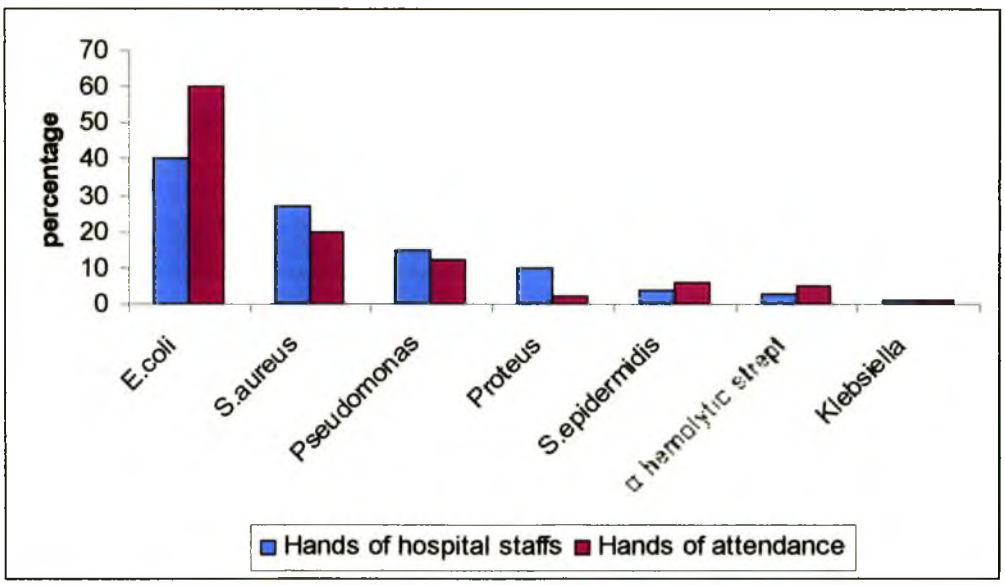


Figure 4.2 Microorganisms isolated from hands of hospital staffs and attendances in public managed hospital (DMCH).

4.? Carriage of Organisms in Anterior Nares of Hospital Staffs and Attendance

Organisms were isolated from anterior nares of hospital staffs and attendances in DMCH and HFRCMCH throughout the study period. In case of private hospital, anterior nares of attendances (50%) contained more *S. aureus* then hospital staffs (35%) and similarly anterior nares of attendances (70%) in public hospital contained more *S. aureus* than Hospital staffs (45%). The prevalence of *Methicillin resistance Staphylococcus aureus* (MRSA) in anterior nares of attendances and hospital staffs of both hospitals were very high. The Prevalence of organisms in anterior nares of hospital staffs and attendances are given in Table 4.1.

Table4.1 Prevalence of organisms in anterior nares of hospital staffs and attendances.

Types of organisms	Private hospital (HFRCMCH)		Public hospital (DMCH)	
	Hospital staffs	Attendances	Hospital staffs	Attendances
S.aureus	7 (35)	10 (50)	9 (45)	14 (70)
MRSA	*5 (71)*	*7 (70)*	*6 (67)*	*12 (86)*
MSSA	*2 (29)*	*3 (30)*	*3 (33)*	*2 (14)*
Pseudomonas	4 (20)	3 (15)	3 (15)	3 (15)
Proteus	2 (10)	4 (20)	2 (10)	2 (10)
No organisms	7 (35)	3 (15)	6 (30)	1 (5)
Total	20 (100)	20 (100)	20 (100)	20 (100)

4.3 Determination of Microbial Load of the Air of Operative and Post Operative Ward of Private and Public Hospital

It was observed that the microbial load of air in the operation theatre of private hospital varied from 30 to 100 and in the operation theatre of public hospital varied from 40 to 130 after 15 min exposure per nutrient agar plate from January 2008 to December 2008 (Figure 4.3). In case of the post operative room, the microbial load of private hospital

varied from 100 to 250 and in public hospital varied from 150 to 270 from January 2008 to December 2008. The highest number of total bacterial load was found in the post operative word of public hospital in June and the lowest number in operation theatre of private hospital in January.

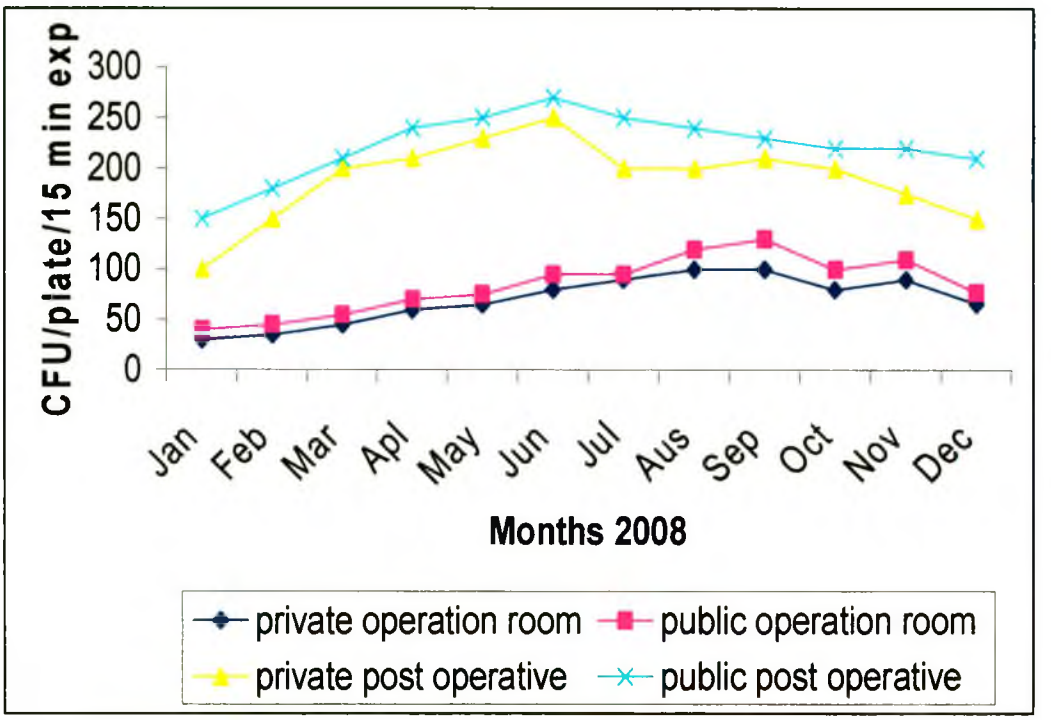


Figure 4.3 Bacterial load in the air of operative and post operative ward.

4.4 Microbiological Status of the Water in Post Operative Room

The qualitative and quantitative analysis of the tap water supply of post operative room was performed through out the year. Total coliform count in the tap water of post operative room was found to be varied from 0.0 to $1.7x10^2$/100 ml of water and faecal colifrom count was varied from 0.0 to $1.1x10^2$/100 ml of water. The highest total and faecal coliform count was observed in December. As the results obtained very similar for both of waters, only the data for HFRCMCH are presented in Figure 4.4. *E. coli* is the most prevalent isolate from the tap water of post operative room.

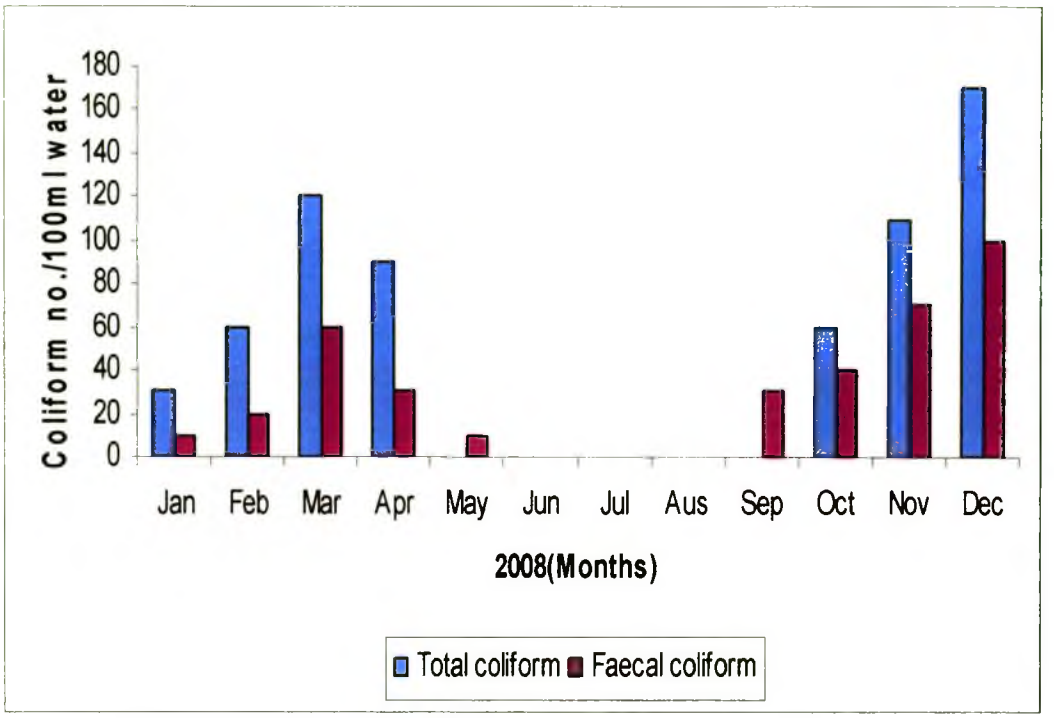


Figure 4.4 Total and faecal coliform count in the tap water of post operative room.

4.5 Organisms in Patients Surroundings of Operative and Post Operative Room

Environmental samples were collected from different sources of surrounding of patients in operative and post operative room of private hospital. A total of 120 samples were collected from the floor, bed sheets, OT instruments, dressing materials (cotton, gauze), nasogastric and endotracheal tube, catheters. *E .coli* (40%) was the predominant organism followed by *S. aureus* (24%), *Pseudomonas* (12%), *Proteus* (10%) (Table 4.5).The commonly prevalent organisms in floor, bed sheet and trolley were *E .coli* and *S. aureus*. *Pseudomonas* was usually found in sucker and tubes and dressing materials was a good resource of *E. coli* and *S. aureus*. *Proteus, Bacillus, Citobacter, Acinetobacter* and *Candida albicans* was also collected from patients surrounding environment. It was also observed that equipments used in the operation theatre contained less organisms than post operative room.

Table 4.2 Organisms in patents surroundings of operative and post operative room in private hospital.

Source (n)	*S. aureus*	*E. coli*	*Pseudomonas*	*Proteus*	Others*
Floor(15)	4	3	2	-	1
linen bed sheet(15)	4	5	1	2	2
Trolley (15)	3	4	-	3	1
Catheter (15)	-	6	-	2	1
Sucker and tubes(15)	2	3	5	-	2
OT instruments(15)	-	1	-	-	2
Oxygen cylinder (15)	3	5	1	-	1
Dressing materials(15)	4	6	1	1	2
Total(n=120)	**20 (24)**	**33 (40)**	**10(12)**	**8 (10)**	**12 (14)**

**Bacillus* spp, *α-haemolytic strep.*spp, *Citrobacter* spp. *Acinetobacter* spp. Candida spp.

Environmental samples were also collected from different sources of surrounding of patients in operative and post operative room of public hospital. The 120 samples were collected from the floor, bed sheets, OT instruments, dressing materials (cotton, gauze), nasogastric and endotracheal tube, catheters, etc. Most of the organisms were *E. coli* (50%) followed by *S. aureus* (24%), *Pseudomonas* (10%), *Proteus* (8%).The highest number *E. coli* was found in dressing materials and catheter and *S. aureus* was highly prevalent in bed sheets and floors. *Pseudomonas* was highly prevalent in sucker and tubes and most of the *Proteus* were found in trolley. Organisms in patient's surrounding of post operative room are shown in Table 4.3.

Table 4.3 Organisms in patents surroundings of operative and post operative room in DMCH.

Source (n)	*S .aureus*	*E.coli*	*Pseudomonas*	*Proteus*	Others*
Floor(15)	4	4	1	-	2
linen bed sheet(15)	4	5	1	2	2
Trolley (15)	3	4	-	3	1
Catheter (15)	2	6	-	2	1
Sucker and tubes(15)	2	3	5	-	2
OT instruments(15)	3	2	-	-	2
Oxygen cylinder (15)	3	5	1	-	2
Dressing materials(15)	4	6	1	-	2
Total(n=120)	**15(17)**	**45 (50)**	**9 (10)**	**7 (8)**	**14 (15)**

E. coli (39%) was the most dominant organisms in the post operative patient's surroundings followed by *S. aureus* (24%) (Figure 4.5)

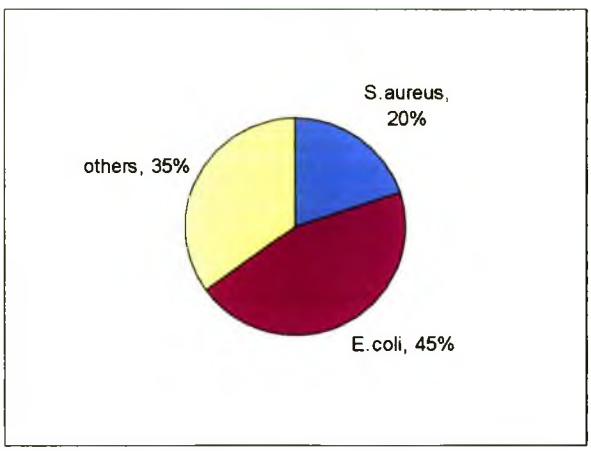


Figure 4.5 Prevalence of E. coli and S. aureus in patient surroundings of OT and postoperative room.

4.6. Patterns of Antimicrobial Resistance of the Microbial Isolates

Antimicrobial suseptibility was determined by disc diffusion method. *Staphylococcus* were tested against Penicillin (P), Ampicillin (AP), Ciprofloxacxin (CIP), Gentamycin (CN), Vancomycin(Va), Tetracycline, Cloxacillin (OB), Cephalexin (CL), Ceftriazone (CRO). *E.coli*, Proteus and for other gram negative bacteria Imipenem (IP) and all the above discs except Penicillin (P), Cloxacillin (OB), Erythromycin (E) were used. Methicillin resistance *Staphylococcus aureus* (MRSA) was detected by 1µg Oxacillin disc andExtended spectrum β lactemases (ESBL) was detected by placing Augmentin discin the centre of the plate and Ceftriazone, Aztreoman, Ceftazidime and Cefotaxime place 30 centimeter away from augmantin disk

4.6.1 Antibiogram of isolated *E. coli* strain

All strains of *E .coli* were found 100% resistance to Ampicillin. More than 90% strains were resistance to Cefalexin and Nalidizic acid. More than 80% strains were sensitive to Gentamicin, Ciprofloxacin and Ceftazidime. The most potent drug against *E.coli* was Imipenem. The result of the antibiogram is presented in the figure:

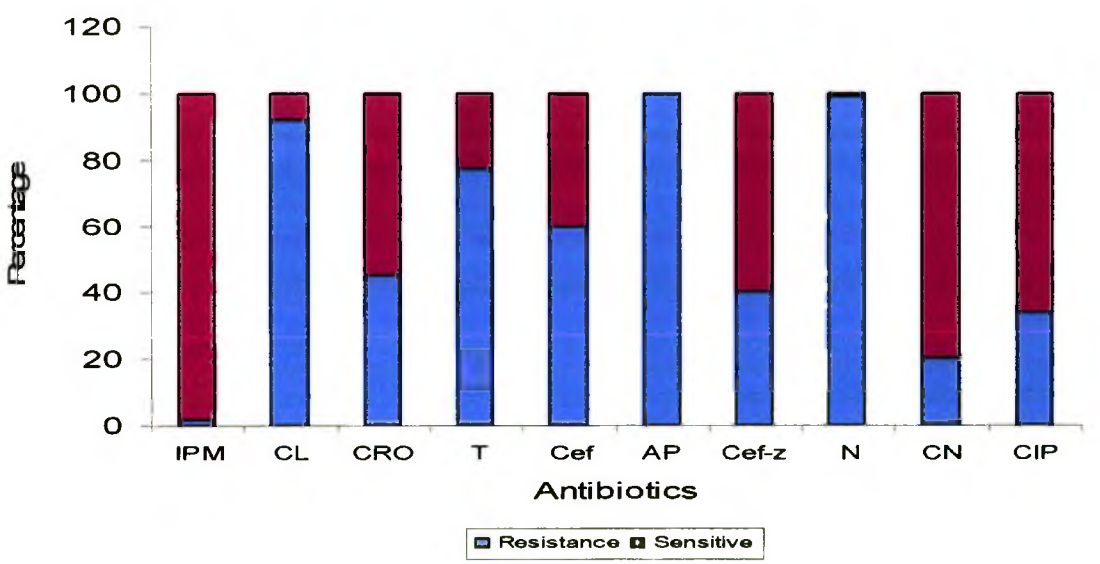


Figure 4.6 Antibiogram of E. coli.

IPM=Imepenem, CL=Cephalexin, CRO=Ceftriazone, T= Tetracycline Cef=Ceftriazone OX=Oxacillin, AP=Ampicillin, Cef-z=Ceftazidime, N=Nalidizic acid, CN=Gentamicin, CIP=Ciprofloxacin.

4.6.2 Antibiogram of the isolated *Staphhylococcus aureus* strains

All strains of *Staphylococcus aureus* were found resistance to Penicillin. More than 75% strains were resistance to Tetracycline, Ampicillin, Oxacillin, Cloxacillin and Erythromycin. More than 80% strains were sensitive to Rifampicina and Fusidic acid. Most potent drug found in this study against *S. aureus* was Vancomycin. The result of the antibiogram is presented in the Figure 4.7.

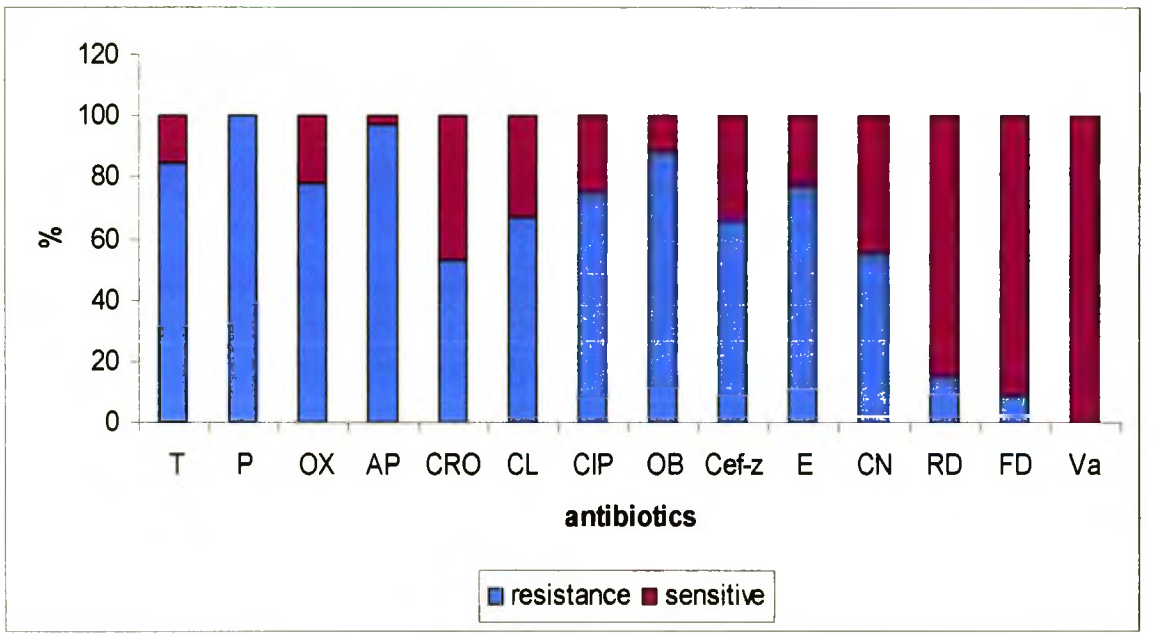


Figure 4.7 Antibiogram of S. aureus.

T= Tetracycline, P= Penicillin, OX=Oxacillin, AP=Ampicillin, CRO=Ceftriazone
CL=Cephalexin, CIP=Ciprofloxacin, OB=Cloxacillin, Cef-z=Ceftazidime,
E= Erythromycin CN=Gentamicin, RD= Rifampicin, FD=Fusidic acid, V=Vancomycin

6.3 Antibiogram of isolated *Pseudomonas* sp.

The strains of *Pseudomonas* were more than 80% resistance to Ampicillin and Tetracycline. Drug of choice is Imepenem. Genatmicin, Ceftriazone, Ciprofloxacin and Cephalexin were found mildly sensitive against *Pseudomanas.* the result of the investigation is presented in Figure:4.8.

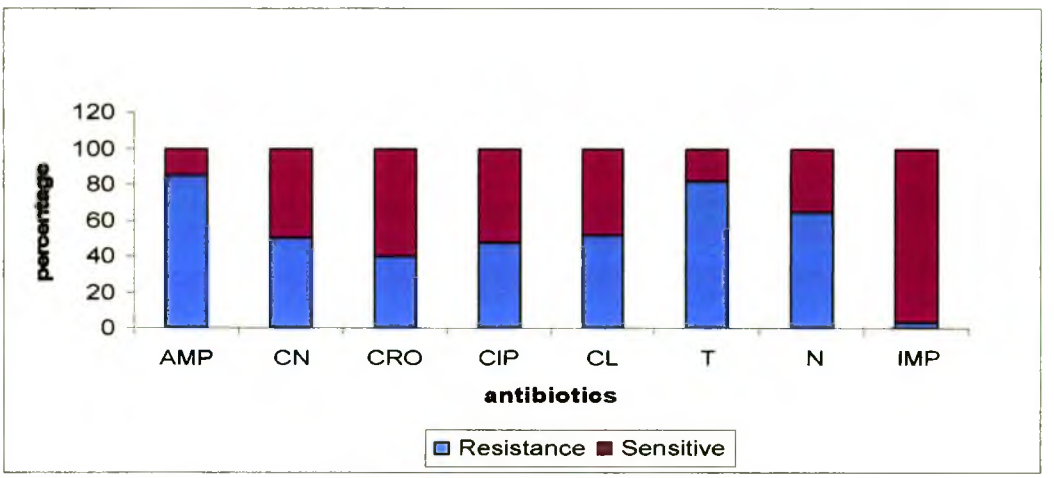


Figure 4.8 Antibiogram of Pseudomonas.

4.6.4 Antibiogram of isolated *Proteus* sp.

Ampicillins are almost 100% resistance to *Proteus.* Imepenem are 100% sensitive and Ceftriazone are more than 80% sensitive to *Proteus.* The effective antibiotics other than Imepenem and Ceftriazone are Gentamycin, Ciprogloxacin.The result of the antibiogram is presente din Figure 4.9.

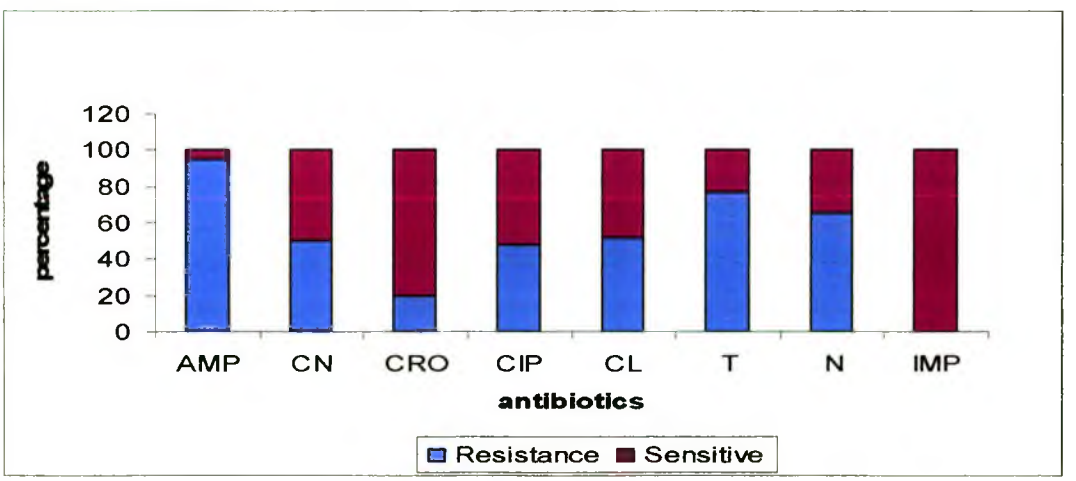


Figure 4.9 Antibiogram of Proteus.

AMP=Ampicillin,CN=Gentamicin,CRO=Ceftriazone,CIP=CiprofloxacinCL=Cephalexin, T=Tetracycline, Cef-z=Ceftazidime, N=Nalidizic acid, IPM=Imepenem

4.7 Antibiogram of the Selected Strains of *E.coli* and *Staphylococcus aureus*

All the selected strain of *E. coli* and *S. aureus* were tested with the common antibiotics to see their resistance pattern. For confirmation of ESBL all *E. coli* were tested with double disc diffusion method and all *S. aureus* were tested with Oxacillin disc diffusion test to detect MRSA

4.7.1 Antibiogram of the 20 selected strains of *E. coli*

All *E.coli* isolates were tested for antibiotic susceptibility pattern against 10-12 different antibiotics. The summary of the results are presented in the Table 4.4

Table 4.4 Antibiogram of the selected *E. coli* strains.

Isolates no.	Resistance to	Sensitive to
E-1	CL, CRO, T, CEF AP, , Cef-Z	IPM, CN CIP, N
E-2	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN CIP
E-3	CL, CRO, T, CEF AP, N, Cef-Z, CIP	IPM CN
E-4	CL, CRO, T, CEF AP, , Cef-Z	IPM CN CIP, N
E-5	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN CIP
E-6	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN
E-7	CL, CRO, T, CEF AP, N, Cef-Z	IPM CIP
E-8	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN
E-9	CL, CRO, T, CEF AP, N, Cef-Z	IPM
E-10	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN CIP
E-11	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN
E-12	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN CIP
E-13	CL, CRO, T, CEF AP, N, Cef-Z	IPM CIP CIP
E-14	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN CIP
E-15	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN CIP
E-16	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN
E-17	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN
E-18	CL, CRO, T, CEF AP, N, Cef-Z IPM CN CIP	IPM CN CIP
E-19	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN
E-20	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN

Twenty *E. coli* strains were examined for antibiogram of which most of them found to be multi drug resistance. These multi drug resistance strains were again tested for ESBLs production by double disc diffusion test. Out of which 13 were ESBL producing are shown in Table.4.5.

Table 4.5 Screening of ESBL producing E. coli by Double Disk diffusion Method.

Isolates	Sensitive to	Resistance to	DD
E-1	CL, CRO, T, CEF AP, Cef-Z	IPM,CN CIP, N	+
E-2	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN CIP	+
E-3	CL, CRO, T, CEF AP, N, Cef-Z, CIP	IPM CN	
E-4	CL, CRO, T, CEF AP, Cef-Z	IPM CN CIP, N	+
E-5	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN CIP	
E-6	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN	+
E-7	CL, CRO, T, CEF AP, N, Cef-Z	IPM CIP	
E-8	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN	
E-9	CL, CRO, T, CEF AP, N, Cef-Z	IPM	+
E-10	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN CIP	+
E-11	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN	
E-12	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN CIP	+
E-13	CL, CRO, T, CEF AP, N, Cef-Z	IPM CIP CIP	+
E-14	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN CIP	+
E-15	CL, CRO, T, CEF AP, N, Cef-Z	IPM, CN, CIP	+
E-16	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN	
E-17	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN	+
E-18	CL, CRO, T, CEF AP, N, Cef-Z IPM, CN , CIP	IPM CN	
E-19	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN CIP	+
E-20	CL, CRO, T, CEF AP, N, Cef-Z	IPM CN	+

IPM=Imepenem, CL=Cephalexin, CRO=Ceftriazone, T= Tetracycline Cef=Ceftriazone OX=Oxacillin, AP=Ampicillin, Cef-z=Ceftazidime, N=Nalidizic acid, CN=Gentamicin, CIP=Ciprofloxacin.

4.7.2 Antibiogram of the 20 selected strains of *S.aureus*

All *S. aureus* isolates were tested for antibiotic susceptibility pattern against 12-15 different antibiotics. The summary of the results are presented in the Table 4.6.

Table 4.6 Antibiogram of the selected S. aureus strains.

Isolates no.	Resistance to	Sensitive to
S-1	T, P,AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va ,RD, OX
S-2	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD FD
S-3	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD FD
S-4	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD FD
S-5	T, P, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD OX
S-6	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va FD
S-7	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD
S-8	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD
S-9	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD
S-10	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD
S-11	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD
S-12	T, P, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va FD OX
S-13	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va FD
S-14	T, P, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD OX
S-15	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va FD
S-16	T, P, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va OX
S-17	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va OX
S-18	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD FD
S-19	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD FD
S-20	T, P, OX, AP, CRO, CL, CIP, OB, Cef-z, E, CN ,RD FD	Va RD FD

T= Tetracycline, P= Penicillin, OX=Oxacillin, AP=Ampicillin, CRO=Ceftriazone
CL=Cephalexin, CIP=Ciprofloxacin, OB=Cloxacillin, Cef-z=Ceftazidime,
E= Erythromycin CN=Gentamicin, RD= Rifampicin, FD=Fusidic acid, V=Vencomycin

4.8. Determination of the Relationship Among Microbes from Clinical and Environmental Samples

The relationship between clinical and environmental samples of *E. coli* and *S. aureus* were determined by molecular and immunological technique.

4.8.1 Immunological (agglutination) tests for MRSA isolates

Two immunological tests were done for all Methicllin resistance Sthaphylococcus *aureus* (MRSA);Methicillin resistance gene (*mecA*) in *S. aureus* was confirmed by detecting the presence of penicillin binding protein 2a(PBP2a) in MRSA latex agglutination test (LAT) and relationship of carrier and patient was determined by coagulase typing.

LAT test is highly sensitive for detection of MRSA. All isolates from patients and carriers were found 100% sensitive to Latex agglutination test (Table 4.7)

Table 4.7 MRSA Latex agglutination test.

Category	MRSA screen latex agglutination test
True positive(TP)	20
False positive(FP)	0
False negative(FN)	0
Sensitivity	100%
Specificity	100%

MRSA strains (13) from wound infections of patients and MRSA strains from nasal samples of carriers (7) were the same Coagulase type VI (Table 4.8).

Table 4.8 Coagulase typing of MRSA isolates.

MRSA isolates	Coagulase type of isolates
From patients	Type -VI
From carriers	Type -VI

4.9 Molecular Determination of *E.coli* from both Clinical and Environmental Samples

E.coli was isolated from clinical wound samples and environmental samples and it is one of the objectives of the study to find out the relationship between them with the help of various molecular techniques like plasmid profile analysis, PCR and PFGE.

4.9.1 Determination of plasmid profile of *E. coli*

Analysis of plasmid DNA by agarose gel electrophoresis revealed that all the isolates contained multiple numbers of plasmid from1-140 MDa, forming multiple banding patterns. Among 14 isolates, Lane 4 contains the most number of band patterns 140, 120, 3.7, 2.7, 2.1 and 1 MDa (Figure 4.10). Besides this there are two sets of plasmid patterns (140, 2.1, 2.7) and (140, 3.7, 2.7, 2.1 and 1MDa) which constitutes 25% of all plasmid. Two plasmid collected from environmental samples (E-15and E-10) are resemble with two plasmid of clinical isolates (E-14and E-19).

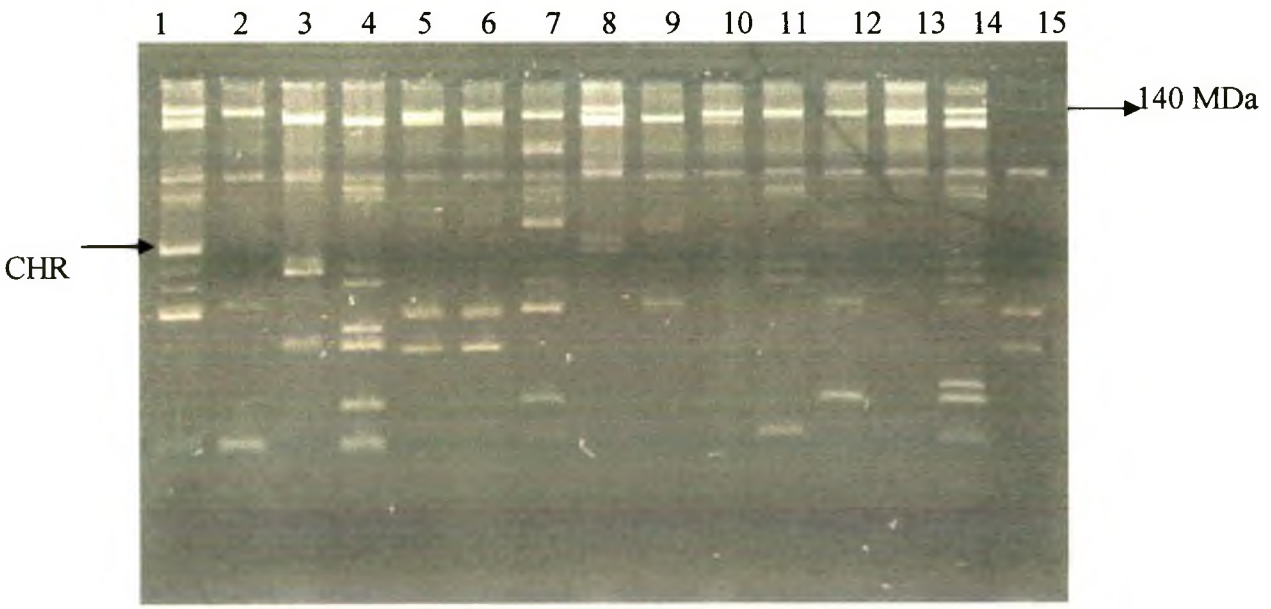


Figure:4.10 Agarose gel electrophoresis of plasmid DNA showing representative pattern of different *E. coli* isolates: Lane 1, *E .coli*-02.CHR indicates the banding position of the chromosomal DNA. Lane-2, *E. coli-01*, Lane-3,*E. coli*-03; Lane 4, *E. coli*-08; Lane 5, *E. coli*-14; Lane 6, *E. coli*- 20, Lane 7, *E. coli*-06; Lane 8, *E.coli*-17; Lane 9, *E.coli*-18;Lane 10, *E. coli*-10; Lane 11, *E. coli*-09; Lane 12, *E. coli*-16; Lane 13, *E. coli*-19; Lane 14, *E. coli-12*; Lane 15, *E. coli* PDK-9 (marker).

4.9.2 Pulsed field gel electrophoresis (PFGE) of *E. coli*

A dendrogram based on similarities of *XbaI*-digested DNA PFGE patterns among *E. coli* strains was created with the Bionumerics software using Dice similarity as described by Beutin *et, al.*, in 2002. PFGE patterns were arranged in two clusters, A and B, which showed 59.74% similarity in their banding patterns (Figure 4.11)

Cluster B again divided into B1 and B2. B1 cluster is again subdivided into B1a and B2a. B1a contains 6 strains (E-02, E-06, E-20, E-04, E-05) of clinical and environmental isolates with clonal relatedness. In B2a, there are 7 strains (E-07, E-11, E-03, E-17, E-14, E-15 and E-12) of clinical and environmental isolates of clonal relatedness. Among them, E-14 of clinical isolate has 100% clonal relationship with environmental isolate E-15. E-14 was isolated from post operative patient of HFRCMCH and environmental sample E-15 was collected from the trolley of the same room. So, may be trolley is the source of that patient's nosocomial wound infection.

B2 is again subdivided into B2a and B2b. B2a contains 4 clinical isolates (E-13, E-16, E-09, E-18) of clonal relatedness. There is no environmental isolates is this group. B2b contains 3 isolates namely (E-10, E-19 and E-01) of clinical and environmental relatedness. The environmental isolates E-10 has100% clonal similarity with clinical isolates E-19. Environmental isolates (E-10) collected from bed sheet of post operative room of HFRCMCH, which may be the cause of the patient's (E-19) nosocomial manifestation of wound infection.

Cluster A contains only one clinical isolate (E-08) collected from DMCH post operative room. All the isolates were collected from clinical and environmental sources of post operative room and its surroundings of HFRCMCH and DMCH.

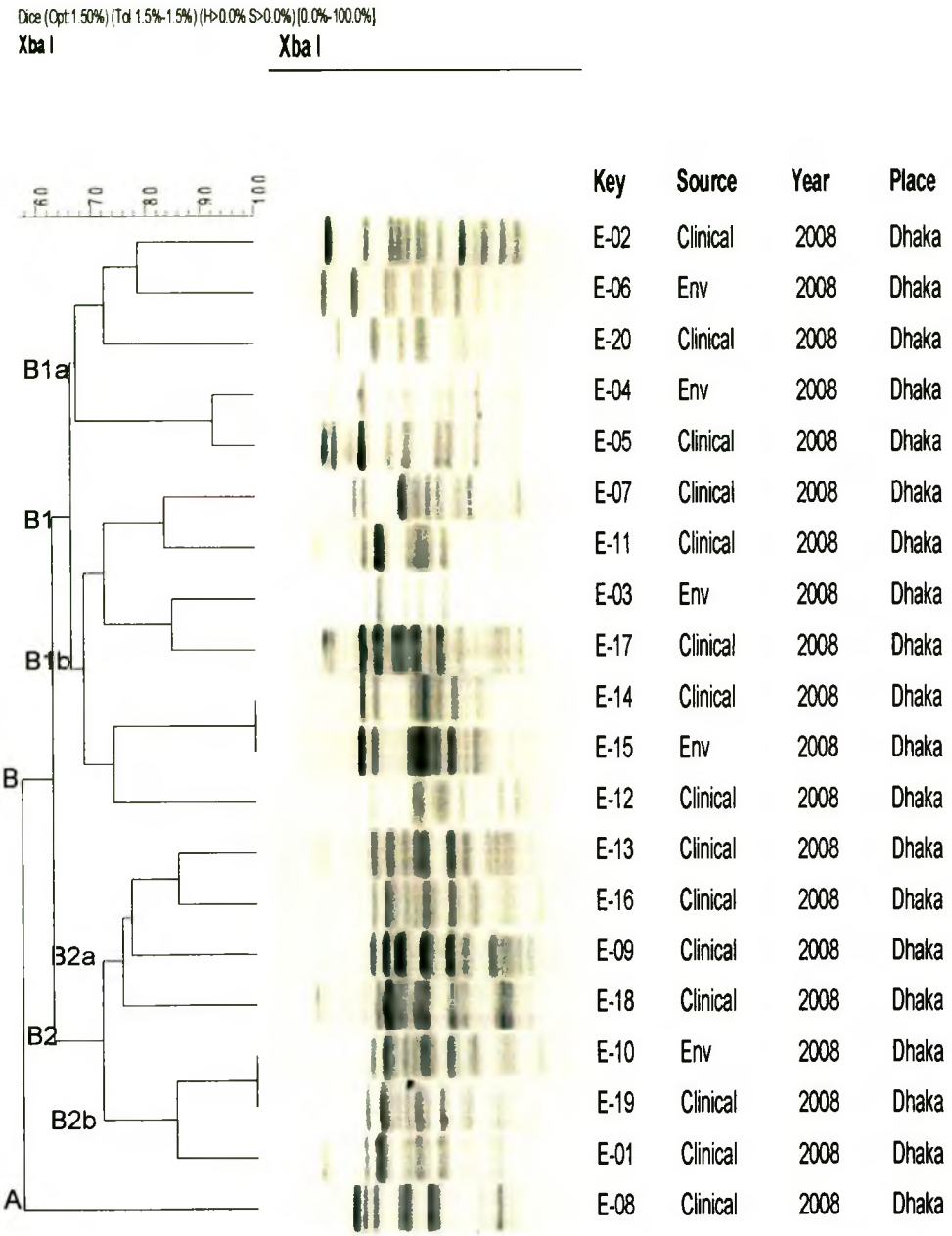


Figure 4.11 Dendogram showing clonal relatedness among E. coli strains.

4.9.3 Resistance gene marker detection by PCR

20 *E. coli* isolates (ESBL phenotypes) were examined for resistance gene marker *intl1* and out of which 14 isolates were posite for *intl1* (70%).

Detection of class I integron (*intl*-l) gene by PCR:

The presence of integrons was explored by PCR to identify class I integron which are specific for the 5' conserved segment containing integrase gene (*intI*-1) (Dalsgaard *et al.*, 2001). In this study, PCR within Int-U and Int-D primers yielded amplicon for 14 of the 20 strains tested including the desired amplicon for the positive control.

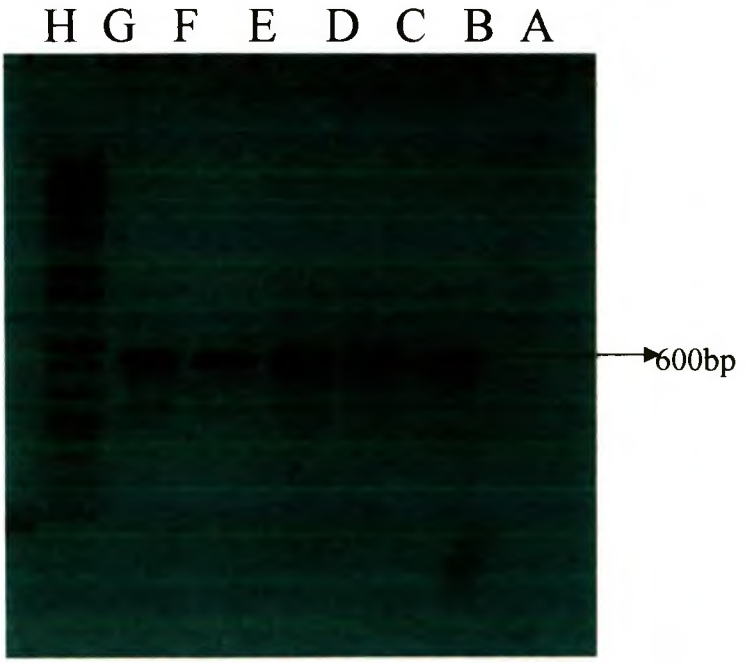


Figure:4.12 Agarose gel electrophoresis showing PCR amplification products of the *intl1*.Lane D E F G representative of E.coli E-19, E-10, E14, E-15. Lane B negative control. Lane C positive control, Lane A reagent blant and Lane H DNA molecular size marker(1kg DNA ladder from GIBCO- BRL).

Sequence analysis of *Intl1*

The chromatogram sequencing files were edited using Chromas 2.32 (Yechnelysium, Queensland, Australia). The homology of the DNA sequences was checked with the DNA sequences of other organisms that had already submitted to GeneBank database using the BLASTN algorithm. (Cole *et al.*, 2003). The sequences were then edited by Chromas software and aligned with clustalx. Finally the sequences were translated using Bioedit and Genedoc softwares. Given below the different species with their access number to compare with *E. coli intl1* strain (E-04)and to find any mutational change in amino acid position causing drug resistance.(Figure 4.13)

>Intl1_Escherichia_coli

RILYLHYSLRTEQAYVHWVRAFIRFHGVRHPATLGSSEVEAFLSWLANERKVSVST
HRQALAALLFFYGKVLCTDLPWLHEIGRPLPSRRLPVVLNPDEVVRILGFLEGEHLLFAQ
LLYGTDMRISEGLQLPLKDLDFDNGTIIVREDKGSKDQALMLPESLATSMHEHLSRARTW
WLKDQAEGRSGVALPDALERKYPRAGHSW
>YP_002415407_Escherichia_coli
RIRYLHYSLRTEQAYVHWVRAFIRFHGVRHPATLGSSEVEAFLSWLAN
ERKVSVSTHRQALAALLFFYGKVLCTDLPWLQEIGRPRPSRRLPVVLTPDEVVRILGFLEGEHRLF
AQLLYGTGMRISEGLQLRVKDLDFDHGTIIVREGKGSKDRALMLPESLAPSLREQLSRARAWWLK
DQAEGRSGVALPDALERKYPRAGHSW
>YP_001350673_Pseudomonas_aeruginosa
RIRYLHYSLRTEQAYVHWVRAFIRFHGVRHPATLGSSEVEAFLSWLAN
ERKVSASTHRQALAALLFFYGKVLCADLPWLQEIGRPRPSRRLPVVLTPDEVVRILGFLEGEHRLF
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KDQAEGRSGVALPDALERKYPRAGHSW
>AAA72104_Shigella_sonnei
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DQAQGRSGVALPDALERKYPRAGHSW
>ABY64756_Klebsiella_pneumoniae
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>YP_002317699_Acinetobacter_baumannii
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ERKVSVSTHRQALAALLFFYGKVLCTDLPWLQEIGRPRPSRRLPVVLTPDEVVRILGFLEGEHRLF
AQLLYGTGMRISEGLQLRVKDLDFDHGTIIVREGKGSKDRALMLPESLAPSLREQLSRARAWWLK
DQAEGRSGVALPDALERKYPRAGHSW

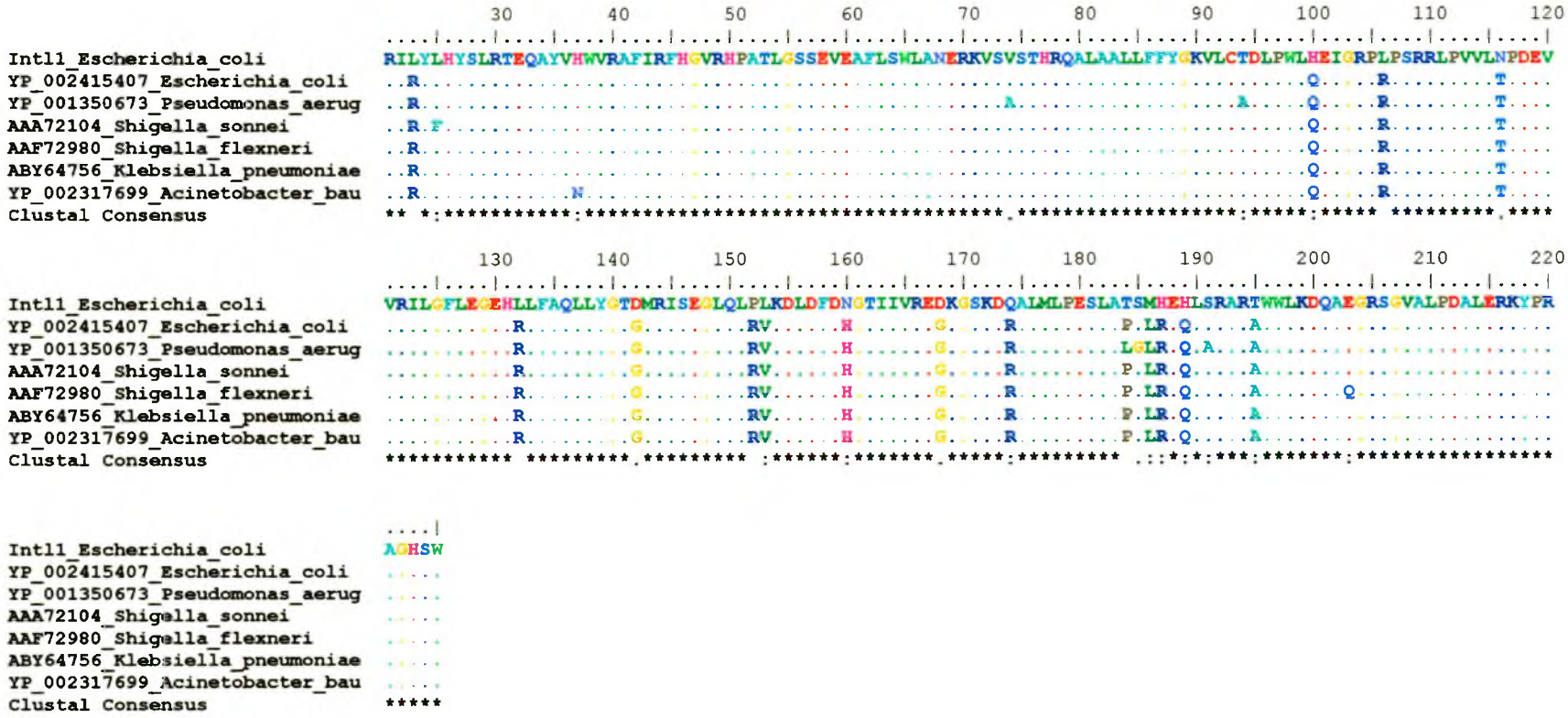

Figure 4.13 Comparison of the *E. coli Intl1* Integrase protein sequence (20-225 amino acids) with other species.

4.10. Summary of the Relationship between Clinical and Environmental sample

Stahphylococcus aureus was isolated from environmental sample (patient's surrounding of post operative ward) and carrier sample from anterior nares of hospital staffs showed same antibiogram, Latex agglutination test positive for both isolates and both has the similar cougulase typing. So, the Table 4.13 shows that carrier sample (S-5) is the causative factor for post operative surgical wound sample (S-1).

Table 4.10 Relationship between clinical and carrier sample of S. aureus.

Isolate no	Isolate from	Anbiogram	LAT	Coagulase typing
S-01	patients wound	MRSA	+	Type VI
S-04	Ant nares of hospital staffs	MRSA	+	Type VI

E. coli isolated from post operative wound and patients surrounding environment (E-15) had shown same antibiogram with similar plasmid pattern, presence of resistance gene marker *Intl1* and in same cluster of clonal relationship proved by PFGE (Table 4.14). Similar pattern of relationship was observed in case of wound sample (E-10) and Environmental sample (E-19)

Table 4.11 Relationship between clinical and environmental sample of E. coli.

Isolate no	Isolated from	ESBL	*Intl1*	Plasmid pattern	PFGE
E-14	wound infection	+	+	similar P3	B-1b
E-15	environment	+	+	similar P3	B-1b
E-10	wound infection	+	+	similar P8	B-2b
E-19	environment	+	+	similar P8	B-2b

4.11. Discussion

The hands of hospital staffs and attendance are of special concern as they are prime source for surgical site as well as post operative nosocomial infection. Without any skin problem, palms and inter-digital spaces and nail folds of health care workers (HCW) and attendances were examined for microorganisms. *E. coli* and *S. aureus* were the predominant organisms in both private and public hospital. Similar observation was also reported by Rotter (2001) and Smith (2008). It was also observed that bacterial growth was higher on the hands of patient's attendance than that of health care workers (HCWs).This is due to the fact that the attendants are usually stayed on the patient's bed and or sometimes on the floor and most of the attendants was ignorant about personal hygiene.

Asymptomatic nasal carriage act as reservoir for the spread of this organism within hospital (Goyal *et al.*,2002). So, detection of carrier is important for control of MRSA infection. Screening of 20 hospital staffs for MRSA carriage in the anterior nares was carried out in this study. Most of the organisms were *S. aureus* in both private and public hospital and resistant organisms (MRSA) were highly prevalent (67-80%). This finding is consistent with the finding of Ahiq (1989) from Pakistan where isolation rate was *S.aureus* 65.3%.

In the present study total and faecal coliform count in supplied tap water of post operative room was examined and it was found that the presence of total and faecal coliform count was excessive and grater then the all recommended international standard.(UNEP/WHO,1996). Highly satisfactory water contains less than one coliform per 100 ml of water by international standard. The World Health Organization (WHO,1971) allows less then 10 coliform /100ml as a maximum for small community supplies.

The importance of airborne transmission as a source of infection in operating theatre is controversial but there is evident to support the view that airborne organisms can cause post operative wound infection (Boyce, 2007 and Shaw *et al.*,1973). Recommended

limits for microbial contamination according to the European Union Good Manufacturing practice is for Environmental grade A should contain less then 1cfu/plate of organism which is also recommended for operation theatre and post operative room. Grade B should contain not more than 10 microbes which are suitable for surgical ward and post operative room (Pasquarel *et al.*, 2000). But in this investigation the rate was much higher than the normal limit in both public and private hospital.

Organisms were found in surroundings of patients of post operative and operative room. OT instruments contained fewer microbes than post operative equipments. Linen bed sheet, Trolley, Sucker, Oxygen cylinder, dressing materials were investigated for the source of nosocomial infection. Previous studies reveled that operative device was a good source of nosocomial infection (Leblebicioglu *et al.*, 2007; Rosenthal, 2004). *E. coli* and *S. aureus* were the dominant pathogens along with *Bacillus* spp, *Klebsiella, Acinetobacter* spp etc. in both private and public hospital.

MRSA screen Latex agglutination test (LAT) detected all MRSA isolates in this study. The sensitivity and specificity of MRSA screen latex agglutination test was 100% in the present study. This result is in agreement with Sakoulas and co-workers (2001) from USA, who reported sensitivity of 100% and specificity 99.1% and also Cavassani and co-workers (1999) from Switzerland where sensitivity of MRSA screen LAT was 100% and 99.2% respectively.

Typing of MRSA strains were done in this study by coagulase typing and this system has been used widely in epidemiological investigation of staphylococcal infection. Coagulase typing showed that all MRSA isolates from patients and carriers were Coagulase type VI. Similar results were found by Khan and co-workers (2007) in Bangladesh. But in Japan the most predominant coagulase type of MRSA was type II and type IV (Nakea *et al.*, 1999). This suggests that predominant coagulase type in Bangladesh (type VI) is different from that of Japan; which might be due to geographical variation. This study shows that Coagulase types of MRSA from patients and hospital staff carrier belonged to the same type. This suggests that most MRSA infection were

exogenous in origin. But in previous studies, typing of *S. aureus* showed that a variable proportion of infections were due to endogenous strains (Masaki *et al.*, 1999).

The susceptibility pattern of MRSA in this study isolated from both hospitals showed that MRSA were highly resistance to the common antimicrobials. The resistance of MRSA to a wide range of antimicrobials is well documented. Vancomycin has been clearly shown to be drug of choice. Rifampicin and Fusiduic acid has been found to have a good activity against MRSA. These drugs could be used in combination with other antimicrobials agent to prevent the development of resistance against these drugs. . This result is in confirmatory with most reports on MRSA by Jahan and coworkers (2004); Qureshi and coworkers (2004); Annupurba and coworkers (2003). MRSA was quite sensitive to Ceftriazone and Genatmycin. Anupura *et al* from India reported lower sensitivity for Ciprofloxacin and Gentamicin. Sensitivity of MRSA was lower for Cephlexin and Erythromycin. Almost similar rate was reported by Quresi *et al* (2004) from Pakistan. MRSA was less sensitive to Penicillin and Ampicillin and Oxacilin as found Vidhani and co-workers (2001) from India. Antibiogram of organisms isolated from the nose of medical staffs had showed the almost same susceptibility pattern of patients MRSA collected from wound.

The strains of *Pseudomonas* were found to be susceptible to Gentamicin (65%), Ciprofloxacin (60%), Ceftriazone (70%). More than 80% strains were resistance to Ampicillin and Tetracyclin. The most useful drug for treating *Pseudomonas* was Imepenem; almost 100% sensitive. William and co-workers (1986) reported that Pseudomonas were moderately susceptible to Gentamycin and Tetracycline but highly resistance to Ampicillin.

Proteus was found 100% sensitive to Imepenem and almost 100% resistance to Ampicillin. More than 80% strains of Proteus were susceptible to Ceftriazone, 55% to Gentamicin. On the other hand, almost 90% strains were resistance to Tetracycline. Almost similar findings were reported by Osoba and coworkers (1994) where *Proteus* was highly susceptible to Ceftriazone but resistance to Ampicillin and Amoxycillin.

A total of 20 *E. coli* strains associated with nosocomial infections were analyzed for the ESBL-producers by double disc diffusion test and 13 were found positive. Detection of ESBL producing bacteria were done by using 3rd generation of Cephalosporins. (Ceftazidime disc), 2nd generation Cephalosporin (Cefoxitin disc), Azotenam disc with an augmentin disc (Amoxycillin plus clavulanic acid). Enhancement of the zone of inhibition was observed towards Amoxyclavulanic acid disc in the centre in case of Ceftazidime and Aztreonam. However, no such enhancement of the zone of inhibition has been seen in case of Cefoxitin, as the ESBL producers are sensitive to this drug. (CDC,1999). In a study, Rahman *et al* (2004) observed different rate of isolation of the ESBL in the different combination of 3rd generation cephalosporin (Ceftriaxone, Cefotaxime and Ceftazidime) discs with Augmentin disc.

Analysis of palsmid DNA of *E. coli* by agarose gel electrophoresis revealed that all the isolates contained multiple number of plasmids ranging from 1 to 140 MDa, forming multiple banding pattern. Among 14 isolates, 10 patterns of plasmid were found. Pattern 1 contains 140, 4,3.7, 2.7, 2.1 and 1 MDa which constitutes 22.71% all plasmid. Pattern 7 contained 140, 3.7, 2.7 and 2.1 and 1 MDa and constitutes 33.28% of all plasmid pattern. Lane 4 contains the most number of band patterns 140, 120, 3.7, 2.7, 2.1 and 1 MDa which constitutes the 15% of all plasmid patterns. Besides this, there are two sets of plasmid patterns (140, 2.1, 2.7) and (140, 3.7, 2.7, 2.1 and 1MDa) which constitutes 25% of all plasmid. Two plasmid patterns collected from environmental samples (E-09 and E-10) had similar band with plasmid patterns of clinical isolates (E-15 and E-14), which may justify the source of clinical infection. Laderberg (1952) showed that different plasmid patterns were responsible for different resistance of drugs.

PFGE profiles of the *E. coli* strains obtained with *Not I* restriction enzyme showed a variety of patterns. Other strains of different locality and period displayed different but closely related restriction profiles. For example, strain E-14 and E-15 had similar restriction patterns. The other strains were distinguishable from each other and have been placed in different clusters. However, the strain, E-10 displayed closely related restriction

fingerprint patterns with E-19, which is an environmental clone and was included in this study for genomic comparison. So, it can be assumed that the clinical isolate E-19 might have originated from related clone to that environmental E-10 strain.

A correlation between antimicrobial resistance and the presence of integrons was explored by PCR of class I integron which are specific for the 5' conserved segment containing integrase gene (*intI1*) (Dalsgaard *et al.*, 2000). In this study, PCR within Int-U and Int-D primers yielded amplicon for 14 of the 20 strains tested. Among the 14 isolates11 were from clinical and 3 were from the environment sample. Pongpech and coworkers (2008) revealed a close relationship with ESBL and class1 integron intregase gene in Thai patient and healthy adults.

Post operative nosocomial infection constitutes a major problem in surgical patients contributing to morbidity, mortality and increased resource utilization and health care costs. Patients in whom SSI develop have an increased number of associated complications, higher risk of requiring a stay in ICU and 2 to 3 times higher risk of mortality. Their hospital stay is increased by 7 to 12 days and they are 5 times more likely to require readmission. Many risk factors have been successfully identified; more important, preventive measures with a significant impact on SSI have also been well defined however, not all risk factors and not all preventive measures seem to affect all surgical patients (Anaya.,2006).

A comparative study on various sources of private and public hospital was examined here and a relationship was drawn between them. Post operative nosocomial infections along with antibiotic resistance are increasing problem in healthcare settings and have a significant impact on the cost effectiveness of its provision. This is true in the developing world, where the introduction of sophisticated and expensive healthcare can outstrip the ability of basic infrastructure such as infection control to support it.

CHAPTER 5

CHAPTER 5

RISK FACTORS AND PREVENTION OF NOSOCOMIAL INFECTION IN POST OPERTAIVE CARE UNIT

Various factors such as overcrowding, restriction of attendances, hand hygiene, personal hygiene, and environment were considered the important risk factors for nosocomial infection of patients carrying surgical wounds. These factors were carefully investigated in the two categories of hospitals (private managed and public managed hospital) and the findings were compared. The effects of various treatments for prevention of the infections were also investigated.

5.1: Assessment of Risk Factors in Public Managed Hospital

Major risk factor for public hospital was overcrowding. For excess of patients, hospital authority could not provide bed for all patients. Photograph of a surgery ward showed in Figure 5.1 that patients were staying in the floor side by side. It was also revealed from the photograph that there were very narrow gap between two patients and environment was not hygienic. It was found to be the classic example of situation in public hospital of Bangladesh. There were no basins for hand washing and only two basins were placed for more than 350 patients. Hand washing was extremely neglected for safety measures. As the patients were laying very closely, transmission based precaution was very much necessary to maintain personal protection. But unfortunately, there was no transmission based precaution in surgery, operation room and post operative ward. Restriction for entry of patients and attendances in DMCH was relapsed. Anyone could enter in the surgery ward and even in the post operative room without any prior permission from the authority. The equipment used in the post operative room was not disinfectant regularly and in this study it was revealed that equipments and surrounding environment of post operative room was a potent source for post operative nosocomail infection. It was also indicated that the patient and the attendance were sleeping in the same bed and sometimes the attendance was using the patient's utensils.It was very unhealthy for the entire environment of post operative room and could be major source for transmission of infection from patient to attendance and vice versa.

Figure 5.1 Photograph showing the placement of patient and attendance in the floor of DMCH.

5.2: Assessment of Risk Factors in Private Managed Hospital

In the study period for collecting the samples from different sources and from patients wounds there were some risk factors identified. First of all, the restriction of entry of visitors. In this study it was already revealed that visitors were the major sources of infections like ESBL, MRSA etc. In HFRCMCH, the restriction of visitors was not well maintained. The entrance of the operation theater was through the post operative room for doctors and nurses. This was also creating a problem in controlling the traffic in post operative room. Hand washing technique was not properly followed. The study has already mentioned that hands were the major sources of nosocomial infection. It is mandatory for nurses and staffs to wear masks in the post operative room. It was observed that nurses and doctors infrequently used masks and if used masks, nostrils were not cover up. The equipments of post operative rooms were not disinfecting everyday or neglect to clean after use. The condition of post operative room is shown in Figure 5.2 and it is revealed from the photograph that the gap between the two beds were very narrow (less the 3 feet).

Figure 5.2 Photograph showing the post operative room of HFRCMCH.

5.3: Comparison and Summary of the Assessment of Risk Factors in Public and Private Hospital

Overcrowding

Public hospital was overcrowded; even patients need to be placed in floor. In private hospital every patient had a bed to place. The gap between a bed was <3 feet (Standard≥3 feet) in private hospital and in public hospital the gap difference was less the 2 feet.

Restriction of entrance of visitors

It was observed that there was very relaxed restriction over the entry of visitors in post operative room of DMCH, whereas in public hospital the entry of visitors was strictly maintained in the duty time.

Hand washing practices

Antimicrobial soap and alcohol based hand rub were frequently used by doctors and nurses in private managed hospital. The basin for hand washing is not properly located and supply of soap and towel was not adequate in HFRCMCH. In public hospital, normal soap along with antimicrobial soap was used infrequently by the doctors and nurses and there were no basin in a ward.

Use of masks and grown

The pressure of visitors, patients, honorary staffs were so intense in DMCH it was really hard for authority to implement masks to prevent transmission based prevention. In private hospital it was experienced that there were a few provisions of masks and gloves for doctors and nurses and even for attendances.

Personal habit and hygiene

Personal habit and hygiene practice in private hospital was slighly improved.

Table 5.1 Comparison of risk factors of government and private hospital.

Variables	DMCH(govt.)	HFRCMCH (private)
Overcrowding	Overcrowded	Not so
Entrance of visitors	Not restricted	Restricted on duty hours
Hand washing practices	Antimicrobial soaps	Alcohol based hand rub
Use of masks and gowns	Not frequently used	Frequently used
Personal hygiene	Not maintained	maintained

5.4: Effect of the Implementation of Hand Washing for Control of Post Operative Nosocomial Infection

Hand washing is one of the major components for infection control in post operative room. Plain soap, antimicrobial soap and alcohol based hand rubs are usually used in clinical settings. In this study it was observed that alcohol based hand rub was the best for reduction of microbial load from hand in private hospital. The first six month (July 2008 to December 2008) in post operative room of HFRCMCH plain soap and antimicrobial soap was used. Then from January 2009 to June 2009 alcohol based hand rub was introduced and there was dramatic reduction in the counts of total and faecal coliform per hand wash of nurse and surgeons; are shown in Figure 5.3.

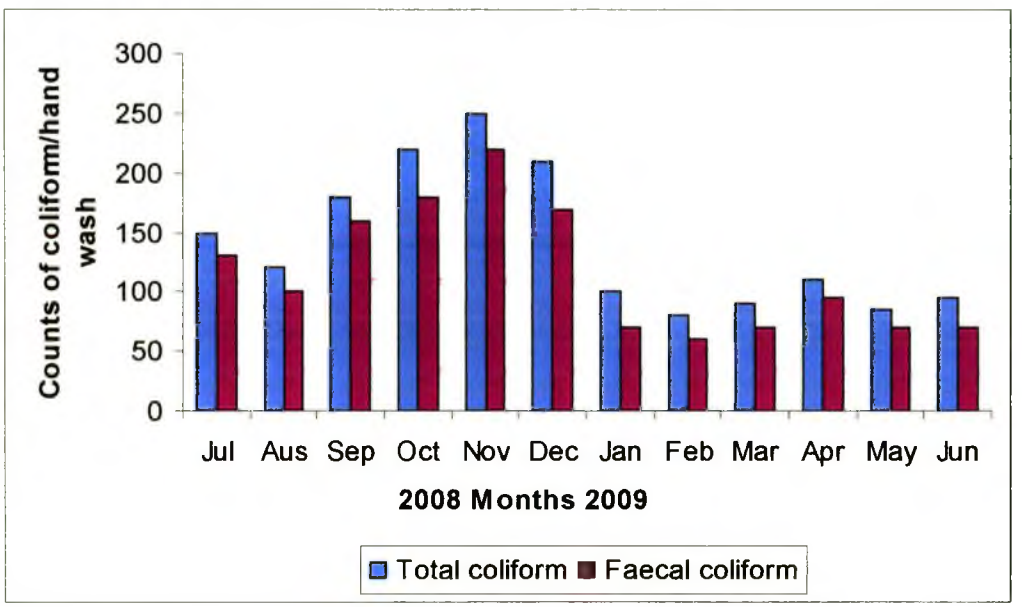

Figure 5.3 Reduction of total and faecal coliform after introducing alcohol based hand rub in private hospital.

On the other hand in DMCH (Figure 5.4), antimicrobial soap was introduced instead of plain soap and effect was observed for six months. It was observed that in DMCH, the reduction of microbes from hands after introducing antimicrobial soap is not significant. It was also concluded that for high turn out of patients and frequent passages of visitors it was not possible to introduce alcohol based hand rub in DMCH properly.

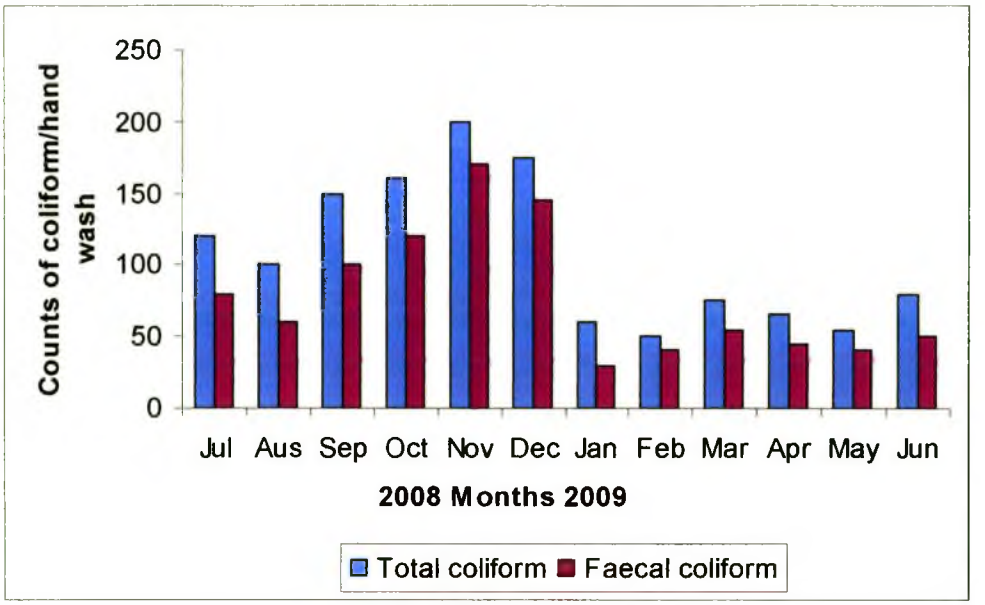

Figure 5.4 Reduction of total and faecal coliform after introducing alcohol based hand rub in public hospital.

5.5 Effect of Implementation of wearing Mask and Gowns for Control of Post Operative Nosocomial Infection

Isolation precaution is one of the major components of control of transmission of nosocomial infection. Here monthly organisms were collected from hospital staffs before introducing proper isolation precaution. In a specific post operative surgical ward of HFRCMCH, in December 2008 number of organisms were high (MRSA) in comparison with proper introduction of standard precaution in January 2009. Only giving emphasis on wearing surgical masks and gowns to health care workers in that particular ward in a month isolation rate of MRSA became nil (Table 5.2).

Table 5.2 Prevalence of organism in anterior nares of hospital staffs before and after isolation precaution in HFRCMCH.

Types of organisms	**Hospital staff** before isolation precaution	**Hospital staff** after isolation precaution
Staphylococcus aureus	5	3
MRSA	4	0
MSSA	1	3
Pseudomonas	1	1
E.coli	1	1
No organism	3	5
Total	10	10

In DMCH, the masks were introduced in February, 2009 as an instrument of isolation precaution to the hospital staffs and attendances and compared the situation with November 2008 when the masks were not properly used. It was observed that by introducing only masks in the post operative ward the prevalence of *Staphylococcus aureus* reduced by half. It was also proved that masks were a useful protector against MRSA (Table 5.3).

Table 5.3 Prevalence of organism in anterior nares of hospital staffs before and after isolation precaution in DMCH.

Types of organisms	**Hospital staff** (before isolation precaution)	**Hospital staff** (after isolation precaution)
Staphylococcus aureus	8	6
MRSA	5	3
MSSA	3	3
Pseudomonas	2	1
E.coli	3	2
No organism	2	6
Total	15	15

5.6 Effect of using Different Disinfectants for Post operative Nosocomial Infection

Three major types of disinfectants have been used for the reduction of microbes on post operative equipments and floors. It was found that after using Hypochlorite solution, Gluteraldehyde and 70% ethyl alcohol there were significant reduction of microbes.

A. Disinfectant used for soiled floor, trolleys, tables, bedside lockers, beds:
Routine cleaning is done by using ordinary detergents (mostly quaternary compounds)
Disinfection is done when spillage of body fluid occurs. Hypochlorite [bleaching liquid (chlorox) 1/10 dilution in water] is used
B. Disinfectant used for semi critical equipments like endotracheal tubes, endoscopes, laryngoscopes etc: Gluteraldehyde (Cidex) is used
C. Unsoiled trolley, ECG machine, Echo machine, stethoscope, thermometer: 70% ethyl alcoho.
In Figure 5.5, it is shown that by using these disinfectants there was slight reduction of microbes from various critical and non critical items of operative and post operative room on DMCH.

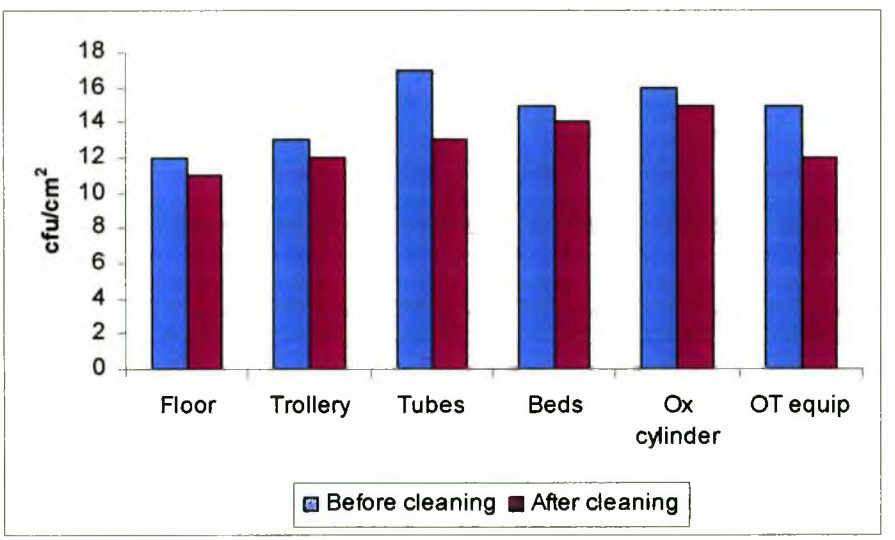

Figure 5.5 The effect of disinfectants on different types of post operative equipments and floors in DMCH.

But on the other hand, by introducing those disinfectants in HFRCMCH there were a significant reduction of microbial load from various critical and non critical items of operative and post operative room (Figure 5.6).

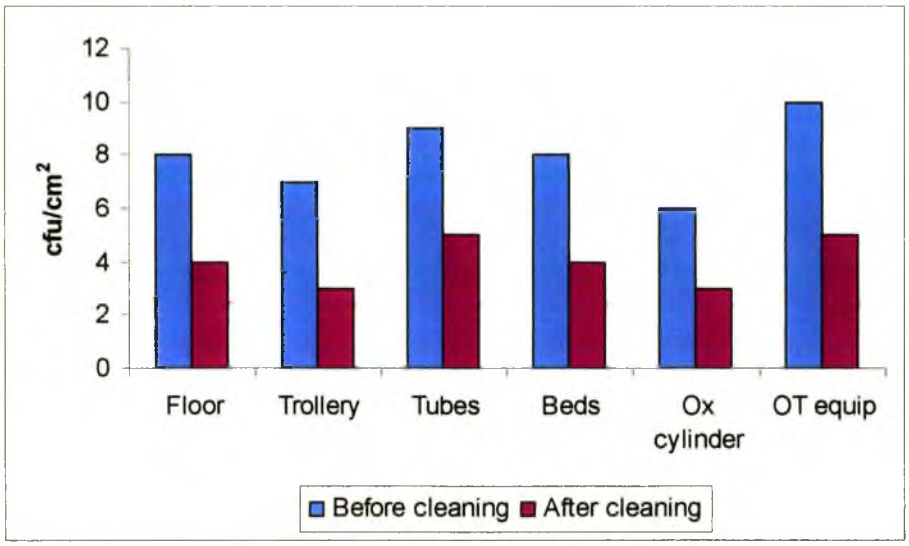

Figure 5.6 The effect of disinfectants on different types of post operative equipments and floors in HFRCMCH.

5.7 Effect of Proper Surveillance for Post Operative Nosocomial Infection

Surveillance is an important factor for controlling infection in post operative ward. After an outbreak of nosocomial infection in a surgery ward by following the below mentioned preoperative, intra operative and post operative risk control measurement; nosocomial infection rate of that ward was reduced.

In preoperative stage, antibiotic prophylaxis and glucose control seemed to be very crucial for all wound patients. Asepsis or preparation against infection was required both in pre operative and operative stage for all patients in both hospitals. In post operative stage,the most important contributors were to be found wound healing and types of closure used (Table 5.4).

Table 5.4 Preoperative post-operative and intra-operative measures were made on the basis of guideline for prevention of surgical site infection.

Preoperative measures	**intra operative measures**	**post operative measures**
Shorten preoperative stay	Asepsis and antisepsis	Wound dressing
antisepsis and asepsis	Good surgical practice	type of closure
antibiotic prophylaxis	fluid resuscitation	tight glucose control
glucose control	Minimize spillage	oxygen supplementation

The Table 5.5 showed that by following appropriate infection control procedures and operative measurements the infection rate in a surgery ward of HFRCMCH was reduced from 5.29% to 3.33%.

Table 5.5 Monthly report of Healthcare associated infection in HFRCMCH.

Private hospital	November 2008	February 2009
Admitted patients	340	270
Total number of Health care associated infection	18	9
Hospital acquired infection rate	5.29%	3.33%

In public hospital like DMCH, it was really hard to implement the basic principals of infection control and assisted all to follow pre-operative, operative and post operative measures. It is shown that by applying some of the measurements of infection control, the hospital acquired infection rate was reduced from 6.8% to 4.1% (Table 5.5).

Table 5.6 Monthly report of Healthcare associated infection in DMCH.

Public hospital	November 2008	February 2009
Admitted patients	220	270
Total number of Health care associated infection	15	9
Hospital acquired infection rate	6.8	3.33%

5.7.1 Waste Management of Post Operative Room of Private and Public Hospitals

Waste management is an important component of post operative room infection control management system. In the selected study period for both the both private and public hospital, two types of waste were collected (Figure 5.7).Most of the wastes were non hazardous (80%) and emphasis was needed for only 15-20% hazardous waste. For proper management of waste in pubic hospital no approved system was functioning whereas in private hospital some of the component of ideal waste management was followed.

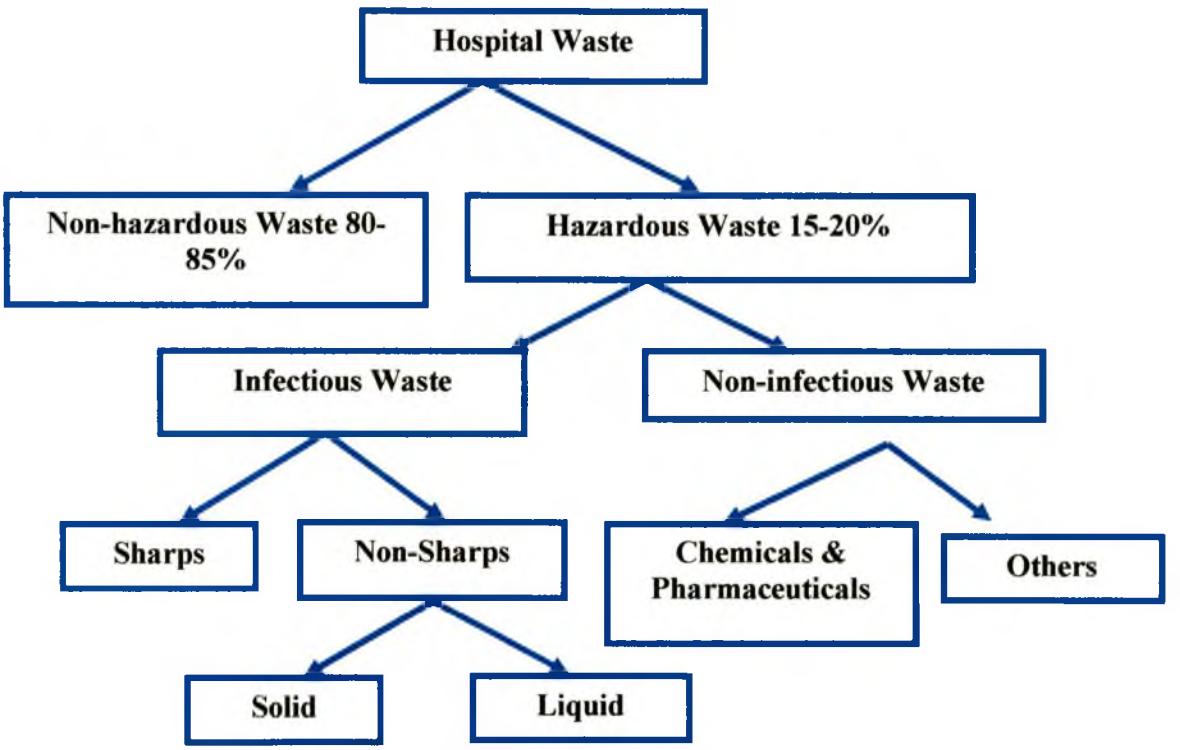

Figure 5.7 Types of wastes collected from post operative room.

Some of the observations regarding waste management in private and public hospitals were performed through out the study period. There were actually no waste management system was functioning in public hospital but in private hospital some of the components of the system were followed.

Figure: 5.8 Photograph showing mixed waste in a bowl a) public managed hospital b) private managed hospital.

5.8. Proposed guideline for the Prevention of Nosocomial Infection for Public and Private hospital

A guideline is proposed by the observation of the present study ewas conducted for both public and private managed hospital to control post operative nosocomial infection. Some of them are only applicable for only private hospital. The elements of guidelines are:

A. Restriction of Visitors

Entrance

1. Visitors will not enter without wearing gown and hospital shoes.
2. No hand carry item is allowed inside. It will be kept at receptionist desk.
3. Alcohol based hand rub will be provided to receptionist who will offer this to each visitor during entrance and exit. (only for private hospital)

Inside the Post operative room

1. In addition to wearing gowns, visitors should also wear Personal protective equipment (PPE) if and when necessary.
2. Visitors must be informed that touching the patient may spread infection and be discouraged to do that.
3. No children (below 7 years) will be allowed inside.
4. Not to provide used gowns to the visitors. After one use each gown will be discarded and sent to laundry.(only for private hospital)

B. Post operative Staff

(Applicable for Doctors, Nurses)

1. To wear prescribed uniforms inside the premises. While going out the premises on temporary basis, to wear a white gown on the top of the uniform.
2. Grown and gloves : To wear grown and gloves when necessary
3. Hand washing is mandatory:
    a. before and after touching each patient
    b. after removing the gloves
4. Hand and finger jewelries are not allowed
5. Not to keep the ID card hanging from the neck. It causes contamination.
6. To wear mask and cap during any suction procedures.
7. To observe good grooming and personal hygiene by all staff.

C. Equipments/ Medications/ Reagents

1. If equipments like ECG, Echo machine, cardiac monitor, portable X-ray, stethoscopes, infusion pumps, thermometer, etc become contaminated disinfection should be done promptly.
2. To write down date of opening of Insulin, Heparin, Xylocaine (reusable items) on the vial. By doing this, use of very old items can be avoided
3. To check the date of expiry of each medicine and to discard outdated ones.
4. To avoid food contamination, pantry should always be kept clean and tidy.
5. No bedpans/urinals should be kept under the patient's bed. Patient's waste materials are to be discarded immediately.

D. Care of the patients

1. If the patient comes from another hospital, he/ she should be kept initially in an isolation room to exclude communicable infection.(only for private hospital)

2. For these referred cases following samples may have to be sent to microbiology lab for culture and sensitivity tests (upon consultant's discretion):

   Example: Bedsore: swab
   Foley's Catheter: urine
   Endotracheal tube: secretions
   CVP line: blood

   If needed, the devices may have to be removed (upon consultant's discretion)
3. For patient who need Contact precaution, Droplet precaution, Airborne precaution
4. Staff assigned to an isolation room/ infectious patient will take special care to avoid contamination of doors knobs/utensils/ equipments etc. She must wash hands before touching such items.

E. After discharge of a patient

5. All items of the room (including chair, bedside equipments, bed, files etc.) should be disinfected with prescribed disinfectants and to keep the room ready for the next admission.
4. Special care for infectious patients: Swab testing (Dept. of Microbiology) is to be done followed by deep cleaning. The room to be kept closed till report from Microbiology lab arrives. Reopening of the room for admission of next patient is done only after getting clearance from microbiology lab.

F Waste Managemant

Four color bins or collection begs can be used instead of normal bins for collection of waste in each ward. These beg were used to separate hazardous and non hazardous waste materials. Any sharp items are placed inside **red** bins. Infectious items or any soaked with body fluids are placed inside **yellow** bins. Non infectious items such as plastic begs, tubes, unbroken vials, liquids are placed inside **blue** bins. Domestic wastes were placed inside **gray** bins. The standard colored bin or beg are shown in Figure 5.9.

Figure 5.9 Ideal colored bins that were introduced in a model ward of HFRCMCH.

Colored bins like that is not possible in public management system of the hospital for expenditure and maintenance in many stations. So, it is important to ensure at least the needle and syringe is separated by using only two separate boxes or bin (Figure 5.10).

Figure 5.10 Needle and syringe collection bin introduced in a model ward of DMCH.

5.9 Discussion

Hand washing is more than half of whole infection control program. After implementing alcohol based hand rub the presence of microbes in hand flora reduced dramatically. It is also observed that alcohol based hand rub is much more effective then antimicrobial soap against microbes of hands. Compliance with hand washing using soap and water by health care workers has been measured below 50% in most observational studies in European and American Hospitals (Rotter, 2001). It is also documented by Rotter (2001) that hand rubbing with alcohol is more efficacious than hand washing with soap in reducing the total microbiological load on health care worker's hands in Intensive and post operative care unit. In public hospital like DMCH, it is not possible to formulate alcohol based hand rub. After introducing antimicrobial soap in a ward of DMCH, the microbial load of hands of health care workers slightly reduced.

The high level of contamination reported on health care equipment in this thesis is of particular concern. A large numbers of potential pathogenic, opportunistic and multi resistant organisms also identified on sampled equipment. Since the emergence of penicillin resistance *S. aureus* in the 1950s, the number of organisms' resistance to antibiotic therapy has increased significantly (Levy, 2002). This increase in microbial resistance has been attributed to the overuse of antibiotics, increasing numbers of immune-compromised and critically ill patients and lapses in infection control, most importantly in terms of hand washing and cleaning of equipment (Schabrum and Chipchase, 2006).Seventy percent alcohol was found to be a highly effective means of reducing contamination on healthcare equipment in the study. Cleaning protocols involving 70% alcohol were more effective than cleaning protocols involving detergent, antiseptic soap, and single and double paper wipes. This finding suggests that regular cleaning of stethoscopes, aurioscopes, diagnostic ultrasound and endoscopes with 70% alcohol is sufficient to reduce the risk of nosocomial infection significantly (Schabrum and Chipchase, 2006).

Healthcare workers are a major route through which patients become infected; microorganisms are transmitted by staff from one patient to another or from the

environment to the patient. In addition, healthcare workers with persistent staphylococcal nasal carriage can also serve as a reservoir from which *Staphylococcus aureus* can be transmitted (Boyce *et al.*,1997). In addition to activities that involve direct contact with patients or body fluids and secretions, touching contaminated environmental surfaces may result in the acquisition of pathogens on the hands (Ray *et al.*,2002).

Isolation, which is proposed by CDC in 1970 as "isolation precaution" in hospitals in 1970 and modified to standard precaution in 1996 (CDC, 1996). In this standard precaution the core object is to prevent patient and protect health care workers from transmission of infection. Masks and gowns are the two most valuable elements of Personal Protective Equipment (PPE) (Corea *et al.*,2003). In this investigation it was proved that after introducing standard precaution (only masks and gowns) the transmission of microbes (especially MRSA) in health care settings (both in DMCH and HFRCMCH) is reduced.

Cleaning and disinfection of environment surfaces are established components of hospital infection control. It is also reasonable to disinfect floors given the minimal cost and added antimicrobial activity. It was found in the investigation that by using disinfectant like hypochlorite solution, gluteraldehyde, the hospital premises are partially free from microbes.Quaternary ammonium compounds and hypochlorite solution used for disinfection of hospital environment and alcohol, chlorhexidine, glutaraldehyde were used for disinfection of hospital equipments in different studies (Dharan *et al.*,1999; Danforth *et al.*,1988). Disinfectants may pose a danger to personnel, patients and the environment and require special safety measures. Today, disinfectants are one of the leading allergens affecting health care personnel (Schnuch *et al.*, 2001).

Environmental surfaces (bedside table, bed nails) in close proximity to the patient have been demonstrated to become contaminated with epidemiologically important microbes such as ESBL and MRSA. This study demonstrate that important hospital acquired pathogens (ESBL, MRSA) can survive on environmental surfaces for an extended period of time and this may allow for environmentally mediated disease transmission. For this

reason, those surface should be disinfected regularly and need to be clean after patient discharge (Rutala and Weber, 2001).

Contact isolation in a single room with barrier precautions, including wearing a grown when entering the room and emphasis on gloves and hand disinfection, are highly recommended. However, in sharp contrast, multi resistant bacteria are also isolated from the environment and would recommend barrier precautions to be taken only when there is close contact with the patient. Hence, single room isolation does not seem to be necessary. Implementing this recommendation would have benefits including cost savings, reduction of stress to the patient and reduced workload for hospital personnel (Lemmen *et al.*, 2004).

The next important component of infection control program is surveillance. A successful surveillance program includes standardized definitions of infection, effective surveillance methods and stratification of the infection rate according to risk factors associated with the development of post operative surgical site infection. Surveillance with feedback of information to surgeons and other relevant staff has been shown to be an important element in the overall strategy to reduce the number of post operative surgical site infection (Bruce *et al.*,2000).

Post operative waste is an important element and reservoir for nosocomial infection. So, a proper management is needed. By introducing color labeled bins or begs for separation of infectious waste from non infectious waste, a good waste management program can be achieved. Before throwing the infectious waste, they need to be disinfected. Syringe and needle has to be separated and with needle cutter machines or other means the sharp end of the needle has to be broken. Otherwise it can be a potent source for transmission of various deadly contagious diseases like, HIV, Hepatitis B etc. In this study it was revealed that private hospital followed some of the components of waste management but unfortunately government hospitals has not yet formulate system for proper management of waste.

A guideline has been proposed to control the transmission of post operative nosocomial infection. Hospital staffs including doctors, nurses and attendances should be aware of the guideline and they need to follow it when it requires.

The present condition of hospitals in Bangladesh in respect of controlling nosocomial infection is in embryonic stage. So, it is necessary to take specific preventing measures by forming infection control team in each institute. The duty of the infection control team will be follow and implement one or two basic infection control measures and advocate the hospital authority as well as hospital staffs to properly follow it. For implementing this infection control program, identification of risk factors will be the primary goal. These risk factors are not prevailing in everywhere in same extent. In public hospital like DMCH it is really hard to implement all components of the program, whereas in private hospital like HFRCMCH it can be followed more accurately.

CHAPTER 6

CHAPTER 6

GENERAL DISCUSSION

Advances in infection control practices include improved operating room ventilation, sterilization methods, barriers, surgical technique, and availability of antimicrobial prophylaxis. Despite these activities, post operative infections remain a substantial cause of morbidity and mortality among hospitalized patients (Hernandez *et al.*,2005).There also are increased numbers of prosthetic implant and organ transplant operations performed. Thus, to reduce the risk of post operative surgical site infection a systematic but realistic approach must be applied with the awareness that this risk is influenced by characteristics of the patient, operation, personnel, and hospital.

Nosocomial pathogens usually are bacteria that survive the high antibiotic selection pressure in hospitals and therefore are multidrug resistance. *E. coli* may collect certain intregron elements that can exist without much need of antibiotic selection and high prevalance of integron elements has a cumulative effect. Although there is a chance of finding the presence of intregron elements in the absence of antibiotic selection pressure, the high percent of integron intregase (70%) observed in this study indicates there has already a strong selection pressure in the communiy (Pongpech *et al.*, 2008). Different study have suggested that integrons are directly responsible for resistance to Trimethoprim and Aminoglycosides and the presence of integrons is significantly associated with multidrug resistance in commensal *E. coli* isolates (Lee *et al.*,2006); which is also obwerved in this study. Class I integron is also widely disseminated among clinical isolates of Gram-positive bacteria (Shi *et al.*,2006).

Since antibiotic resistance is a major phenotypic trait particularly for the clinical isolates, it has a potential interest in exploring the characteristic of these ESBL producing isolates of *E. coli*. In the present study, most ESBL producers collected from patients in the post operative room and surgical ward. These patients were exposed to great antibiotic pressure. The susceptibility test results showed that all the ESBL producing isolates were resistance to 3rd generation Cephalosporin, This reflected the relationship between the

ESBL and to 3rd generation Cephalosporins. Increased resistance might be due to extensive use of 3rd generation Cephalosporins and other β- lactum drugs. All the ESBL producing isolates were resistance to 4th generation Cephalosporins and Monobactams (Aztreonam). All the ESBL producing *E. coli* was resistance to all available antibiotic discs used except one isolates that was found to be sensitive to Ciprofloxacin and two isolates that were sensitive to Gentamycin. This finding justifies that ESBLs producing *E.coli* are multidrug resistance. Infection caused by ESBL producing organisms have currently been treated with Carbepenems such as Imipenem (Pitout *et al.*, 1998). Resistance pattern of antibiotics to microbial flora of nosocomial infection revealed in the present study that most of the isolates were resistance to at least 3 drugs and maximum isolates were resistance to 6 to 7 drugs whereas some of them were found to be resistance to all drugs. *E. coli, S. aureus*, *Proteus* and *Pseudomonas* all of the organisms of nosocomial infection showed multidrug resistance.

Gene coding ESBL are usually located on conjugative plasmids although many of the most recently described ESBL genes are frequently found within integron like structure. (Canton *et al.*, 2003 and Bonnet *et al.*, 2004). Integrons are naturally efficient recombination and expression systems able to capture genes as part of genetic elements known as gene cassettes. Out of five integrons, Class 1 integrons are the most commonly prevalent in nosocomial and community environments. ESBL located on integrons-like structure are being increasingly reported world wide (Rowe Mangus and Mazel,2002). Besides this, molecular characterization of plasmids isolated from different clinical strains showed that the resistance gene was located together with the other resistance determinants near class1 integrons (Jones *et al.*, 2005).

Class 1 integrons of *E. coli* is closely associated with human related environments that are more likely affected by antibiotic selective pressures. Two pressures might be at work. The presence of antibiotics selecting some genes associated to integrons, such as Sulfonamide resistance genes or any mobile cassette acting as a facilitator and promoting their retention. As gene cassettes within *E. coli* integrons appear to be mostly related to antibiotic resistance traits, it can be presumed that antibiotics exert the main pressure that

selects and or maintains integrons in *E. coli.* Together this indicates that lifting the selection pressure posed by antibiotics might not reduce the prevalance of resistance itself, but instead that of some of the mobile genetic elements by which resistance can be transferred (Diaz-Mejia *et al.*, 2008) .The second possibility is that the sparse distribution of the *intl1* PCR positive isolates among non human *E. coli* strains might be a consequence of the lesser ability of those strains to acquire integrons, or simply that do not interact with integron bearing organisms rather than a higher loss rate. Furthermore, integrons have been found in a wide variety of environments with and without human influences, so that these elements are readily available for non human strains (Diaz-Mejia *et al.*,2008).

Plasmid profile analysis is well documented and meaningful in epidemiological studies of enteric pathogens, particularly if there is a broad spectrum of plasmid patterns in bacterial population (Mayer, 1988). When used as a fingerprint for strains, the plasmid profile may aid in differentiation of strains, identifying a source of infection or evaluating the efficacy of control measure (Takeda *et al.*, 1991). In this study, similar pattern plasmid could establish a definite relationship between plasmid pattern and antimicrobial resistance. Plasmids are responsible for transfer of drug resistance to other organisms in hospitals and community. A number of studies reported that the ESBL marker was also transferred through plasmid. *E. coli* j53 strain was used successfully for transfer of Ceftazidime resistance in different strains (Hernandez *et al.*, 2005).

The development of DNA fingerprinting schemes for the typing of bacteria has in part been stimulated by the view that phage typing or Serotyping no longer occupies the ''gold standard'' position as a high-resolution discriminator between strains. Thus, for *E. coli*, PFGE of genomic DNA digested by rare cutter restriction enzymes has been widely used as a DNA fingerprinting method and has shown itself to have advantages over other DNA-based methods, such as plasmid analysis, which in essence tracks plasmids rather than their hosts; conventional RFLP analysis, which requires the analysis of complex banding patterns; and ribotyping, which is labor-intensive and slow (Bannerman *et al.*, 1995).

Clinical samples *E. coli* (E-14 and E-10) were similar in term of ESBL pattern, plasmid pattern , presence of resistance gene *IntI1* and PFGE pattern with the environmental samples (E-14 and E-19). So, it correlates between the clinical and environmental sample. Similarly, *S. aureus* isolated from patient wound (S-1) showed similar antibiogram pattern (MRSA), similar coagulase typing (Type VI) and Latex agglutination test positive with the sample isolated from anterior nares of staffs (S-4).

Responsible administration of antibiotics in medical facilities and regular monitoring of bacterial resistance is the basis for sound antibiotic policy. Using this approach, it is sometimes possible to predict adverse trends in resistance and to limit the spread of resistance organisms (Kolar and Latal,1999). In this study it was observed that multidrug resistance was the central problem of post operative patient and it is really hard to find a suitable drug for treating those patients. Antibacterial prophylaxis of post operative infection is now accepted as a routine part of surgical practice in clean contaminated operations as well as some clean procedures. In the case of contaminated or dirty operations, bacterial contamination or infection is already established before the start of surgery and the peri-operative administration of antibiotics is actually therapeutic and for a prophylactic measure (Lalla, 2002).

Surgical antimicrobial prophylaxis (AMP) refers to a very brief course of an antimicrobial agent initiated just before an operation begins. Bumpous and Johnson (1995) mentioned that AMP is not an attempt to sterilize tissues, but a critically timed adjunct used to reduce the microbial burden of intra operative contamination to a level that cannot overwhelm host defenses. AMP does not pertain to prevention of surgical infection caused by postoperative contamination. Intravenous infusion is the mode of AMP delivery used most often in modern surgical practice (Nooyen *et al.*, 1994).AMP is sometimes indicated for operations that entail incisions through normal tissue and in which no viscus is entered and no inflammation or infection is encountered. Two well-recognized AMP indications for such clean operations are: (1) when any intravascular

prosthetic material or a prosthetic joint will be inserted, and (2) for any operation in which an incisional or organ/space SSI would pose catastrophic risk (Page *et al.*,1993).

Cephalosporins are the most thoroughly studied AMP agents. These drugs are effective against many gram-positive and gram-negative microorganisms. They also share the features of demonstrated safety, acceptable pharmacokinetics, and a reasonable cost per dose. In particular, Cefazolin is widely used and generally viewed as the AMP agent of first choice for clean operations. If a patient is unable to receive a cephalosporin because of penicillin allergy, an alternative for gram-positive bacterial coverage is either Clindamycin or Vancomycin (Nichols, 1995).The routine use of Vancomycin in AMP is not recommended for any kind of operation However, Page and coworkers (1993) mentioned that Vancomycin may be the AMP agent of choice in certain clinical circumstances, such as when a cluster of MRSA mediastinitis or incisional Surgical site infection due to methicillin-resistant coagulase-negative staphylococci has been detected.

Sterile surgical gowns and drapes are used to create a barrier between the surgical field and potential sources of bacteria. Gowns are worn by all scrubbed surgical team members and drapes are placed over the patient. Gowns and drapes are classified as disposable (single use) or reusable (multiple use). Regardless of the material used to manufacture gowns and drapes, these items should be impermeable to liquids and viruses (Moylan *et al.*,1987).

In both private and government hospitals sterile gloves are put on after donning sterile gowns. A strong theoretical rationale supports the wearing of sterile gloves by all scrubbed members of the surgical team. Sterile gloves are worn to minimize transmission of microorganisms from the hands of team members to patients and to prevent contamination of team members' hands with patients' blood and body fluids. If the integrity of a glove is compromised (e.g., punctured), it should be changed as promptly as safety permits (Dodds *et al.*,1988).

The wearing of surgical masks during operations to prevent potential microbial contamination of incisions is a longstanding surgical tradition (Mitchell *et al.*,1991). Nevertheless, wearing a mask can be beneficial since it protects the wearer's nose and mouth from inadvertent exposures (i.e., splashes) to blood and other body fluids. Masks in combination with protective eyewear, such as goggles or glasses with solid shields, or chin-length face shields are worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious material may be generated and eye, nose, or mouth contamination can be reasonably anticipated (Tunevall and Jorbeck, 1992). It is found that the practice of wearing surgical masks during operation is more frequent in private hospital than that of public one.

Asepsis by all scrubbed personnel is the foundation of surgical site infection prevention. Others who work in close proximity to the sterile surgical field, such as anesthesia personnel who are separated from the field only by a drape barrier, also must abide by these principles. Post operative infections have occurred in which anesthesia personnel were implicated as the source of the pathogen (Bennett et al., 1995). Herwaldt and coworkers (1996) observed that lack of adherence to the principles of asepsis during such procedures including use of common syringes and contaminated infusion pumps, and the assembly of equipment and solutions in advance of procedures have been associated with outbreaks of postoperative infections.

Surveillance of nosocomial infections, by itself, may decrease the incidence. When each surgeon is provided with their own wound-infection rates and with other surgeons' rates for comparison, the institutional surgical-wound infection rate decreases. Outbreak control includes early identification of potential outbreaks, as well as evaluation and intervention if an outbreak is identified. Continuing education of hospital staff about the importance of, and their role in, preventing nosocomial infections is necessary (Mertens, 1994).

The type of postoperative incision care is determined by whether the incision is closed primarily (i.e., the skin edges are re-approximated at the end of the operation), left open

to be closed later, or left open to heal by second intention. When a surgical incision is closed primarily, as most are, the incision is usually covered with a sterile dressing for 24 to 48 hours. Beyond 48 hours, it is unclear whether an incision must be covered by a dressing or whether showering or bathing is detrimental to healing (DuMortier,1933). When a surgical incision is left open to heal by second intention, it is also packed with sterile moist gauze and covered with a sterile dressing (Kravitz , 1996).

Excellent surgical technique is widely believed to reduce the risk of surgical site infection (Zacharias and Habib,1996). Such techniques include maintaining effective hemostasis while preserving adequate blood supply, preventing hypothermia, gently handling tissues, avoiding inadvertent entries into a hollow viscus, removing devitalized (e.g., necrotic or charred) tissues, using drains and suture material appropriately, eradicating dead space, and appropriately managing the postoperative incision. In the study observed that experienced surgeons had less chance of developing post operative infection then junior registers. Any foreign body, including suture material, a prosthesis, or drain, may promote inflammation at the surgical site and may increase the probability of post operative infection (Smilanich *et al.*,1996).

Surgical personnel who have active infections or are colonized with certain microorganisms have been linked to outbreaks of post operative infection.It is important that healthcare organizations implement policies to prevent transmission of microorganisms from personnel to patients. These policies should address management of job-related illnesses, provision of post exposure prophylaxis after job-related exposures and, when necessary, exclusion of ill personnel from work or patient contact (Viglionese *et a*l.,1991).

Drains placed through an operative incision increase incisional surgical site infection risk. It appears in the study that post operative infection risk also decreases when closed suction drains are used rather than open drains. Closed suction drains can effectively evacuate postoperative hematomas or seromas, but timing of drain removal is important. Bacterial colonization of initially sterile drain tracts increases with the duration of time the drain is left in place (Dougherty and Simmons, 1992).

Several antiseptic agents are available for preoperative preparation of skin at the incision site (Ritter *et al.*, 1980). Through out the study period the iodophors (e.g , povidone-iodine), alcohol-containing products, and chlorhexidine gluconate were the most commonly used agents and they have tremendous effect on reducing bacterial load from different inanimate and animate objects of post operative room of DMCH and HFRCMCH.

An effective infection-control program requires dedicated staff with appropriate training and sufficient resources. The number of personnel is determined by the size and complexity of the facility. Infection-control practitioners are responsible for program activity. In larger hospitals, program leadership is provided by a physician with training in epidemiology and infection control. Smaller facilities may obtain such expertise by contractual arrangement with outside experts. Oversight of the infection-control program is usually provided by a multidisciplinary infection-control committee. The program director, however, should report directly to senior hospital management to ensure optimal program effectiveness.

Institutional policies and practices must be developed and adhered to. In particular, optimal hand washing and glove use must be facilitated and reinforced, as transmission of organisms between patients occurs primarily on the hands of staff members. Isolation guidelines to identify and segregate patients who have an increased risk of transmitting infection to other patients or staff are also essential. Other important policies include: for urinary infection, the use and care of the indwelling catheter; and for surgical wound infection, optimal surgical technique including preoperative preparation and prophylactic antimicrobials. Many national or local standards and regulations will also prevent nosocomial infection, and institutions must be in compliance. These regulations cover hospital construction, municipal water supply, laundry management, food handling, waste disposal, sterilization and other reprocessing procedures, as well as standards for pharmacy and microbiology laboratory practice.

It can be concluded that prevention of nosocomial infections requires a systematic, multidisciplinary approach which is usually achieved under the leadership of an

institutional infection-control program. The principle activities of such a program will be surveillance, risk factors identification, outbreak management, policy development, expert advice, and education. In both government and private hospital the practice of infection control is not adequate. The proposed guideline of the study will help to crate a formula on controlling post operative nosocomial infection in both government and private hospital. In government hospital, gradually the integral part of infection control should be formulated and always try to implement first two or three basic principals like; hand washing, waste management, proper use of disinfectants, etc. It is necessary for health care workers to keep in mind that patients come to hospitals to get rid of infection, not to get the infection from hospitals.

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APPENDIXES

Appendix- A

Media composition

The composition of the media used in this thesis work is given below:

Unless otherwise mentioned all media were autoclaved at 121^0C for 15 minutes at 15 lbs pressure.

1.1 Muller-Hinton Agar (DIFCO)

Beef infusion	10.0g
bacto casammo acid	10.0g
Starch	30.0g
Bacto agar	10.0g
Distilled water	1000ml
P^H	8.4

1.2 MacConkey agar (DIFCO)

Peptone	17.0g
Protease peptone	3.0g
lactose	10.0g
Bile salt no.3	1.5g
Sodium chloride	5.0g
Agar	13.5g
Neutral red	0.03g
Crystal violet	0.001g
Distilled water	1000ml
P^H	7.1±0.2

1.3 Blood Agar base (DIFCO)

Tryptone	14.0g
Peptone	4.5g
Yeast extract	4.5g
NaCl	5.0g
Agar	12.5g
Distilled water	1000ml
P^H	7.3±0.2

1.4 Nutrient Agar (DIFCO)

Peptone	5.0g
Bef extract	3.0g
NaCl	5.0g
Agar	15.0g
Distilled water	1000ml
P^H	7.2

1.5 Mannitol Salt Agar (DIFCO)

Protease peptone	10.0g
Beef extract	10.0g
D -Mannitol	10.0g
NaCl	75.0g
Phenol red	0.025g
Agar	20.0g
Distilled water	1000ml

1.6 T_1N_1 broth (DIFCO)

Trypticse	10.0g
NaCl	10.0g
Distilled water	1000ml
P^H	7.5

1.7 Sabouraud dextrose agar (DIFCO)

Neopeptone	10.0g
Dextrose	20.0g
Agar	17.0g
Distilled water	1000ml
P^H	6.8-7.0

1.8 Loeffler's serum (DIFCO)

To make 15ml slopes

Sterile glucose broth	10ml
Sterile sheep, bovine and horse serum	30ml
P^H	7.0-7.4

1.9 Nutrient agar slant (Oxoid)

Dehydrated blood agar base	40.0g
Distilled water	1000ml
P^H	7.4

1.10 MFC agar (DIFCO)

Tryptose	10.0g
poly peptone	50.0g
Yeast extract	03.0g
Sodium chloride	05.0g
Lactose	12.5g
bile salt	1.5g
Analine blue	0.1g
Distilled water	1000ml
Agar	115g
pH	7.4

Distribute into small 1"x ½" size screw-capped vials and autoclaved at 121^{0}C fro 15 minutes under 15 lbs pressure. The vials were kept in slanting position till media were solidified

Appendix- B

Buffers and reagents

M Tris-Cl

121.1 g of Tris-base was dissolved in 800 ml of distilled water. The pH was adjusted to the desired value by adding concentrated HCl and the final volume was made up to 1 liter with distilled water. The solution was sterilized by autoclaving and was stored at room temperature.

Phosphate buffered saline (PBS)

PBS was prepared by dissolving 8.0 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 2.0 g of KH_2PO_4 in 800 ml of distilled water. The pH was adjusted to 7.4 with HCl. The final volume was adjusted to 1 liter by distilled water. The solution was sterilized by autoclaving and was stored at room temperature.

Normal saline

8.5 gm of Nacl mixed and staired with 1 liter distilled water. pH was adjusted to 7.8

0.5 M EDTA

37.24 g of EDTA was dissolved in 150 ml of distilled water and adjust the pH to 8.0 with pellets of NaOH. Adjust the volume up to 200 ml and autoclaved.

TE buffer

TE buffer (10 mM Tris-Cl/1mM EDTA, pH 8.0) was prepared was prepared by diluting concentrated stocks of 1 M Tris-Cl and 0.5 M EDTA in distilled water. The buffer was autoclaved and was stored at room temperature.

10 x TBE

54.0 g of Tris-base, 27.5 g of boric acid and 20ml of 0.5 M EDTA (pH 8.0) were taken and distilled water was added to the mixture to make 500 ml. The buffer was stored at room temperature.

Sodium pentobarbital solution

250 ml Sodium pentobarbital solution contains 16.19 g of sodium pentobarbital, 25 ml of absolute ethyl alcohol, 50 ml of propylene glycol and 5 ml of benzyl alcohol

Gel loading buffer

10 x concentrated loading buffer consisted of 800µl of 20% Ficoll 400, 400 µl of 0.1 M EDTA (pH 8.0), 10 µl of 0.25% bromophenol blue and 200 µl of 1% SDS in 590 µl of distilled water. It was stored at 4°C in 1 ml aliquot.

Ethidium bromide solution

2.5 mg of ethidium bromide (Sigma, USA) was dissolved in 5 ml of distilled water at a concentration of 0.5 mg/ml. This solution was covered with aluminum foil and stored at room temperature.

Methyl red reagent

0.01 g of methyl red was dissolved in 30 ml of 95% ethanol. Then distilled water was added to make the final volume 50 ml. This reagent was covered with aluminum foil and stored at 4°C.

Oxidase reagent

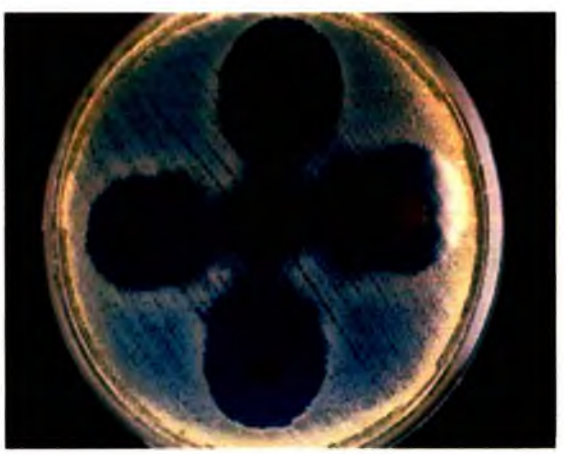
100 mg of N,N,N^{1},N^{1}-tetramethyl-p-phenyldiamine-dihydrochloride was dissolved in 10 ml of distilled water and covered with aluminum foil. Then the solution was stored at 4°C.

Appendix C

3.1 Detection of ESBL by double disk synergy technique

1. Inoculate the test organism on Mueller- Hinton media
2. Place Augmentin disk (amoxicillin 20 micro gm and clavulanic acid 10 micro gm) in the center of the plate
3. Place cefotaxime (30), ceftazidime (30), aztreoman (30) and ceftriaxone (30) 30 centimeter away from augmantin disk (from center to center)
4. Incubate at 37^0C overnight
5. Look for band formation between augmentin and any other disc.

Detection of ESBL
Double Disc Diffusion Technique



Key

AMC-Amoxycillin and clavulanic acid. CAZ-Ceftazidime. ATM-Aztreonam, CTX-Cefotaxime, CRO-Ceftriaxone,

3.2 Zone size interpretation chart

Zone size interpretation chart for some commonly used antimicrobial agents based on results obtained using Muller Hilton Agar.

Antimicrobial agent	Zone diameter nearest whole mm			
	Disc content	Resistant	Intermediate	Sensitive
Ampicillin	10 µg	11 or less	-	14 or more
Bacitricin	10 µg	8 or less	9-12	13 or more
Ceftazidime	30 µg	14 or less	15-17	18 or more
Cephalexin	30 µg	14 or less	14-17	18 or more
Chlotamphenicol	30 µg	12 or less	13-17	18 or more
Gentamycin	10 µg	12 or less	13-14	15 or more
Nalidixic acid	30 µg	13 or less	14-18	18 or more
Novobiocin	10 µg	17 or less	18-21	22 or more
Ceftriazone	30 µg	13 or less	14-20	21 or more
Tetracycline	30 µg	14 or less	15-18	19 or more
Penicillin- G	10 µg	14 or less		15 or more

Appendix D

Solution I:

500 ml preparation of stock solution (needs to be autoclaved)

50mM glucose 94.504 gm/500ml)

25mm Tris HCl P^H -8.0 (1.5137 gm)

10mm EDTA P^H- 8.0(1.8612 gm)

↓

Add these three in a beaker, strire well after adding 300 ml distilled water.

↓

Adjust PH:8 using concentarted HCl

↓

volume upto 500ml

Solution II:

Freshly prepared (not to be autoclaved)

0.2 N NaOH (from 10N stock)

1%SDS (from 20% stock0

Preparation: Add 1 ml to 49 ml distilled water to reach 0.2 N

Discard 2.5ml. Then add 2.5ml of 20% SDS

Solution III:

250 ml preparation of 3M K- Acetate (need to be autoclaved)

Preparation: 7.6 gm K-acetate

↓

Add 150 ml distilled water

Adjust P^H with 5 M Acetic acid to 4.8

↓

Volume up to 250m

Appendix E

Data collection form (English and Bengali)

Hospital		__ __
Unit		__ __
Patient Identification		__ __ __
Age	(years)	__ __ __
Gender	☐ male ☐ female	__
Date of admission (in the hospital)	(dd/mm/yy)	__ __ __ __ __ __
Date of discharge (from the unit)	(dd/mm/yy)	__ __ __ __ __ __
Date of operation	(dd/mm/yy)	__ __ __ __ __ __
Main procedure	(code)	
Wound class	☐ Clean ☐ Contaminated ☐ Clean-contaminated ☐ Dirty/infected	__
ASA score	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5	__
Duration of operation	(minutes)	__ __ __
Urgent	☐ Yes ☐ No	__
Prosthesis/implant	☐ Yes ☐ No	__
Multiple procedures	☐ Yes ☐ No	__
Coeliosurgery	☐ Yes ☐ No	__
Antimicrobial prophylaxis	☐ Yes ☐ No	__
Starting date	(dd/mm/yy)	__ __ __ __ __ __
Duration	(days)	__ __
Surgical site infection	☐ Yes ☐ No	__
Date of infection	(dd/mm/yy)	__ __ __ __ __ __
Infection site	☐ superficial ☐ deep ☐ organ/space	__
Microorganism 1		__ __ __
Microorganism 2		__ __ __
Date of last contact	**(dd/mm/yy)**	__ __ __ __ __ __

সার্জিক্যাল ক্ষতের সংক্রামন সর্তকীকরণের জন্য ফরম

হাসপাতালের নাম

বিভাগ

রোগী ঃ

নাম ঃ

বয়স ঃ (বৎসর)

লিঙ্গ ঃ ☐ পুরুষ ☐ মহিলা

ভর্তির তারিখ ঃ (হাসপাতালে) দিন / মাস / বৎসর

ছাড়পত্রের তারিখ ঃ (বিভাগ হতে) দিন / মাস / বৎসর

অপারেশন ঃ

অপারেশনের তারিখ - দিন / মাস / বৎসর

অপারেশনের পদ্ধতি ঃ

ক্ষতের শ্রেণীবিভাগ ☐ পরিস্কার ☐ সংক্রামক

☐ পরিস্কার - সংক্রামক ☐ দূষিত / সংক্রামক

আসা স্কোর - ☐ ১ ☐ ২ ☐ ৩ ☐ ৪ ☐ ৫

অপারেশনের স্থায়িত্ব - মিনিট

অত্যাবশ্যক ☐ হ্যাঁ ☐ না

প্রসথেসিস / ইমপ্ল্যান্ট ☐ হ্যাঁ ☐ না

বহুবিধ কার্যপ্রণালী ☐ হ্যাঁ ☐ না

কোলিত্ত সাজরী ☐ হ্যাঁ ☐ না

এন্টিবায়োটিক

রোগ নিবারক হিসাবে জীবানু নাসক ঔষধের প্রয়োগ ☐ হ্যাঁ ☐ না

শুরুর তারিখ ঃ দিন / মাস / বৎসর

স্থায়িত্ব ঃ দিন

সার্জিক্যাল ক্ষতের সংক্রামন ঃ

সার্জিক্যাল ক্ষতের সংক্রামন ঃ ☐ হ্যাঁ ☐ না

সংক্রামণের তারিখ দিন / মাস / বৎসর

সংক্রামণের অবস্থান ☐ উপরিভাগে ☐ গভীরে ☐ অঙ্গ / স্পেইস

অনুবীক্ষণীক জীবানু ১

অনুবীক্ষণীক জীবানু ২

শেষ সাক্ষাতের তারিখ দিন / মাস / বৎসর