A STUDY ON NOSOCOMIAL INFECTIONS AND ITS CONSEQUENCES

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Abstract

Nosocomial infection is an endemic problem encountered in hospitalized patients all over the world including Bangladesh. The present prospective study was carried out on 152 patients who were admitted in Dhaka Medical College Hospital and BIRDEM Hospital, Dhaka from November 1997 to June 1999 to investigate the organisms responsible for nosocomial infection. The main objectives of the study were to determine the common microorganisms responsible for nosocomial infection and to determine the antibiogram pattern of the organisms. Samples were collected from the postoperative wounds, post catheterized urinary tract infection (UTI) and diabetic wounds. The patients without postoperative wound infection were taken as control group. An attempt was made to trace the sources of infection by collecting the samples from the different objects of the hospital environment. The collected samples were cultured and isolated organisms were identified by colony morphology, gram staining and necessary biochemical tests. The identified organisms were tested for antibiogram pattern, plasmid profile and restriction fragment length polymorphism (RFLP).

The predominating organisms responsible for nosocomial infection in this study was *Esch. coli.* (55.9%). The other organisms were *Pseudomonas* sp. (33.3%), *Proteus* sp. (12.7%), *Staphylococcus* sp. (15.9%), *Klebsiella* sp. (4.9%) and *Acinetobacter* sp. (3.9%). The sensitivity pattern of the isolated organisms to the commonly used antibiotics ranged from 0 to 80.0%. It was also observed that the resistance pattern of the organisms isolated from the second samples (i.e. the samples collected 5-7 days after the collection of the first sample) markedly increased. In case of *Esch. coli,* the increased rate of resistance to ceftriaxone (from 43.9 to 73.9%; $p<0.01$), ceftazidime

(from 28.1 to 65.2%; $p<0.002$), ciprofloxacin (from 70.2 to 100.0%; $p<0.005$) and gentamicin (from 64.1 to 91.3%; $p<0.008$) was statistically significant. In case of *Pseudomonas* sp., there was significant increase of resistance to ciprofloxacin (from 58.8 to 92.0%; $p<0.004$). It was also observed that resistance pattern of *Esch. coli* and *Pseudomonas* sp. isolated from different sites of infection varied. In the study, it was evident that the mean duration of hospital stay increased significantly ($p<0.001$) in infected patients as compared to that of postoperative non-infected patients. The duration of hospital stay also increased significantly ($p<0.001$) in case of infection by multiple organisms than that of single type of organisms.

An attempt was made to find out the sources of nosocomial infection by analyzing the antibiogram, plasmid profile and RFLP pattern of the organisms isolated from the patients and different objects of the hospital environment. In the present study, the source of nosocomial infection could not be determined by above methods due to inadequacy of resources, time and technical facilities.

The findings of the present study, therefore, revealed the prevalence of multi-drug resistant organisms in the hospital environment causing nosocomial infection. Among the various contributory factors, the indiscriminate use of several antibiotics in the hospitals could be an important factor for development of multi-drug resistant organisms in hospital environment. The findings of the study will help to formulate a guideline for taking some preventive programme and shall also serve as a useful basis for future research and planing for the control of nosocomial infection at different hospitals in Bangladesh.

Chapter I

Introduction

Nosocomial infection is derived from the Greek word "nosos" means disease and " Komeo" mean attend to. It is often referred to as hospital acquired infection. It is defined as infection which is acquired by the patients while they are in hospital or by staffs who are working in the hospital. If a patient acquire infection more than 72 hours after admission, it is considered to be a hospital-acquired infection (Caddow.1989). The National Nosocomial Infection Surveillance (NNIS) system of the U.S.A. defines nosocomial infection as a localized or systemic condition that results from adverse reaction to the presence of agent (s) or its toxin (s) and that was not present or incubating at the time of admission in the hospital (Garner *et al.,* 1988). For most bacterial nosocomial infection, this means that the infection usually becomes evident 48 hours (i.e. typical incubation period) or more after admission in the hospital. However, because the incubation period varies with the type of pathogen and to some extent with the patients underlying condition, each infection must be assessed individually for evidence that links it to the hospitalization. There are two special situations in which an infection is considered nosocomial- a) infection that is acquired in the hospital but does not become evident until after hospital discharge and b) infection in a neonate that results from passage

through the birth canal. There are two special situations in which an infection is not considered as nosocomial. A) Infection that is associated with a complication or extension of infection already present on admission, unless a change in pathogen or symptoms strongly suggests the acquisition of a new infection and b) in an infant, an infection that is known or proved to have been acquired transplacentally (e.g. Rubella, Cytomegalo virus or syphilis) and becomes evident at or before 48 hours after birth. (Garner *et al.,* 1988).

Nosocomial infections are problems of increasing importance in hospital practice. In 1980 the first national prevalence study of infection was undertaken in England and Wales (Meers *et al.,* 1981). The study showed that 19.1% of the patients were infected. Half of these (51.9%) were community-acquired infections and the rest (48.1%) were hospital-acquired infections. In America, the National Nosocomial Infection Surveillance (NNIS) system began in 1970 and selected US hospitals began routinely reporting their nosocomial infection surveillance data to Centre for Disease Control (CDC) for aggregation into national database. It is currently the only source of national data on the epidemiology of nosocomial infections in the United States. In America, the nation wide nosocomial infection rate was approximately 5.7 per hundred admissions (Haley *et al.,* 1985). A surveillance study conducted by Westwood *et al.,* (1974) on infections at the Ottawa general hospital showed that the overall infection rate was 13.5% of which 5.6% was community acquired while

7.9% was nosocomial origin. It also showed that Urinary Tract Infection (UTI) accounted for 44.8% of all the nosocomial infection and clearly dominated the picture. The post operative wound infection rate was 3.9% and accounted for only 18% of nosocomial infection. Westwood (1974) reported that the risk of hospital acquired infection was increased three fold by carrying of an operative procedure. Mayonwhite *et al.* (1988) stated that the prevalence rates of nosocomial infection in many countries ranged from 9.2 to 21.4%. In Thailand prevalence rate was 11.7%, in Ethiopia 17%, UK 9.2% and in Norway it was 9%. As for the site of infection urinary tract infection (UTI) was the commonest infection (60%) followed by Pneumonia and surgical site infection (A WHO manual 1989). The annual rate of nosocomial infection was 7% by a new surveillance system (kardex review) conducted in the University of Virginia Hospital in 1972-75. Despite advances in operative techniques, better understanding of pathogenesis of wound infection and widespread use of prophylactic antibiotics, post operative surgical site infections continued to be a major causes of morbidity and mortality for patients undergoing operative procedures. It was estimated that surgical site infection developed in 2-5% of the 16 million patients undergoing surgical procedures each year (Cruse 1981, Graves 1987). Pitaksiripien *et al.* (1995) conducted a surveillance study on nosocomial infection in Lampang hospital and showed that the incidence rate of nosocomial infection in 58,355 patients was 3.5%. A cross sectional study conducted by Hussain *et al.* (1991) in the surgical wards of Dhaka Medical

College Hospital showed that out of 240 patients, 72 (38.0%) suffered from nosocomial infection of which maximum number i.e. 26 (36.1%) suffered from surgical wound infection followed by 17 (23.6%) urinary tract infection. Prevalence of nosocomial infection was found to be higher (49%) in post operative patients than pre operative patients (15.9%) in this study. Western *et al.* (1982) reported that the nosocomial infection rate could be as high as 26-65% in developing countries. In England, Meers *et al.* (1980) reported that prevalence rate of nosocomial infection was of 11%. The lower nosocomial infection rate in the hospitals of the developed countries indicated an increased awareness about nosocomial infections. Based on the study of the efficacy of Nosocomial Infection Control (SENIC) by the Centers for Disease Control (CDC) and prevention it was expected that over 5,00,000 surgical site infection would occur among adults each year in the united states. These infections prolonged hospital stay by an average of 7.4 days at a cost of between $400 and $2600 per wound infection resulting in an annual cost of $130 to $845 million per year. (Haley *et al.*, 1985).

Many investigators elaborated etiology, source and mode of transmission of nosocomial infections in their studies. Adams *et al.* (1960) noted that environmental air of the operating room contained pathogenic bacteria, which were constantly being deposited upon the walls, floors, tables and other exposed surfaces. Bacteria might then reach the operative wounds. Blowers *et*

al. (1955) and Adams *et al.* (1959) demonstrated that the immediate vicinity of the operative wound was exposed to large number of bacteria when the personnel of the surgical team talked, sneezed or coughed. A clean operative wound might in this way become contaminated by microorganisms. Douglas *et al.* (1973) stated that air borne bacteria were potential source of contamination and infection of surgical wounds in operation theatre. Blowers *et al.* (1955) reported that following improved theatre ventilation in a thoracic surgery unit, there was a fall in wound sepsis from 10.9% to 3.9 %. Charnley and Iftekhar in 1969 reported that when operations were carried out in unventilated operation theatre, the rate of infection involving prosthetic hip joint was 8.7% and this was reduced to 1.3% when operations were performed in an enclosure with a high rate of air flow and other stringent precautions were taken. Cruse *et al.* (1980) found a direct relationship between duration of surgery and infection rate. Among clean wounds, the infection rates for operations lasting 1,2 and 3 hours were 1.3%, 2.7% and 3.6% respectively. Garibaldi *et al.* (1991) found that the duration of surgery greater than 2 hours was associated with relative risk of 3 fold for the surgical site infection. Kaiser (1990) stated that pathogens that cause surgical site infections were acquired either endogenously from patient's own flora or exogenously from contact with operating room personnel or the environment. It was noted that most pathogens either exogenously or endogenously acquired were implanted at the time of surgery. The nares and oropharynges of the operating room personnel were colonized with

microorganisms, which could shed large droplets into the surgical sites. Ford *et al.* (1967) isolated *Staph. aureus, Staph. epidermidis* and *Streptococci* frequently from the nasopharynges of operating room personnel and recovered the same organisms from the exudates of infected wounds. Wiley *et al.* (1979) stated that patients' endogenous flora at a distant sites might be a source of surgical site infection. It was reported that surface microflora could migrate from distant sites and gained entrance to the operative site despite distance and the use of clothes and adhesive drapes as barriers. Edwards (1976) and Valentine *et al.* (1986) stated that microorganisms causing infection at remote sites might gain access to the operative site by haematogenous or lymphogenous seeding. Untreated urinary tract infections, skin and respiratory tract infections were the three most common remote infections that had been associated with increase in the rate of surgical site infections.

Aman(1982) stated that in the operation theatre the source of infection may be physical environment of the theatre due to polluted air coming from the wards through the staffs working in the theatre and incompletely sterilized instruments used in the surgery.

Visitors had a role in the development of nosocomial infection. Over crowding in the wards and in front of operation theatre might be one of the important cause of increased frequency of nosocomial infection. (American Hospital

Association Infection Control in the hospital, Chicago,1968). The National Research Council study in 1964 reported that the rate of surgical site infection rose from 6% for preoperative stay of one day to 14.7 % when preoperative stay was 21 days or more. Cruse and Foord in 1980 reported that the overall infection rate was 1.1% for the patients whose preoperative stay was one day versus 2.1 % in patients who remained in the hospital for one week before their operation.

In bacterial analysis of wound infection in Mayo hospital, Lahore, Aman in 1982 found that the causative organisms of infection were *Staph. aureus* (28.6%), *Esch coli* (24.7%), *Pseudomonas* sp. (23.7%) and *Proteus* sp. (10.4%). Zaman *et al.* (1992) found that the commonest microorganisms were *Esch. coli* (60%) followed by *Staph. aureus* (20%) in postoperative wound infections in 8 medical college hospitals of Bangladesh. The predominant causative organisms for the postoperative wound were *Esch. Coli* (37.5%), *Staph, aureus* (21.7%), *Pseudomonas* sp. (15.1%), *Streptococcus* sp. (8.4%), *Proteus* sp. (2.7%) in the study conducted by Ashraf *et al.* (1973) in the surgery ward of Dhaka Medical College Hospital. Ahmed (1982) found that the common organisms isolated from the cases of urinary tract infection were *Esch.coli* (55.6%), *Staph. aureus* (18.6%) *Pseudomonas aeruginosa* (9%), *Proteus* sp. (4.8%) and *Klebsiella* sp. (6.7%), It was shown that there was a rise

in the frequency of infection by *Esch. coli* from 1975 to 1979 (46.4% Vs 57.6%).

Westwood (1974) found that the organisms responsible for nosocomial infections were *Esch.coli.* (25.3%), *Enterobacter* and *Klebsiella* sp. (16.8%), *Proteus* sp. (7.9%), *Pseudomonas aeruginosa* (6.8%), *Staph. aureus* (12.5%) *Streptococcus faecalis* (7.3%) and *Diplococcus pneumoniae* was (2.7%). Jinnah *et al.* (1998) observed that the most frequently encountered pathogen among non-hospitalized diabetic wound was *Staph. aureus* (42.0%). For hospitalized diabetic group the frequency of *Staph. aureus* isolation was (13.6%), the other pathogens were *Pseudomonas sp.*. (36.9%), *Esch. coli* (21.9%), *Proteus* sp. (21.6%), *Klebsiella* sp. (9.5%) and *Streptococcus* sp. (3.2%). A single infecting species was responsible for about 80.0% of the urinary tract infection in patients with short term catheters but most patients with long term catheters had polymicrobial infection with spontaneous turn over of individual species Warren *et al.* (1982). *Esch. coli* caused 80.0% or more of the cases of out patients (Hooton *et al.,* 1991) and less than 50.0% of the nosocomial ones.

A number of investigators showed sensitivity patterns of the isolated organisms. Aman in 1982 conducted a study on 500 specimens of Mayo Hospital, Lahore and found that *Staph. aureus* (28.6%) was the commonest organism, which was followed by *Esch.coli* (24.7%), *Pseudomonas* sp (23.7%).

and *Proteus* sp.(10.4%). Antimicrobial sensitivity test revealed that gentamicin was sensitive to 83.4% of cases, cephalexine was sensitive to 22.7% of cases. It was noted that cephalexin was less sensitive because of its extensive use. Maniatis *et al.* (1997) found that *Staph. aureus* and coagulase negative *Staphylococcus* did not display any significant variation in isolation rate but showed an alarming increase in resistance rate to oxacillin from 11% and 21% in 1986 to 51% and 75% in 1994 respectively .

Sharafi *et al.* (1991) conducted a study with a total of 180 patients of complicated and uncomplicated nosocomial urinary tract infection. The predominating infecting organisms were *Esch. coli.* and *Proteus* sp. Cefepenie (4^{th} generation of cephalosporin) and ceftazidime produced a clinical response in 89.0% and 86.0% respectively. A study conducted by Coronado *et al.* (1995) showed that there was an increase in resistance to ciprofloxacin among nosocomial pathogens especially *Pseudomonas aeruginosa* and *Staph. aureus.* Ciprofloxacin resistance was more common among *Staph. aureus* isolated from urinary tract and respiratory tract infection than any other sites of infection isolation. It was concluded that resistance to ciprofloxacin was more frequent among nosocomial *Staph. aureus* than *Pseudomonas aeruginosa* and resistance was increasing rapidly among *Staph. aureus* isolates. Ahmed (1982) observed that there was a definite fall in the sensitivity of *Esch.coli* and *Klebsiella* sp. to ampicillin. Zaman *et al.* (1992) observed that the commonly used antibiotics

like ampicillin, cotrimoxazole, and cephalexin were markedly resistant to *Esch. coli* and *Staph. aureus*.

Ashraf *et al.* (1973) observed that prevalence of gram positive cocci (*Staph. aureus, Streptococcus* sp.*)* were greatly reduced but gram negative organisms came to emerge infecting the wounds of surgery wards. Both of these organisms were resistant to most of the common antibiotics of that time. Jinnah *et al.* (1996) found that *Esch. coli* isolated from hospitalized diabetic patients suffering from urinary tract infection were more resistant to antibiotics compared to isolates from the outdoor diabetic and nondiabetic patients. Jinnah *et al.* (1998) found that resistance rate of *Staph. aureus* isolated from hospitalized diabetic and outpatient diabetic wounds to ampicillin 74.5% Vs 81.0%, cloxacillin 37.2% Vs 21.6%, cephalexin 41.1% Vs 21.6%, gentamicin 38.2% Vs 16.2%, ceftriaxone 33.3% Vs 16.2%, ciprofloxacin 26.4% Vs 13.5%, cotrimoxazole 57.8% Vs 32.4%and tetracycline 55.8% Vs 21.6% respectively. This suggested the possibility of increased resistance rate among the hospitalized patients because these antibiotics were frequently used to the hospitalized patients.

Nosocomial infection prolongs hospital stay of the patients and adds to economic burden for the underlying diseases. Zaman *et al.* (1992) stated that the mean post operative hospital stay in case of patients with nosocomial infection was 25 days which was 3 times than that of the cases without

infection (8.6) days. This resulted in unnecessary monetary loss and bed occupancy.

Haley *et al.* (1981) conducted a study and showed that hospital stay was prolonged by 3.1 to 4.5 days due to nosocomial infection. Sudsukh (1988) showed that nosocomial infection cost was about 40 million US Dollars in a year in Thailand. In comparison to this a full preventive program required one million US dollars. Westwood *et al.* (1974) found that patients with nosocomial infection spent on average, 22 extra days in the hospital. The cost of these extra days would represent 2.5 million US dollars in a year for the Ottawa general hospital alone whose entire annual budget was only about 14 million US dollars. Cruse (1970) concluded that post operative wound infection delayed patient's discharge by on an average of 7.7 days and it would represent 800000 US dollars per annum for a 600-bed hospital.

Leroyer *et al.* (1997) stated that the mean extra cost per infected case was 10440 US dollars and extra days of hospital staying was 5.2 days in case of neonates acquiring hospital acquired infection. Jervis (1996) stated that approximately 2 million nosocomial infections occurred annually in the United States. The excess duration of hospital stay secondary to nosocomial infection had been estimated 1 to 4 days for urinary tract infection, 7 to 8.2 days for surgical site infections. The estimated average cost of these infections were 558

to 583 US dollar for each urinary tract infection, 2734 US dollars for each surgical site infection.

Detection of source of infection is an important prerequisite factor for designing a control programme of nosocomial infection. In this regards Plasmid Profile Analysis (PPA) and Restriction Fragment Length Polymorphism (RFLP) were used by some investigators to identify the source of infections.

Girgis *et al.* (1992) found that aminoglycoside resistant strains of *Acinetobacter* in an intensive care setting was identical to that of environment and thus suggested a means of transmission. Markowitz *et al.* (1983) found that two outbreaks of infection caused by *Enterobacter cloacae* occurred 6 years apart in the same burn unit were attributed to two different strains by plasmid fingerprinting. A study conducted by Eisenstein *et al.* (1986) found that all epidemic isolates of gram negative bacilli were the same where as controls showed different DNA patterns and even the antibiogram failed to show a difference. Plasmid can spread from one bacterial species or strains to another by conjugation. It was occasionally the plasmid rather than the bacterial strain that was epidemic (Farmer, 1983). These epidemic plasmids could enter a hospital in one or a few strains and subsequently spreaded by conjugation to other strains present in the flora of the hospitalized patients (Farmer, 1983).

Restriction fragment polymorphism (RFLP) was proved useful for strain identification for detecting cross contamination and for tracing epidemics (Stratton *et al.*, 1993)

It is evident from the above discussion that nosocomial infection is an alarming problem throughout the world. In America National Nosocomial Infection Surveillance System began in 1970 and now it is the only source of national data on epidemiology of nosocomial infection in the United States. In Bangladesh, no study on nosocomial infection with elaborate information was undertaken except a few scattered studies with inadequate information. So, the present study- "nosocomial infections and its consequences" has been designed with the following objectives.

Objectives of the Study

- To determine the pattern of organisms causing nosocomial infection;
- To determine the antibiotic resistance pattern of the organisms;
- To determine the risk factors of nosocomial infection;
- To assess the impact of nosocomial infection;
- To determine the possible source of nosocomial infection using different techniques like antibiogram plasmid profile and RFLP.

Chapter II

Literature Review

2.1 Nosocomial infection

Hospital is a vital part of the health care system. But by the very nature of its work, a hospital constantly exposes patients to microbiological risks. When a person enters hospital, he exchanges his secure home environment for a bed in a small, possibly restricted, hostile area -the ward. From the moment of admission to the hospital the person is potentially put at a disadvantage by his illness, environment, medication, surgery and other treatment. It may seem strange that, in an effort to cure, we take people from the safety of their homes and expect them to make progress in the comparatively unsafe environment of a hospital.

Nosocomial infection is derived from the Greek word "nosos"-means disease and "Komeo"-means attend to. It is often referred to as hospital acquired infection (HAI). It is defined as infection, which is acquired by patient while they are in hospital or by staff who are working in the hospital. If a patient acquire infection more than 72 hours after admission, it is considered to be hospital-acquired infection (Caddow, 1989). The national nosocomial infection surveillance system defines nosocomial infection as a localized or systemic

condition that results from adverse reaction to the presence of agent (s) or its toxin (s) and that was not present or incubating at the time of admission in the hospital (Garner *et al.*, 1988).

For most bacterial nosocomial infections, this means that the infections usually become evident 48 hours (i.e. typical incubation period) or more after admission in the hospital. However, because the incubation period varies with the type of pathogen and to some extent with the patient's underlying condition, each infection must be assessed individually for evidence that links it to the hospitalization. There are several other important principles upon which nosocomial infection definitions are based. (Garner *et al.*, 1988). First, the information used to determine the presence and classification of infection should be a combination of clinical findings and results of laboratory and other tests. Second, a physician's or surgeon's diagnosis of infection derived from direct observation during a surgical operation, endoscopic examination or other diagnostic studies or from clinical judgement is an acceptable criterion for an infection unless there is compelling evidence to the contrary.

There are two special situation in which an infection is considered nosocomial (a) infection that is acquired in the hospital but does not become evident until after hospital discharge and (b) infection in a neonate that results from passage through the birth canal. There are two special situation in which an infection is

not considered as nosocomial (a) infection with a complication of infection already present on admission, unless a change in pathogen or symptoms strongly suggests the acquisition of a new infection and (b) in an infant, an infection that is known or proved to have acquired transplacentally (e.g. Rubella, toxoplasmosis, cytomegalovirus or syphilis) and become evident at or before 48 hours after birth.

2.2 HISTORICAL ASPECTS

Nosocomial infections have been existing as long as there have been hospitals. But attention was not paid until the middle of the nineteenth century. During that time, Florence Nightingale improved hospital design and higher standards of nursing care. During the World War I and II, nosocomial infections were usually caused by *Streptococcus* sp. and *staphylococcus* sp. (Eickhoff 1983, Gardner *et al.,* 1987). After the discovery of penicillin, *Streptococcal* infections were dramatically reduced and it had only temporary effect on *staphylococcal* infections (Eickhoff, 1983). The frequency of gram negative bacilli were increasing since 1960. The predominance of infections caused by gram negative bacilli were established in 1970 and subsequently increasing of methacillin resistant *staph. aureus* were also recorded (Eickhoff, 1983). Methacillin resistant *staph. aureus* is still one of the important causal agent of nosocomial infection (Rao *et al.,* 1988) and gram negative bacilli being the predominant organisms (Kapur *et al.,*1985 , Gardner *et al.* 1987). Ashraf *et al.*

(1973) observed that gram positive cocci (*Staph. aureus*) were greatly reduced but gram negative organisms came to emerge in infecting the wounds of surgical wards. Both of them were resistant to most of the antibiotics of common use at that time. Ashraf *et al.* (1973) suggested that "days are not far off when most of the antibiotics in common use today will become less and less effective against the common types of pathogenic organisms around us. We should care more for the prevention of infection with the help of aseptic techniques and antiseptics rather than letting contamination occur, then look for antibiotics".

2.3 EPIDEMIOLOGY

Nosocomial infections are problems of increasing importance in hospital practice. They occur at a rate of 5 to 10 per hundred admissions and represents a leading cause of death and add to economic burden of underlying diseases (Wenzel, 1987). In 1980, the first national prevalence study of infection in England and Wales (Meers *et al.,* 1981) showed that 19.1% of patients were infected. Over half of these (51.9%) were community-acquired infections and the rest (48.1%) were hospital acquired infections. In America, the nationwide nosocomial infection rate was approximately 5.7 per hundred admissions (Haley *et al.*,, 1985). A surveillance study conducted by Westwood *et al.* (1974) on infections at the Ottawa general hospital showed that the overall infection rate was 13.5% of which 5.6% was community acquired while 7.9%

was nosocomial origin. It also showed that Urinary Tract Infection (UTI) accounted for 44.8% of all the nosocomial infections and clearly dominated the picture. The postoperative wound infection rate was 3.9% and accounted for only 18% of nosocomial infections. Westwood (1974) also showed a nosocomial superinfection rate of 14.3% whereas patients not infected on admission showed a nosocomial infection rate of 6.9%. This indicated that the chances of acquiring infection of hospital origin were doubled in patients with pre-existing infection. The overall nosocomial infection rate on the medical service was 9.3% and post operative infection rate for all services was 13.5% of which 27.4% was due to UTI. When no surgery was performed, the infection rates were-surgery 4.3%, gynecology 4.4%, maternity 2.1%, paediatrics 6.7%. The risk of nosocomial infections was increased three fold by carrying out of an operative procedure. The most important single finding was the dominance of UTI which represented 44.8% of all the nosocomial infections. Many authors showed that post catheterization infection was responsible for the majority of nosocomial UTI (Pyrah *et al.,* 1955, Kass *et al.,* 1957, Sanford 1963, Lindan 1969, Moody *et al.,* 1972). Britt *et al.* (1978) conducted a prospective study in Salt Lake City V.A hospital and showed that severity of underlying diseases at the time of admission indicated medical patients at the unusual risk of nosocomial infections. The nosocomial infection rate was 23.6% in patients with fatal underlying disease, 9.6% in those with ultimately fatal disease and 2.1% in those with non-fatal disease. Nosocomial infections represented an

important endemic problem affecting 3 to 5% of hospitalized patients. (Eickhoff, 1973). The severity of a patient's underlying disease affects both the risk of acquisition of selected infections and their associated morbidity and mortality (Dupont *et al.,* 1962, McCabe *et al.,* 1968, Bryant *et al.,* 1971, Schecker *et al.,* 1977). A new surveillance system (Kardex review) was described for detecting hospital acquired infection (HAI) in the University of Virginia Hospital in 1972-1975. The annual rate of nosocomial infection was 7% and in the succeeding year of surveillance it was 6% (Wenzel *et al.,* 1976). This was higher than rates reported by the university hospital participating in the National Nosocomial Infection Study (NNIS) which was sponsored by the Center for Disease Control (CDC). Their median monthly rates ranged from 3% to 5.5% (Center for Disease Control, National Nosocomial Infection Study report 1st and 2nd quarter 1972 –1973). The special prevalence studies from Boston city hospital it was 11.5% (Kislak *et al.,* 1964, Barrett *et al.,* 1967, Adler *et al.,* 1970). Despite advances in operative technique, better understanding of pathogenesis of wound infection and wide spread use of prophylactic antibiotics, post operative surgical site infection continued to be a major cause of morbidity and mortality for patients undergoing operative procedures. It was estimated that surgical site infection developed in 2-5% of the 16 million patients undergoing surgical procedures each year (Vital statistics 1989- Graves, 1987 and Cruse, 1981). They accounted for about 24% of all nosocomial infections, making surgical site infection as the second most

common site of nosocomial infections, second only to UTI, (Hughes *et al.*, 1980-82, MM WR 1983). Burns *et al.* (1982) showed that the overall infection rate in clean wounds was (2.5%), clean contaminated (6.7%) and dirty wounds (13.3%). It was showed that the highest infection rate in the general surgery was (8.2%) followed by plastic surgery (5.6%) and it was (5.2%) in Gynecology. Edwards (1976) stated that the risk of acquiring wound infections in gynecological surgery was 1.56% and that the risk of orthopaedic surgery was 5.61%. Jogerst *et al.* (1981) showed that use of prophylactic and therapeutic antibiotic might have contributed to be the lower rate of postoperative infection. Pitaksiripen *et al.* (1995) conducted a surveillance study on nosocomial infection in Lampang hospital and showed that the incidence rate of nosocomial infection in 58,355 patients was 3.5%. The highest infection rate (16.8%) was found in intensive care units followed by the surgery department (9.0%) and Orthopaedic department (8.5%). UTI was the commonest site of infection (19.4%) followed by surgical wound and lower respiratory tract infection. The infecting organisms were *pseudomonas* sp., *Esch coli* and *Klebesiella* sp.

A cross sectional study conducted by Hussain *et al.* (1991) in the surgical wards of Dhaka Medical College Hospital showed that out of 240 patients 72(38%) suffered from nosocomial infections of which maximum number i.e. 26(36.1%) suffered from wound infection followed by UTI 17(23.6%). The

frequency of other types of infections encountered in this study were respiratory tract infection 11(15.3%), gastrointestinal tract infection 9(12.5%), skin infection 3(4.1%), and fever 6 (8.3%). Prevalence of nosocomial infections was found to be higher (49%) in the postoperative patients than pre operative patients (15.9%) in the study. High frequency of infection especially in postoperative wards and other wards indicated a breach of asepsis some where in the operation theatre, post operative wards and /or other wards.

A surveillance study was conducted by Zaman *et al.* (1992) in eight Medical College Hospitals of Bangladesh and found that the prevalence rate of nosocomial infections ranged from 6 to 18%. All cases in the study were postoperative surgical wound infection and the commonest organisms were *Esch.coli*. The prevalence rate of nosocomial infections in many countries ranged from 9.2 to 21.4%. In Thailand the prevalence rate was 11.7%, in Ethiopia 17%, U.K 9.2% and in Norway it was 9%. (Mayon White *et al.*, 1988).

Western *et al.* (1982) reported that the nosocomial infection rate be as high as 26 to 65% in the developing countries. In England Meers *et al.* (1980) reported a prevalence rate of nosocomial infection was 11%. The lower nosocomial infection rate in the hospital of the developed countries indicated an increasing awareness about nosocomial infection. The study on the efficacy of

nosocomial infection control (SENIC) project, the comprehensive hospital infection project (CHIP) and the National Nosocomial Infection Studies (NNIS) play an important role in reducing the nosocomial infections.

Vincent (1995) determined the prevalence rate of intensive care unit acquired infection and risk factor for these infections and found that out of 10038 ICU patients, 4501 patients (44.8%) were infected. Of the infected patients pneumonia was (46.9%), lower respiratory tract infection was 17.8% and blood stream infection was 12%. The risk factors for ICU acquired infection were (1) increasing length of ICU stay (>48 hours) (2) mechanical ventilation (3) central venous catheterization, (4) Pulmonary artery catheterization (5) Urinary catheterization (6) stress ulcer prophylaxis.

Graves *et al.* (1987) and Cruse *et al.* (1981) noted that surgical site infection developed in 2-5% of the 16 million patients undergoing surgical procedures each year. Based on the study of the Efficacy of Nosocomial Infection Control (SENIC) by the centers for disease control and prevention (CDC), it was expected that over 5,00,000 surgical site infections would occur among adults each year in the United States. These infections prolong hospital stay by an average of 7-4 days at a cost of between $400 and $2600 per wound infection, resulting in an annual cost of $130-$845 million per year.

2.4 Etiology, Source and mode of Transmission of Nosocomial Infections

Many investigators elaborated etiology, source and mode of transmission of nosocomial infections in their studies. Adams *et al.* (1960) noted that environmental air of the operating room contained pathogenic bacteria, which were constantly being deposited upon all the walls, floors, tables and other exposed surfaces. Bacteria might thus reach the operative wounds. Blowers *et al.* (1955) and Adams *et al.* (1959) demonstrated that the operative wounds were exposed to a large number of bacteria when the personnel of the surgical team talked, sneezed, or coughed. A clean operative wound in this way become contaminated by microorganisms. Letts *et al.* (1983) stated that microorganisms became air borne as a result of conversation which created droplet nuclei from the respiratory tract or as a result of shedding from hair or exposed skin. Douglas *et al.* (1973) stated that air borne bacteria were potential source of contamination and infection of surgical wounds in operation theatre. Blowers *et al.* (1955) reported that following improved theatre ventilation in a thoracic surgery unit, there was a fall in wound sepsis from 10.9% to 3.9%. Charnely and Ifthekhar (1969) reported that when operations were carried out in unventilated operation theatre, the rate of infection involving prosthetic hip joint was 8.7% and this was reduced to 1.3% when operations were performed in an enclosure with a high rate of air flow and other stringent precautions were taken. Cruse *et al.* (1980) found a direct relationship between duration of

surgery and infection rate. Among the clean wounds the infection rates of operations lasting for 1, 2 and 3 hours were 1.3%, 2.7% and 3.6% respectively. Garibaldi *et al.* (1991) applied step wise logistic regression to the analysis of 1852 procedures and found that the duration surgery more than 2 hours was associated with relative risk of 3 for the surgical site infections. Wiley *et al.* (1979) stated that patients' endogenous flora at distant sites might be a source of surgical site infection. It was suggested that surface microflora could migrate from distant sites and gain entrance to the operative site despite distance and the use of clothes and adhesive drapes as barriers. Edwards (1976) and Valentine *et al.* (1986) stated that microorganisms causing infections at remote sites might gain access to the operative sites by haematogenous or lymphogenous seeding. Untreated UTI, skin and respiratory tract infections were the 3 most common remote infection that had been associated with the increase in the rate of surgical site infections. Kaiser (1990) stated that pathogens that cause surgical site infections were acquired either endogenously from the patients own flora or exogenously from contact with operating room personnel or the environment. It was reported that most pathogens either exogenously or endogenously acquired, were implanted at the time of surgery. The nares and oropharynges of the operating room personnel were colonized with microorganisms, which could be shed in large droplets into the surgical sites. Ford *et al.* (1967) isolated *Staph. aureus*, *Staph. epidermidis* and *Streptococci* frequently from the nasopharynges of the operating room

personnel and recovered the same organisms from the exudates of the infected wounds. Basset *et al.* (1970) noted that contaminated solutions had been the source for surgical site infections caused by *Pseudomonas aeruginose*, *Pseudomonas multivorans* and *Serratia marcescens*. Ahmed (1982) stated that almost all the infections of UTI were autoinfection from the gut. It was stated that *Esch. coli* of the gut might colonize at the pre urethral area from where ascending infection started. Due to indiscriminate use of ampicillin the gut *Esch. coli* might be a resistant type of beta lactamase producers or R-plasmid type, which gave a high percentage of resistance .

The most common pathogens responsible for surgical site infection as reported to National Nosocomial Infection Surveillance (NNIS) between 1986-1989 and 1990-1992 are as follows.

Percentage of distribution of Nosocomial pathogens for surgical site infection (NNIS), 1986-1989 and 1990-1992.

Pathogens	1986-89 N= 16727	1990-92 N=11724
Esch. Coli	10	8
Enterococci	13	12
Ps. aeruginosa	08	08
staph. aureus	17	19
Coagulase-ve staph.	12	14
Enterobacter sp..	08	07
Klebsiella pneumonia	03	03
Proteus mirabilis	04	03
Streptococcal sp.	03	03

Jinnah *et al.* (1998) observed that the most frequently encountered pathogen among non-hospitalized diabetic wound was *Staph. aureus* (42%). For the hospitalized diabetic group the frequency of *Staph. aureus* isolation was (13.6%) and ranked 4th position. Other pathogen in order of frequency of isolation were *Pseudomonas* sp.(20.4%), *Esch coli (15.9%)*, *Proteus* sp (5.7%), *Klebsiella* sp (7.9%) and *Streptococcus* sp (7.9%).

The frequency of individual pathogens causing nosocomial UTI has changed markedly in the last 2 decades. The most important factor influencing the distribution of infecting species in the hospital was the use of antimicrobial agents. The organisms usually responsible for catheter associated UTI were gram negative organisms derived from the faecal flora, (Lohr *et al.,* 1989). It was also noted that *Esch.coli* was the infecting organism responsible for about 80% of the UTI in patients with short term catheters.

In bacterial analysis of wound infection in Mayo hospital, Lahore, Aman (1982) found that the causative organisms were *Staph. aureus* (28.6%), *Esch. coli* (24.7%), *Pseudomonas* sp (23.7%) and *Proteus* sp (10.4%).

Zaman *et al.* (1992) found that the commonest microorganisms were *Esch. coli* (60%) followed by *Staph. aureus* (20%) and *Pseudomonas* sp (20%) in postoperative wound infections in 8 medical colleges of Bangladesh.

The predominant causative organisms for the postoperative wounds were *Esch. coli* (37.5%), *Staph. aureus* (21.7%), *Pseudomonas* sp (15.1%), *Streptococcus* sp. (8.36%), *Proteus* sp (2.7%) in the study conducted by Ashraf *et al.* (1973) in the surgery wards of Dhaka Medical College Hospital. Ahmed (1982) found that the common organisms isolated from the cases of UTI were *Esch. coli* (55.6%), *Staph. aureus* (18.6%), *Pseudomonas aeruginosa* (9%), *Proteus* sp. (4.8%) and *Klebsiella* sp. (6.7%). It was shown that there was rise in the frequency of infection by *Esch. coli* from 1975 to 1979 (46.4% Vs 57.6%). Westwood (1974) found that in Ottawa general hospital urinary tract infections accounted for (44.8%) of all nosocomial infections and post operative wound infection rate was (18%). The organisms responsible for nosocomial infections were *Esch. coli* (25.3%), *Enterobacter* and *Klebsiella* sp (16.8%), *Pseudomonas aeruginosa* (6.8%) and *Staph. aureus* (12.5%), *Streptococcus faecalis* (7.3%) and *Diplococcus pneumoniae* (2.7%). Vincent *et al.*, (1995) found that the Prevalence of nosocomial infection in the intensive care units in Europe was frequently caused by *Enterobacteriaceae* (34.4%), *Staph. aureus* (30.1%), *Pseudomonas aeruginosa* (28.7%), coagulase negative *Staphylococcus* (19.1%).

Aman (1982) stated that the wounds become infected through many variable sources. In the operation theatre the source may be physical environment of the theatre due to polluted air coming from the wards through the staffs working in the theatre and incompletely sterilized instruments used in surgery.

Visitors had a role in the development of nosocomial infection. Over crowding in the wards and in front of the operation theatre might be one of the important causes of increased frequency of nosocomial infection (American Hospital Association Infect. Control in the Hospital, Chicago, 1968). Curse and Foord (1980) reported that the over all infection rate was 1.1% for the patients whose pre operative stay was one day versus 2.1% in patients who remained in the hospital for one week before operation.

2.5 Antimicrobial Susceptibility

Aman (1982) conducted a study on 500 specimens in Mayo hospital, Lahore and found that *Staph. aureus* was the commonest organisms which was followed by *Esch. coli*, *Pseudomonas* sp and *Proteus* sp. Antimicrobial susceptibility test revealed that gentamicin was sensitive to (83.4%) of cases, cephradine was sensitive to (22.72%) of cases. It was observed that cephalexin was less sensitive because of extensive use of this drug.

Barbee *et al.* (1975) reported that *Staph. aureus* was the single most important pathogen in hospital infections and resistant strains of *Staph. aureus* emerged from indiscriminate use of antibiotics. Maniatis *et al.* (1977) found that *Staph. aureus* and coagulase negative *Staphylococcus* did not display any significant variation in isolation rate but showed an alarming increase in resistance rate to oxacillin from (11%) and (21%) in 1986 to (51%) and (75%) in 1994 respectively. Sharafi *et al.* (1996) conducted a study with a total of 180 patients of complicated and uncomplicated nosocomial infections of UTI and the predominating infecting organisms were *Esch.coli* and *Proteus* sp. Both cefepine (4^{th} generation of cephalosporin) and ceftazidime were administered intramuscular and intravenous route. Cefepine and ceftazidime produced a clinical response of (89%) and (86%) respectively.

A study conducted by Cornado *et al.* (1995) showed that there was an increase of resistance to ciprofloxacin among the nosocomial pathogens especially *Pseudomonas aeruginosa* and *Staph. aureus*. It was observed that (27.1%) isolates of *Staph. aureus* and (4.7%) isolates of *Pseudomonas aeruginosa* were resistant to ciprofloxacin. It was also observed that ciprofloxacin resistant was more common among *Staph. aureus* isolated from urinary tract and respiratory tract infection than any other sites of isolation. The author concluded that resistance to ciprofloxacin was more frequent among nosocomial *Staph. aureus* than *Pseudomonas aeruginosa* and resistance was increasing rapidly among

Staph. aureus. Ahmed in 1982 conducted a study on antibiotic sensitivity pattern of common causative organisms of urinary tract infections for four years and reported a definite fall in the sensitivity of *Esch. coli* and *Klebsiella* sp to ampicillin.

Zaman *et al.* in 1992 conducted study on hospital acquired infections in 8 medical college hospitals of Bangladesh and found that the commonest microorganism was *Esch. coli* (60.0%) which was followed by *Staph. aureus* (20.0%). The commonly used antibiotics like ampicillin, cloxacillin, amoxycillin, gentamicin, co-trimoxazole and cephalexin were markedly resistant to *Esch coli. coli* and *Staph. aureus*. Ashraf *et al.* (1973) observed that prevalence of gram positive cocci (*Staph. aureus, Streptococcus*) were greatly reduced but gram negative organisms (*Esch. coli, Proteus* sp, *Pseudomonas* sp,) came to immerge infecting the wounds of surgery wards. Both gram positive and gram negative organisms were resistant to most of the common antibiotics of that time. Jinnah *et al.* (1996) found that *Esch.coli* isolated from hospitalized diabetic patients suffering from urinary tract infections were more resistant to antibiotics as compared to isolates from outdoor diabetic and non diabetic patients. Jinnah *et al.* (1998) found that the resistance rate of *Staph. aureus* isolated from hospitalized diabetic and out patient diabetic wounds to ampicillin 74.5% Vs 81.0%, cloxacillin (37.2% Vs 21.0%), cephalexin (41.1% Vs 21.6%), gentamicin (38.2% Vs 16.2%), ceftriaxone (33.3% Vs 16.2%),

ciprofloxacin (26.4% Vs 13.5%), cotrimoxazole (57.8% Vs 32.4%) and tetracycline (55.8% Vs 21.6%). This suggested the possibility of increased resistance rate among the hospitalized patients because these antibiotics were frequently used to hospitalized patients.

Plasmid Profile Analysis (PPA) and Restriction Fragment Length Polymorphism (RFLP) were used by some investigators to identify the source of infections.

Girgis *et al.* (1992) found that aminoglycoside resistant strains of *Acinetobacter* in an intensive care setting was identical to that of environment and thus suggested a means of transmission. Markowitz *et al.* (1983) found that two outbreaks of infection caused by *Enterobacter cloacae* occurred 6 years apart in the same burn unit were attributed to two different strains by plasmid fingerprinting. A study conducted by Eisenstein *et al.* (1986) found that all epidemic isolates of gram negative bacilli were the same where as controls showed different DNA Patterns and even the antibiogram failed to show a difference. Plasmid can spread from one bacterial species or strains to another by conjugation. It was occasionally the plasmid rather than the bacterial strain that was epidemic (Farmer 1983). These epidemic plasmids could enter a hospital in one or a few strains and subsequently spreaded by conjugation to other strains present in the flora of the hospitalized patients (Farmer,1983).

2.6 Mechanisms of antimicrobial resistance in microorganisms

Inactivation of antimicrobial agents

β-lactamases
Chloramphenicol acetyltransferases
Aminoglycoside modifying enzymes

Decreased drug accumulation (membrane impermeability antimicrobial efflux)

Intrinsic resistance
Acquired resistance : chromosomally mediated
plasmid-mediated

Alteration in target site

Penicillin binding proteins
DNA gyrase
RNA polymerase
Ribosome

Metabolic bypass

Altered dihydrofolate reductases
Altered dihydroperoate synthetases

Overproduction of target enzyme inhibited by antimicrobial agents

Gene amplification
Mutation in regulatory gene

Tolerance

Defect in autolysins

Auxotrophic change

Thymine auxotrophy

Genetic basis of resistance of microorganisms to antimicrobial agents: Microbial resistance to antimicrobial agents is a matter of great importance, if sensitive strains are supplanted by resistance ones, then the valuable drugs become useless. Resistance to antimicrobials may be an acquired property or an intrinsic naturally occurring trait that is a characteristic of a species.

Acquired resistance may develop either from changes or mutation to genomic DNA or by acquisition of antimicrobial resistance genes through mobile genetic elements (Plasmids, bacteriophages, integrons and transposons) by gene transfer mechanisms such as conjugation, transformation, transduction, site specific integration or conjugative transposition. After each new antimicrobial agent becomes widely used, antimicrobial resistance gene eventually emerge and spread through the world's bacterial population. Antimicrobial agent everge either by being mobilized through obscure strains or by evolving from obscure ancestral gene (Amyes *et al.,* 1992). About one hundred resistance genes have been recognized to date. Each encodes a protein that either inactivate antimicrobial agents or prevents them from blocking their target function or provides a new function to substitute for the function blocked

by the agents. Resistant genes may be situated on chromosomes, plasmids, integrons or on transposons. A resistant gene may emerge and spread through a sequence of events: mutation to enhance resistance, transposition to a plasmid, recombination with other plasmids, linkage with other resistant genes, transfer of plasmid to new strains, and migration of resistant strains from one host to colonize or infect others. Continuos use of the antimicrobial gents amplifies the resistant bacterial population and tends to distribute the resistant gene in progressively larger population of bacteria. DNA hybridization and polymerase chain reaction are two major technique which can be used to detect antimicrobial resistant genes.

Plasmid and antimicrobial resistance: A plasmid is a naturally occurring, autonomous, extrachromosomal, circular duplex and self-replicating DNA molecule. It ranges in size from 1kb. to greater than 400 kb. and is extremely common in bacteria and even present in eukaryotes. Bacteria house plasmids and strictly regulate their copy number i.e. the number of plasmids per chromosome is 1 to 20. Single copy plasmids maintain parity with chromosome but multi copy plasmids exist in a characteristic number of 10-20 per bacteria cell. Copy number is the result of an existing replication control mechanism in the bacteria. Plasmids that share the same replication control system tend to as incompatible. Thus, incompatibility groups of plasmids have been defined which can be used for epidemiological investigations. Recently plasmid

replicon typing has been developed to classify plasmid (Couturier *et al.*, 1988). Plasmids can exists either in an autonomous, extra-chromosomal state or they can be inserted into bacterial chromosome and can be carried as a part of it and called an episome. A plasmid is a major class of mobile genetic elements. The mobile conjugation plasmid has the necessary genes known as tra genes, required for the conjugation process, which is a natural and probably most important genes transfer mechanism. The plasmids may transfer themselves into different species and even into different genera. A conjugative plasmid can mobilize a non-conjugative plasmid when they are present in the same host. Plasmid may have a co-valantly closed circular (CCC), open circular or linear form of DNA. Plasmid DNA usually encodes no essential but rather acts as a dispensible accessory source of DNA that provides many unique functions to bacteria without overloading the main genome. They carry genes for the inactivation of antibiotics, the production of toxin, the break down of natural products and invasion genes. Some plasmids have no known phenotypic function and are known as cryptic plasmids. Plasmids mediated resistance occurs much more frequently than chromosomal resistance in clinically important bacteria. It is responsible for most of the resistance phenomena and in association with transposon greatly increases the rapid spread of resistance factors or genes.

The first R-factor or R-plasmid was detected in Japan in S.Dysenteriae type –I in mid 1950s. Plasmids and chromosomes can themselves exchange genes by general recombination or by transposition, thus, increasing the spread of resistant genes even in the single bacterial cell. Plasmids are involved in the dissemination of antibiotic resistance in many ways. A single clone of a specific bacterium may spread antibiotic resistance by clonal spread or by plasmid transfer or by plasmid and transposon spread or by mobilization of resistance genes from plasmid to chromosome or vice versa. Resistant plasmids may contain any number of individual resistant genes and in recent years "Super" plasmid encoding resistance to 8 or more antimicrobials have been reported.

Chromosome and antimicrobial resistance: Chromosomal resistance is the result of mutation in the genome. It may occur spontaneously or by selective pressure of antibiotics on the organisms, resulting in clonal spread of resistance with suppression of susceptible organism. A chromosomal resistant gene may be transfer to a plasmid by transposition. It is a less frequent cause of emergence of clinical significant drug resistance in a given patient because of low frequency of mutation (10^{-7} to 10^{-12}). Chromosomal mutants are resistant in most of the cases by virtue of changes in the drug receptors or target.

Transposon and antimicrobial resistance: A transposon is a small mobile genetic element of specific DNA sequence which is able to transpose it self between unrelated DNA sequence. It is not self-replicating and therefore, must exists on a chromosome, plasmid or bacteriophage. Transposon often known as jumping genes, can move between plasmids, between chromosome and plasmid, as well as to and from a phage. The simplest transposon is an insertion sequence (Is), typically about 1 kb long and consists of a gene for a transposase bounded by inverted terminal repeats. Many antimicrobial resistant genes reside within transposons and it is probably this fact that explains the evolution of R plasmids from ones with few resistance genes to ones with many. Some transposons contain many antibiotic resistant genes i.e. Tn 2571. Transposition is a continuous and ongoing process in bacterial populations. Recently, a new class of transposons has been described that has the capability to move from the chromosome of one bacterium to another without being part of a plasmid or bacteriophage. These are known as "conjugative" transposons and have been detected in aerobic and anaerobic gram-positive bacteria (Cohen, 1976).

Intergrons and antimicrobial resistance: Recently, a family of potentially mobile DNA elements called intergrons has been described within some bacterial chromosomes. Intergrons consists of two conserved DNA segments separated by various acquired antibiotic resistant gene cassettes. The whole intergron is flanked by a short (25 base pairs) imperfect inverted repeat base

pair. (Bissonnette *et al.,* 1992, Stokes *et al.,* 1989). The intergron does not encode the proteins necessary for transposition. The site specific integration function (the insertion site and integrase production) is performed by the conserved DNA segment. A majority of the integrons appear to depend on host genome for their movement. Independent transposition of intergron Tn 402 has been observed. The intergron probably acts as an expression cassette by supplying the promoter for the inserted genes. The presence of a specific site for integration on the intergron will favour tandem integration of antimicrobial resistant genes resulting in a multiple resistant intergron (Bissonnette *et al.,* 1992, Stokes *et al.,* 1989). Intergrons have been shown to encode trimethoprim resistant dihydrofolate reductase and sulfonamide resistant dihydrofolate synthetases.

2.7 Socioeconomic Impact

Hospital acquired infection has socioeconomic impact. Zaman *et al.,* (1992) stated that the mean post operative hospital stay in case of patients with hospital acquired infection was 25 days which was 3 times than that of the cases without infection (8.6 days). This resulted in unnecessary monetary loss and bed occupancy.

Haley *et al.* (1981) conducted a study and showed that hospital stay was prolonged by 3.1 to 4.5 days due to hospital acquired infection. Sudsukh (1988) showed that nosocomial infection cost was about 40 million US dollars in a year in Thailand. In comparison to this a full preventive program required one million US dollars. Westwood (1974) found that patients with nosocomial infection spent, on an average, 22 extra days in the hospital. The cost of these extra days would represent the huge amount of 2.5 million US dollars in a year for the Ottawa general hospital alone, whose entire annual budget was only about 14 million US dollars. Cruse (1970) concluded that the postoperative wound infection delayed patients discharge by an average of 7.7 days and it would represent 800000 US $ dollars per annum for a 600 beds hospital.

Leroy *et al.* (1997) stated that the mean extra cost per infected case was 10,440 US $ dollars and extra days of hospital staying was 5.2 days in case of neonates acquiring hospital-acquired infection. Jervis (1996) stated that approximately 2 million nosocomial infections occurred annually in the United States. These infections resulted in substantial morbidity, mortality and cost. These excess duration of hospitalization secondary to nosocomial infections was 7 to 8.2 days for surgical site infections, 7 to 21 days for blood stream infections 6.8 to 30 days for pneumonia. The estimated mortality associated with nosocomial bloodstream infection and Pneumonia were 23.8% to 50% and 14.8% to 71%

respectively. The estimated average cost of these infections was $ 2734 for each surgical site infection, US $ 3061 to 4000 for each blood stream infection and US$ 4947 for each pneumonia. In countries with prospective payment system based on diagnosis related groups hospital lose from US$ 583 to US$ 4886 for each nosocomial infection.

Chapter III

Materials and Methods

3.1 Study place and period

The clinical specimens for this study were collected from the selective patients who were admitted in Dhaka Medical College Hospital (DMCH) and Bangladesh Institute of Rehabilitation for Diabetes, Endocrine and Metabolic disorders (BIRDEM), Dhaka. The required investigations were done in the department of Microbiology, BIRDEM and University of Dhaka. The study was conducted from November 1997 to June 1999.

3.2 Selection of cases and collection of samples

Hospital admitted patients of different age groups and of sexes were included in this study. The study population was categorized in 4 groups. Among them, 3 groups included the patients with different types of infections while the 4th group included the postoperative non-infected patients. The patients showing clinical symptoms of bacterial infection were selected as study population. The non-infected group was included in this study to compare the impact of nosocomial infections in terms of hospital stay with postoperative infected patients.

The four categories of patients were as follows:

a) Patients with postoperative wound infections: The postoperative patients who developed pus or purulent discharge forming wounds were selected for

sample collection. The first sample was collected from the operated wound area by a sterile cotton swab stick on the 1st day of dressing (in between 5^{th} to 8^{th} postoperative day). A second sample was collected from the same wound 5-7 days after the first sample collection when the patient was available.

b) Patients with post catheterized urinary tract infections (UTI): Urine samples were collected aseptically from the patients who were catheterized after admission. 1^{st} sample was collected immediately after catheterization and second simple was collected at least 3 days after catheterization. Culture was done for both the samples. When the 1^{st} sample showed significant growth of bacteria, it was excluded from the study. Only those cases were included in this study where 1^{st} sample showed insignificant or no growth but the subsequent 2nd sample showed significant growth of bacteria on culture.

c) Newly admitted diabetic patients with wounds: Newly admitted diabetic Patients with preexisting diabetic wounds were considered as study population. 1^{st} sample was collected under aseptic condition from the diabetic wound immediately after admission with a sterile cotton swab stick. A 2^{nd} sample was collected from the same wound 5 to 7 days after the collection of 1^{st} sample.

d) Postoperative patients without wound infection: This group of post operative patients manifested healed wound or yielded no growth on culture of the discharge from the wound (when available).

3.3 Collection of specimens for detection of source of infection

For detection of the source of infection, samples were collected from the different objects of the hospitals as described by (Weissfeld, 1980; Pelczar *et al.,* 1994). These samples were collected in the 1st and 3rd weeks of every month consecutively for 6 months.

a) Sample collection from the O.T Table, Floor and Wards: Samples were collected randomly from the O.T. table, floor during operation by swabbing 8 times of an area of (15cm X 15cm) with sterile cotton swab sticks which were pre moistened with sterile saline water. Samples were also collected randomly from floor of wards in the same way. The samples were then brought immediately for inoculation on to the selective culture media.

b) Sample collection from bed sheets: Samples were collected from one used bed sheet and one unused clean bed sheet of the surgical ward and of the operation theatre. Samples were collected from the bed sheets by swabbing 8 times of an area of (15cm x 15cm) with sterile swab sticks which were premoistened with sterile saline. The samples were then brought immediately for inoculation on to the selective culture media.

c) Sample collection from the tools of operation theatre: Samples were collected from different tools of operation theatre like forceps, scissors, distal end of suction tube during operation by swabbing 8 times with sterile swab sticks which were pre moistened with sterile saline. The samples were then brought immediately for inoculation on to the selective culture media.

d) Sample collection from dressing materials of ward: Samples were collected from dressing materials like gauge, bandage and cotton, by swabbing 8 times with a sterile cotton swab sticks pre moistened with sterile saline. In case of liquid samples like savlon, Acroflavin, containers were agitated frequently and then sterile swab sticks were impregnated aseptically into the liquid to make it wet with the liquid sample. The samples were then brought immediately for inoculation on to the selective culture media.

e) Sample collection from nasal cavity of ward and O.T personnel (doctors, nurses and wardboys): Before the start of operation in the O.T. the sterile swab sticks pre moistened with sterile saline was gently passed along the floor of the nose directing the swab downwards and backwards. Then it was taken out after rotating it on the nasal mucosa and it was immediately inoculated on to the selected agar media. In this way, the nasal samples were collected from the doctors, nurses and ward boys of the ward before dressing of the wounds of the patients. The samples were then brought immediately for inoculation on to the selective culture media.

f) Sample collection from the nail folds, interdigital spaces and palms of wards and OT personnel (doctors, nurses and wardboys): Samples were collected from nails folds, inter digital spaces and both palms of doctors, nurses and ward boys of O.T. during operation by sterile swab sticks which were pre moistened with sterile saline. In this way, the samples were collected from doctors, nurses and ward boys before dressing of the wounds of the patients in

the wards. The samples were then brought immediately for inoculation on to the selective culture media.

g) Air sampling from Operation Theatre and Ward: The settling plate technique was used for air sampling. One blood agar and one MacConkey agar plate were exposed for 30 minutes in the operation theatre during operation and in the ward during dressing of the wound of the patients. The plates were then brought immediately for incubation.

3.4 Method of isolation and identification of the organisms from the specimens

The organisms were isolated from the specimen by the following methods –

1. **Inoculation:** - The specimens were immediately inoculated on to the Blood agar and MacConkey agar media and incubated at 37^0 C for 24 hours.
2. **Sub culture:** The representative colonies were picked up by sterilized wire loop and further streaked on to the Blood agar and MacConkey agar media and incubated at 37^0 C for 24 hours for obtaining pure culture.

Identification of isolated organisms: Pure cultures were obtained through subcultures of the test isolates on to the selected agar plate (Blood agar and Mac conkey agar) depending on the type of bacteria and identification of the organisms were done by (i) colony morphology, (ii) Gram staining, (iii) necessary biochemical tests.

Colony morphology: In pure culture the size, shape, edge, elevation, opacity and colour were studied on plating media for morphological studies of the bacterial colony (Appendix-F).

Gram staining: This technique was used for observation of morphological characteristics of bacterial strains. Gram positive organisms were purple and gram negative organisms were pink to red in colour.

Biochemical tests: The following biochemical tests were performed for the identification of the gram-positive bacteria.

Coagulase test: A drop of physiological saline was take on each end of a clean slide. A colony of the test organisms was emulsified in each of the drop to make suspensions. Then a drop of plasma was added to one of the suspensions and it was mixed. No plasma was added to the second suspension. Clumping of the organisms within 10 seconds indicated positive coagulase test (Cruickshank *et al.*, 1975).

Catalase Test: 2-3 ml of hydrogen peroxide was poured into the test tube and it was shaken well to expel the dissolved oxygen. Then a growth of the test organisms was immersed into the hydrogen peroxide by a sterile glass rod. Immediate bubbling in the hydrogen peroxide indicated positive test (Cruickshank *et al.*, 1975).

The following biochemical tests were performed for identification of gram negative bacteria:

Oxidase test: A piece of filter paper was soaked with a few drops of oxidase reagent (Phenylene diamine). A colony of the test organism was picked up with a sterile toothpick and streaked on to the filter paper with impregnated reagent. A dark purple colour development within 5-10 seconds was considered positive test (Cruickshank *et al.*, 1975).

Motility and Urease test: MIU media contained in tubes were inoculated with smooth colonies of test organisms, using sterile straight wire by stabbing the media to a depth not touching the bottom. The tubes were inoculated at 37^0 C for 24 hours. The motile organisms dispersed through the medium leaving the stab line and made the media turbid. pink colour of the media indicated positive urease.

Indole test: The test organisms were cultured in 5 ml of peptone water and it was incubated at 37^0 C for 24 hours. Then a few drops of Kovac`s reagent was added. Positive indole test was indicated by production of red colour at the junction of the culture and the reagent.

Citrate test: Tubes of Simon's citrate agar media were inoculated with smooth colonies of the test organisms by stabbing the butt and streaking the slant by straight wire. Then the tubes were incubated at 37^0 C for 24 hours. After 24 hours a change in colour of the indicator from light green to blue indicated citrate utilization.

Kligler Iron Agar (KIA) test: Tubes of KIA media were inoculated with smooth colony of the test organisms by stabbing the butt and streaking the slant. Then the tubes were incubated for 24 hours at 37^0C. Yellow colour formation in the butt and slant indicated acid formation, hydrogen sulfide production were indicated by blackening of the media, and production of bubbles in the media indicated gas formation.

Sugar Fermentation tests: Fermentation tests were done with the following carbohydrates i.e. Glucose, Fructose, Sucrose, Maltose, Lactose, Manitol. The results were interpreted as per Cruickshank *et al.* (1975).

3.5 Preservation of the identified organisms

After identification, the isolates were subcultured on Muller Hilton agar media to obtain profuse growth. Then these were collected from the media and preserved in 15% Glycerol broth and stored at -20^0C for antibiogram, Plasmid profile analysis (PPA) and RFLP.

3.6 Antimicrobial susceptibility test for isolated strains by disc diffusion method

All the isolates were tested for sensitivity towards the following antimicrobial agents- ampicillin (AM), cotrimoxazole (TS), cephalexin (CFX), tetracycline (T) ceftriaxone (CRO), ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (GM). Susceptibility test was performed by disc diffusion method of Kirby

Bauer *et al.* (1966) and commercially available antimicrobial discs were used for the test. These discs were procured from Mast Group Ltd, U.K.

Method for sensitivity test: Preserved organisms were cultured on Muller Hilton agar media. One loop-full of the organism was taken from each of the strain to be tested from the Muller-Hilton agar plate and was inoculated in 4.5 ml of peptone water. The culture was then allowed to incubate at 37^0C until slightly visible turbidity appeared (usually 2-5 hours). The turbidity of the actively growing broth cultures was then adjusted with peptone water to obtain turbidity visually comparable to that of turbidity standard of one half of MacFarland tube no.1 (Appendix-D) which approximately correspond to 1.5x 10^8 organisms.

Freshly prepared Muller Hilton medium (Appendix-B) was used for sensitivity test. Within 15 minutes of adjustment of density of the organism in the inoculated tubes, a sterile cotton swab was dipped into the bacterial suspension. Excess of 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 Muller Hilton agar plate in three different planes (by rotation the plate at 60^0 angle each time) to get uniform distribution of the inoculum. and it was at left at room temperature for 10-15 minutes to dry. Then the antibiotic discs were placed on the inoculated surface of the plate with the help of a pointed sterile forceps. The discs were applied firmly to ensure

complete contact with the agar. The discs were placed 15 mm away from the edge of the petridish and 24 mm away from the adjacent discs. The plates were then incubated at 37^0 C for 18 to 24 hours.

Quality control: In order to standardize the disc potency, discs from each batch were tested against reference strain of ATCC *Esch. coli.* No 25922 and *Pseudomonas aeruginosa* No 27853. Zones of inhibition were compared with the standard value (Figure 1 and 2). A control was set up for every batch.

Reading of sensitivity tests: Each plate was examined after over night (18- 24 hours) incubation at 37^0 C and the diameters of complete zone of inhibition were measured with the help of a scale placed on the undersurface of the petridish. Zone of inhibition was measured in two directions at right angles to each other through the center of the disc and average of the two readings was taken (Figure 3 and 4).

Interpretation of zone sizes: Inhibition zone produced by each drug was translated into 3 susceptibility categories namely-sensitive (S), moderately sensitive (M) and resistant (R). (According to National Committee for clinical laboratory standards. Voluntary consensus standards for clinical laboratory testing. Villanova, PA, NCCLS, 1998) as shown in antibiogram interpretation chart below.

BACTERIOLOGICAL INVESTIGATIONS

Interpretative chart of zone sizes

Diameter of zone inhibition (mm)

Antibiotic or Chemotherapeutic agent	Disc Potency	Susceptibility		
		Resistant	Intermediate/ moderately	Susceptible
Ampicillin	10 µg	≤ 13	14-16	≥ 17
Ceftriaxone	30 µg	≤ 13	14-20	≥ 21
co-trimoxazole	25 µg	≤ 10	11-15	≥ 16
Gentamicin	10 µg	≤ 12	13-14	≥ 15
Tetracycline	30 µg	≤ 14	15-18	≥ 19
Ceftazidime	30 µg	≤ 14	15-17	≥ 18
Ciprofloxacin	5 µg	≤ 15	16-20	≥ 21
Cephalothin (cephalexin)	30 µg	≤ 14	15-17	≥ 18

Source: National Committee for Clinical Laboratory Standards, Voluntary Consensus standards for clinical laboratory testing. Villanova, PA, NCCLS, 1998.

3.7 EXTRACTION AND ANALYSIS OF PLASMID DNA

Plasmid profile analysis (PPA) was done to determine the possible sources of nosocomial infections. PPA was done with the selected bacterial strains which were isolated from the samples of the admitted patients and from the isolates from possible sources of hospital environment.

a) **Method of extraction of plasmid**

The plasmid was extracted from *Esch.coli, Pseudomonas* sp., *Klebsiella* sp. which were isolated from the patients and the possible sources of hospital environment by Miniprep method as described by Frederick *et al.* (1995). The details of the procedure was given below:

1. A single colony of bacteria was inoculated in an antibiotic (Tetracycline–16μg/ml) containing Luria broth (12 ml) and it was incubated for 18–24 hours at 37^0C
2. 1 ml of overnight culture was harvested in 1.5 ml of micro centrifuge tube and it was centrifuged at 13000 rpm for 5 minutes.
3. The supernatant was discarded and the pellet was re-suspended in 100 μl of solution I and it was incubated at room temperature for 5 minutes.
4. 200 μl of freshly prepared solution II was added and it was mixed gently by inverting the tube two or three times and was put on ice for 5 minutes.
5. 150 μl of ice cold solution III was added and was mixed gently and it was vortexed for 10 seconds and then it was incubated on ice for 5 minutes.
6. Then it was centrifuged for 5 minutes at 13000 rpm to precipitate the cellular remnants that included protein, dodecyl sulphate and chromosomal DNA.

7. The supernatant was transferred to fresh tube and 2 volumes (900 µl) of 95% ethanol was added and it was mixed well by vortex and incubated for 2 minutes at room temperature.
8. Then it was centrifuged for 10 minutes at 13000 rpm to precipitate the plasmid DNA.
9. The supernatant was then discarded and the lid of the tube was kept open for a few minutes to air dry.
10. 20 µl of TE buffer was added & it was stored at 4^0C.
11. The extracted plasmid DNA was then analyzed by Agarose gel electrophoresis

b) Agarose gel electrophoresis (In Vertical electrophoresis apparatus)

In agarose gel electrophoresis plasmid DNA move from negative (Cathode) to positive (anode) pole and produce bands which can be visualized by staining with ethidium bromide. The migration of different DNA species is related inversely to their molecular weight i,e. larger the molecular weight, the slower the rate of migration.

Equipments and reagents used :

i) Gel tank, ii) Glass plates, iii) Spacers, iv) Comb, v) Power source, vi) Clamp, vii) Microsampling pipettes, viii) 0.7% Agarose gel, ix) Electrophoresis buffer (TBE buffer) : containing 10.8 gm. Tris base, 0.93 gm. Sodium EDTA, 5.5 gm boric acid, made up to 1000 ml with distilled water) x). Ethidium bromide 10 gm./ml (stock solution).

Aagarose gel electrophoresis

Test procedure:

1. 0.7gram of agarose was dissolved homogeneously in 100ml of TBE buffer (pH 8.9) by boiling. The molten agarose was cooled down to 50^0C before pouring the gel on the gel slab.
2. The sample wells were prepared with a comb (made of Lucite, PVC Teflon) by inserting it into the molten agar. The comb was adjusted before pouring the gel on the gel slab. After the gel was completely set the comb was removed carefully. Then gel with wells and plates were placed into the electrophoresis (gel) tank. The top and bottom tanks were filled with electrophoresis buffer. 10µl of loading buffer (0.15%, W/V bromophenol blue, 0.5% SDS, 0.15% M EDTA, 50% glycerol) was added to the 20µl of samples. After mixing with loading buffer, 10µl of the samples were loaded into the wells. A standard molecular weight marker having plasmids of known molecular weight was also included in the gel run procedure.
3. Sufficient amount of 1 x TBE buffer was poured on the electrophoresis chamber to flood the agarose gel and electrophoresis was run at 60–120 volts for 2-3 hours or until the front line of the dye had run through the gel.
4. The gel was stained after the run by soaking the gel in 0.5µg per ml of ethidium bromide in TBE buffer for about one hour.
5. The stained gel was then destained by soaking in distilled water for half an hour.

6. The seperated bands of DNA were visualized by UV transilluminator and photograph was taken by Bio documentation system. (Figure 5).

Determination of molecular weight of unknown plasmids:

A standard curve was drawn by plotting the mobility of known plasmid DNA along the X-axis and its molecular weight along the Y-axis. The molecular weight of the unknown plasmids were determined from this curve. (Figure 6).

3.8 EXTRACTION OF GENOMIC DNA FOR RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) ASSAY. Restriction fragment length polymorphism (RFLP) was also done to determine the possible sources of nosocomial infection. RFLP was done with the selected bacterial strains which were isolated from the samples of admitted patients and those of the possible sources of infection.

Method of extraction of genomic DNA: The genomic DNA was extracted from *Esch.coli, Pseudomonas* sp., *Klebsiella* sp. isolated from the infected sites of the admitted patients and possible sources of hospital environment by Miniprep method as described by Frederick *et al.,* (1995). The details of the procedure was given below:

1. A single colony of bacteria was inoculated in 20ml of Luria broth.

2. 2 ml of culture was centrifuged in eppendorps tube at 14000 rpm for 02 minutes and it was repeated for 5 times to have a big pellet. The supernatant was discarded.
3. The pellet was resuspended at 200 µl solution-1. (Glucose, EDTA, Tris).
4. 75µl of 0.25 M EDTA and 75µl of 0.25 M Tris Hcl was added.
5. 3 mg of lysozyme was also added into the cell containing solution-1 and it was kept at 40^0C for 5 minutes.
6. 140 µl of proteinase-k buffer (0.5M EDTA + 0.5% SDS) + 5.0 µl proteinase-k (stock conc. H_2O dissolved).
7. It was kept at 45^0C for 2 hours.
8. It was then treated with equal volume of phenol and centrifuged for 10 minutes and the supernatant was taken.
9. It was then treated with equal volume of phenol, chloroform, isoamyl alcohol solution (25:24:1) and then centrifuged for 10 minutes and supernatant was taken.
10. DNA was precipitated with double volume of ethanol and then centrifuge for 10 minutes. The supernatant was discarded and the pellet was taken and it was dried.
11. The pellet was dissolved in 400µl of T E buffer.
12. 5µl of RNase A (DNase free) was added and kept at 37^0C for 2 hours.
13. The procedure No. 8-10 was repeated and the DNA was dissolved in 40-50µl of T E buffer.

Digestion of Isolated genomic DNA:

The concentration of isolated genomic DNA was measured by spectrophotometer. Then 1μg of DNA was digested with restriction enzymes *Eco* R_I, *Hin* dIII, *Bam* H_I for 6 hours (at 37^0C. The digested product was then analyzed by agarose gel electrophoresis as described in para 3.7.

3.9 Data management and analysis: The collected data were checked, verified and edited daily. After proper verification, the data were coded and entered into computer by using SPSS Data Entry II program. After the entry, range and consistency of data were checked by using Epi Info version 6 software program. Data were analyzed according to the objectives of the study. Statistical analysis were carried out by using SPSS 7.5 software program. Statistical significance was tested at the appropriate probability level.

Fig 1:Antimirobial sensitivity pattern of *Esch.coli* ATCC 25922 strain in Muller –Hilton medium

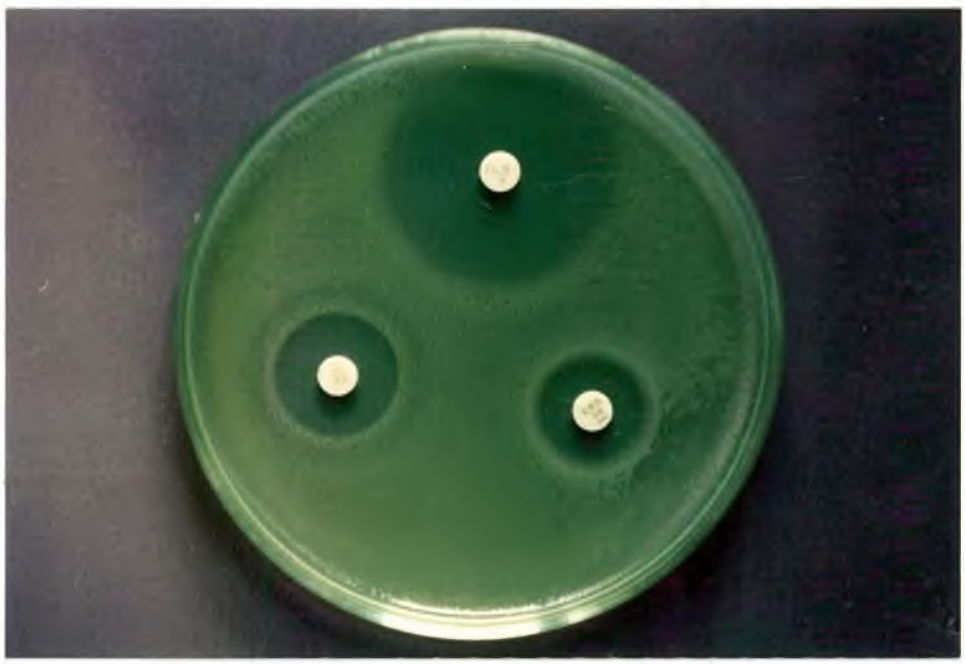


Fig 2: Antimirobial sensitivity pattern of *Pseudomonas* ATCC 27853 strain in Muller –Hilton medium

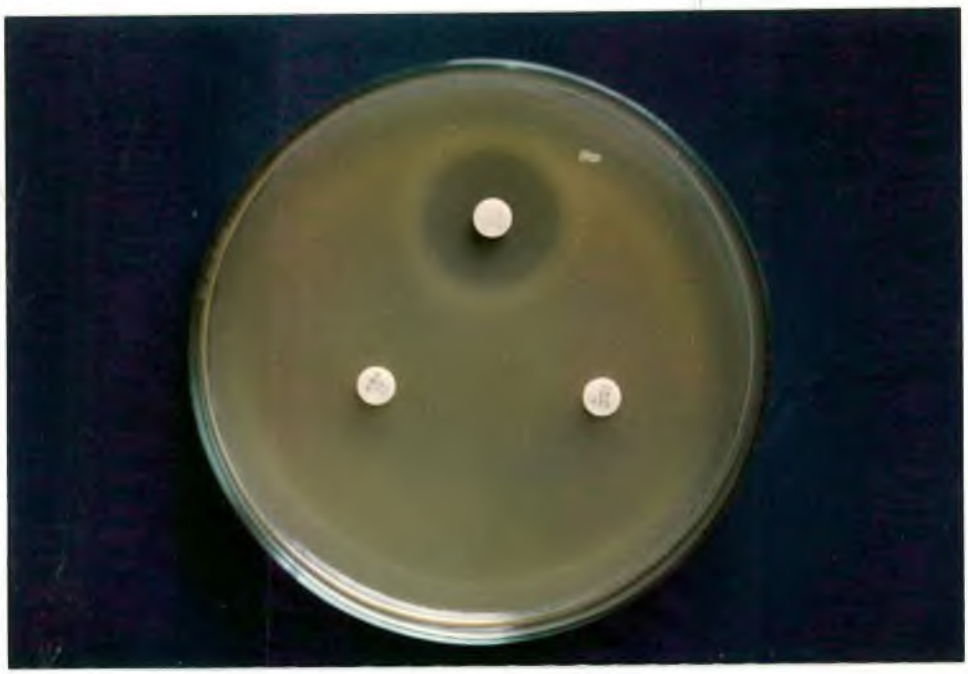


Fig 3:Antibiogram of *Esch.coli* in Muller –Hilton medium showing sensitive to CAZ and resistant to AMP, SXT (Cotrimoxazole).

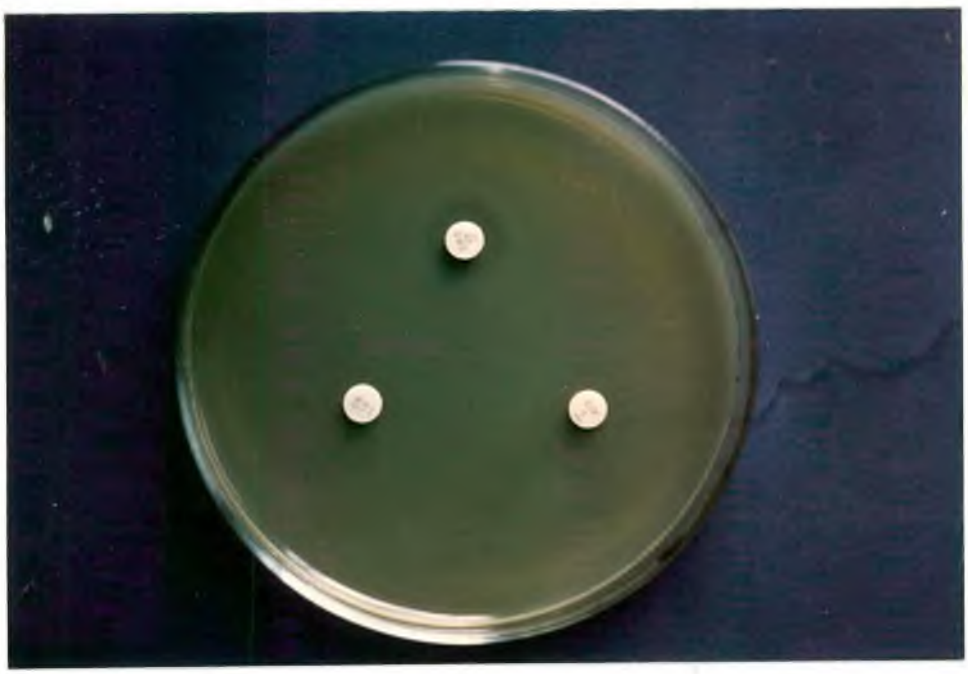


Fig 4: Antibiogram of *Pseudomonas* in Muller –Hilton medium showing resistant to CIP,CRO and CN (gentamicin).

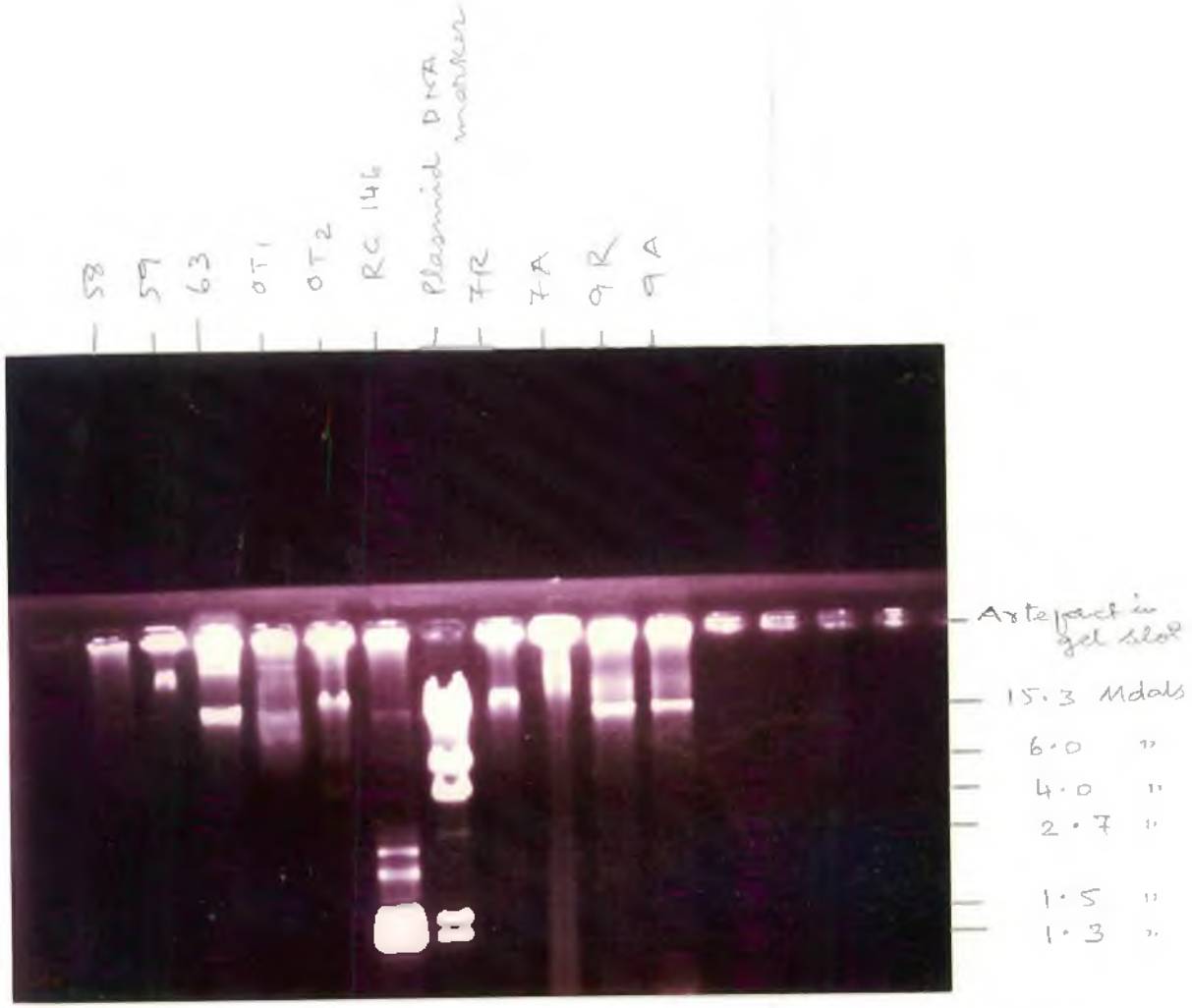


Fig 5: Agarose gel electrophoresis of plasmids extracted from different samples

From left to right:

Sample 58 showing no plasmid band.

Sample 59 showing 1 plasmid band.

Sample 63 showing 2 plasmid bands.

Sample OT 1 showing 4 plasmid bands.

Sample OT 2 showing 3 plasmid bands.

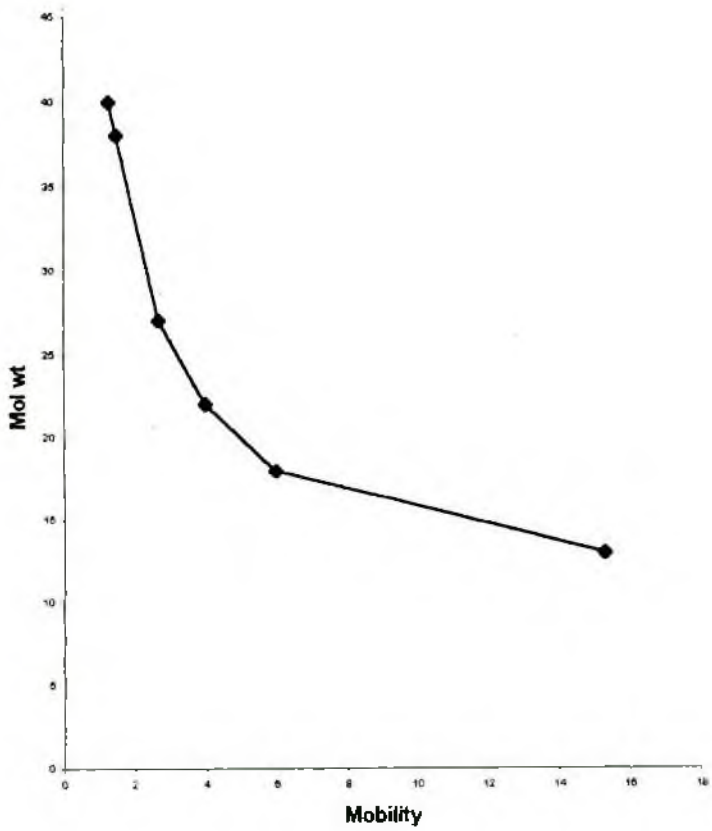
Sample 7R showing 1 plasmid band.

Sample 7A showing 2 plasmid bands.

Sample 9R showing 1 plasmid band.

Sample 9A showing 1 plasmid band.

Fig 6: Determination of molecular weight of unknown plasmid



Chapter IV

Results

A total of 152 patients were studied in different categories as showed in ***Table 1***. Among them 102 (67.1%) were infected cases and the remaining 50 (32.9%) were postoperative non-infected cases. Among the infected cases 74 (48.7%) had postoperative infection and 16 (10.5%) were cases with post catheterized UTI and the remaining 12 (7.9%) patients were with diabetic wound infection.

Table 2 shows age distribution of infected and non-infected cases. It was observed that the highest percentage (31.6%) of the patients were in the age group of 20-29 years followed by age group of 30-39 years (21.7%). No significant age difference was observed between infected and non-infected cases ($p>0.05$).

Table 3 shows the distribution of patients by sex. Most of the patients were male 114(75.0%) and the remaining 38 (25.0%) were female. It was evident that infected cases were more in female than male ($p<0.001$).

Table 4 shows that 14 different types of operations were performed on 124 patients of this study population. It was observed that out of 124 operated cases

74 (59.7%) were postoperative infected and 50 (40.2%) postoperative non-infected cases. It was also observed that laparotomy 42 (33.9%)cases topped the list of performed operation, followed by caesarean section operation 22 (17.7%), appendisectomy 18 (14.5%), inguinal herniorrhaphy 9 (7.2%), wound toileting 7(5.6%). Other operations were fistulectomy 4 (3.2%), plastic surgery 3(2.4%), amputation 2(1.6%), mastectomy 2(1.6%), cystostomy 1(0.8%), sequestrecstomy 1(0.8%), nephrostomy 1(0.8%). It is to be noted that in case of caesarean section operation, postoperative non infected group could not be included due to circumstantial constrains.

Rate of infection according to the type of operative procedures is shown in **Table 5**. It was observed that out of 42 laparotomy cases 26(61.9%) suffer from postoperative wound infection, while 16(38.1%) did not develop postoperative wound infection. In case of caesereans section operation 22(100.0%) were postoperative infected patients. Postoperative non infected group of caesarean operation could not be included in this study due to circumstantial constrains. In case of appendisectomy, inguinal herniorrhaphy, cholecystectomy postoperative infected and postoperative non infected cases were 2(11.1%) VS 16(88.9%), 1(11.1%) VS 8(88.9%), 1(14.3%) VS 6(85.7%) respectively.

Table 6 shows the rate of isolation of organisms from different types of infections. It was found that *Esch. coli* was the predominating organisms in case of postoperative wound infection (56.7%) and post catheterized urinary tract infection (68.7%). This was followed by *Pseudomonas* sp. which was (27.0%) in case of postoperative wound infection and 18.7% in case of post catheterized UTI. In case of diabetic wound infection, *Pseudomonas* sp. was the predominating organism (91.7%) followed by *Esch.coli* (33.3%).

Figure 7 and 8 shows the antibiogram pattern of all the isolated organisms from 1st and 2nd samples of infected cases. It was observed that the resistance of the organisms to the used antibiotics markedly increased in the 2nd samples. *Pseudomonas, Klebsiella, Proteus* species included all the species isolated as mentioned in Table 6.

Table 7 shows the antibiogram pattern of *Esch. coli* isolated from the 1st and 2nd samples of infected cases. It was observed that ampicillin was resistant to 100.0% of *Esch. coli* isolated from the 1st and 2nd sample. It was also observed that sensitivity of *Esch. coli* to other antibiotics markedly reduced in 2nd samples. There was significant increase of resistance to ceftriaxone from 43.9% to 73.9% ($p<0.001$), ceftazidime from 28.1% to 65.2% ($p<0.002$), ciprofloxacin from 70.2% to 100.0% ($p<0.005$) and gentamicin from 61.4% to 91.3%

($p<0.008$) in 2nd samples. But no significant difference of the rate of resistance was found between 1st and 2nd samples to ampicillin, cephalexin, tetracycline and cotrimoxazole ($p>0.05$).

Table 8 shows the antibiogram pattern of *Pseudomonas* sp. isolated from the 1st and 2nd samples of infected cases. It was observed that resistace of *Pseudomonas* sp. to all the used antibiotics increased in 2nd samples, but it was not statistically significant ($p>0.05$) except ciprofloxacin ($p<0.004$). The resistance activity of *Pseudomonas* sp. to ciprolloxacin increased in 2nd samples significantly ($p<0.05$).

Table 9 shows the antibiogram pattern of *Proteus* sp. isolated from the 1st and 2nd samples. It was observed that sensitivity of *Proteus* sp. to all the used antibiotics reduced in 2nd samples but none were statistically significant ($p>0.05$). The resistance pattern of *Proteus* sp. to antibiotics increased except ampicillin and cotrimoxazole but the difference was not statistically significant ($p>0.05$).

Table 10 shows antibiotic resistance pattern of *Esch.coli* isolated from postoperative infected wounds, post catheterized UTI and diabetic wounds and were compared to cephalexin, ceftriaxone, ceftazidime, ciprofloxain and

gentamicin. It was found that resistance pattern of *Esch.coli* isolated from different sites of infection were dissimilar. *Esch.coli* isolated from diabetic wound infections showed the highest resistance to cephalexin (100.0%) followed by ceftriaxone (75.0%), ciprofloxacin (75.0%), ceftazidime (50.0%) and gentamicin (50.0%). However, *Esch. coli* isolated from post catheterized UTI showed the highest resistance to cephalexin (90.9%) followed by ciprofloxacin (81.8%) and gentamicin (81.8%), ceftriaxone (72.7%) and ceftazidime (63.6%). It was also observed that *Esch. coli* isolated from postoperative wound infections showed lower rate of resistance to all the used antibiotics except gentamicin.

Table 11 shows the variation of antibiotic resistance pattern of *Pseudomonas* sp. isolated from the different sites of infection. It was found that *Pseudomonas* sp. isolated from the post catheterized UTI showed the highest resistance to cephalexin (100.0%), followed by ceftrioxone (66.7%) and ciprofloxcin (66.7%). *Pseudomonas* sp. isolated from the diabetic wound infection showed the highest resistance to gentamicin (45.5%). *Pseudomonas* sp. isolated from the post operative wound infection showed the lower rate of resistance to all the used antibiotics except ceftazidime (35.0%)

Table 12 shows the pattern of antibiotics prescribed in infected and non infected cases. It was observed that rate of ciprofloxacin prescription was the highest 31(30.4%) in postoperative infected cases followed by ampicillin+gentamicin 13(12.7%), ampicillin+ciprofloxacin 11(10.8%). On the other hand in postoperative non-infected cases, rate of amoxicillin prescription was the highest 29(58.0%) followed by ceftriaxone 8(16%) and ciprofloxacin 4(8.0%).

Table 13 shows the antibiogram pattern of isolated organisms to the prescribed antibiotics. It was observed that resistance rate of isolated *Esch.coli* and *Pseudomonas* sp. to the prescribed ampicillin, cotrimoxazole, cephalexin was 100.0%. However, resistance rate of *Esch.coli* to prescribed gentamicin, ceftriaxone and ciprofloxacin was (91.7%), (66.9%) and (85.7%) respectively. Resistance rate of *Pseudomonas* sp. to gentamicin, ceftriaxone and ciprofloxacin was (100.0%), (100.0%) and (94.1%) respectively.

Table 14 shows the involvement of single and multiple type of organisms on the causation of infection. It was observed that (85.3%) infection was caused by single organism, while 12.7% was caused by two types of organisms and only 2.0% of infection was caused by three types of organisms.

Duration of hospital stays in infected and non-infected cases was show in ***Table 15.*** It was found that mean duration of hospital stay in infected patients was 23.5±13.3 days and that of postoperative non-infected patients 9.5±6.1 days indicating that the mean duration of hospital stay was significantly higher in infected patients ($p<0.001$).

Table 16 shows the relationship between type of infection and duration of hospital stay. It was found that the mean duration of hospital stay was 26.1±9.9 days in case of diabetic wound infection followed by 23.9±14.3 days in postoperative wound infection. In case of post catheterized UTI it was 19.9±10.1 days, while in case of postoperative non-infected cases the duration of hospital stay was 9.5±6.1 days. This suggested that the longer duration of hospital stay happened in case of infected patients ($p<0.001$).

Table 17 depicts the results of the duration of hospital stay in relation to the infection caused by single and multiple organisms. It was found that the highest duration of hospital stay 33.5±20.6 days was in multiple organisms whereas it was 21.8±10 days in case of single organism indicating that mean duration of hospital stay was significantly higher in cases of infections caused by multiple organisms. ($p<0.001$)

Table 18 shows relationship between type of infecting organisms and duration of hospital stay. It was observed that the longest duration of hospital stay 33.5±20.6 days was in case of infection caused by mixed organisms (i.e polymicrobial infections of *Esch.coli+Pseudomonas* sp.+*Proteus* sp.) followed by infection caused by *Staph.aureus, Klebsiella* sp. *and Acinetobacter* sp. Mean duration of hospital stay was 21.2±9.1 days in infection caused by only *Esch. coli*, whereas it was 18.7±8.7 days in *Pseudomonas* sp. infection alone. In case of non-infected cases it was 9.5±6.1 days indicating a significantly longer duration of stay in hospital in case of infections caused by mixed organisms ($p<0.001$)

Table 19 shows the relationship between the length of operation time and causation of infection in case of operative patients. It was observed that mean length of operation was 53.2±20.3 minutes in case of postoperative infected patients and 52.3±21.0 minutes in case of postoperative non-infected group. No significant mean difference of length of operation time was observed between postoperative infected and non-infected group ($p>0.05$).

Organisms isolated from different objects of hospital environment on different dates was depicted in ***Table 20 and Figure 9-14***. It was observed that among the organisms seven isolates of *Esch. coli* were isolated from OT air, table,

floor, tap water, distal end of suction tube and ward floor. Two isolates of *Pseudomonas* sp. were isolated from OT air and boiled water. Two isolates of *Klebsiella* sp. were isolated from OT floor. One isolate of *Proteus* sp. was isolated from OT gloves. However, six isolates of *Strep. viridans* were isolated from the nasal cavity of the OT personnel. Moreover, 15 isolates of coagulase negative *Staphylococcus* were isolated from nasal cavity, palm, nail bed and inter digital spaces of the OT and ward personnel and OT air. *B. subtalis* were isolated from the OT floor, ward floor and bed sheets.

Table 21(a-e) shows antibiogram pattern of the organisms isolated from the different objects of hospital environment. It was observed that different isolates of the hospital environment showed different pattern of antibiogram to the used antibiotics in this study.

The molecular weight and the number of extracted plasmid DNA of the representative isolates of the patients and the hospital environment was shown in ***Table 22***. The number of plasmid DNA and its corresponding molecular weight of the isolates were also compared. It was observed that the pattern of plasmid DNA of the patient and that of the hospital environment was dissimilar.

Table 1: Categories of study population (n = 152)

Categories of cases	Number of Patients	Percentage
Patients with postoperative surgical wound infection	74	48.7
Patients with post catheterized urinary tract infection	16	10.5
Patients with diabetic wound infection	12	7.9
Total	**102**	**67.1**
Patients without postoperative wound infection	50	32.9
Total	**152**	**100.0**

Table 2: Age distribution of infected and non-infected cases (n=152)

Age in Years	Infected cases (%)	Non-infected cases (%)	Total (%)
< 20	11	6	17
	(10.8)	(12.0)	(12.2)
20-29	31	17	48
	(30.4)	(34.0)	(31.6)
30-39	23	10	33
	(22.5)	(20.0)	(21.7)
40-49	14	10	24
	(13.7)	(20.0)	(15.8)
50+	23	7	30
	(22.5)	(14.0)	(19.7)
Total	102	50	152
	(67.1)	(32.9)	(100.0)

N.B. Figure in parenthesis indicate percentages

$\chi^2 = 2.39$;p-value = 0.609.

Table 3: Distribution of patients by sex

Category	Sex		Total
	Male	Female	
Infected cases	68 (59.6)	34 (89.5)	102 (67.1)
Non-infected cases	46 (40.4)	4 (10.5)	50 (32.9)
Total	**114 (75.0)**	**38 (25.0)**	**152 (100.0)**

$\times^2$=11.5; p-value=0.001;

N.B. Figures in Parenthesis indicate Percentages.

Table 4: Types of operative procedures performed in infected and non-infected cases

Type of operation	Postoperative Infected cases		Postoperative non-infected cases		Total infected	
	No.	%	No.	%	No.	%
Laparotomy	26	21.0	16	12.9	42	33.9
Caesarean section operation	22	17.7	--	--	22	17.7
Appendisectomy	2	1.6	16	12.9	18	14.5
Inguinal herniorrhaphy	1	0.8	8	6.4	9	7.2
Cholecystectomy	1	0.8	6	4.8	7	5.6
Wound toileting	7	5.6	0	0	7	5.6
Abscess excision	5	4.0	0	0	5	4.0
Fistulectomy	1	0.8	3	2.4	4	3.2
Plastic surgery	3	2.4	0	0	3	2.4
Amputation	2	1.6	0	0	2	1.6
Mastectomy	1	0.8	--	0.8	2	1.6
Cystostomy	1	0.8	0	0	1	0.8
Sequestrectomy	1	0.8	0	0	1	0.8
Nephrostomy	1	0.8	0	0	1	0.8

Table 5: Rate of infection according to the type of operative procedures

Type of operation	Infected cases		non-infected cases		Total	
	No.	%	No.	%	No.	%
Laparotomy	26	61.9	16	38.1	42	100.0
*Caesarean Section operation	22	100.0	0	0	22	100.0
Appendisectomy	2	11.1	16	88.9	18	100.0
Inguinal herniorrhaphy	1	11.1	8	88.9	9	100.0
Cholecystectomy	1	14.3	6	85.7	7	100.0
Wound toileting	7	100.0	0	0	7	100.0
Abscess excision	5	100.0	0	0	5	100.0
Fistulectomy	1	25.0	3	75.0	4	100.0
Plastic surgery	3	100.0	0	0	3	100.0
Amputation	2	100.0	0	0	2	100.0
Mastectomy	1	50.0	1	50.0	2	100.0
Cystostomy	1	100.0	0	0	1	100.0
Sequestrectomy	1	100.0	0	0	1	100.0
Nephrostomy	1	100.0	0	0	1	100.0

N.B. In Caesarean section operation postoperative non-infected cases could not be included due to circumstantial constraints

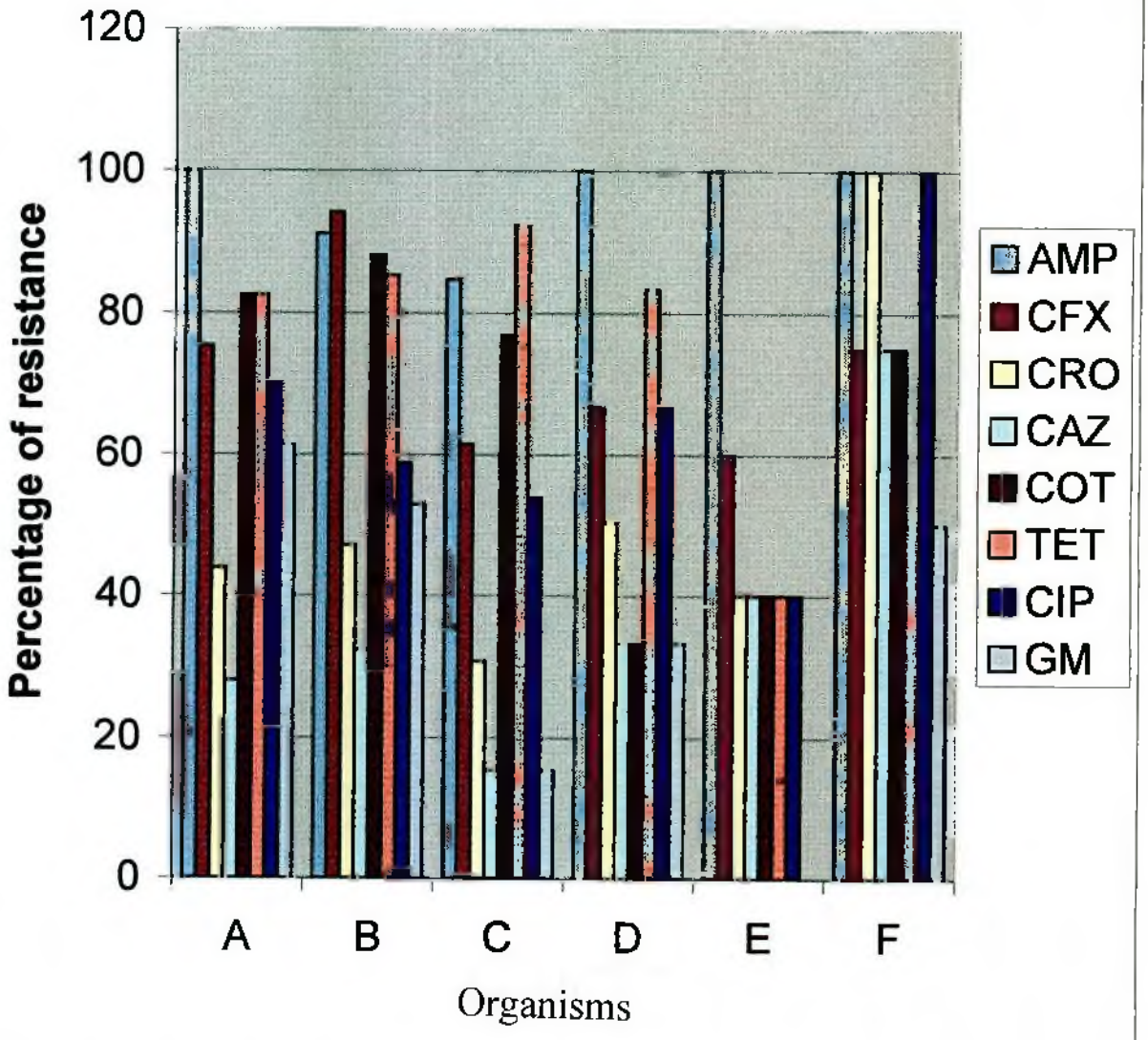
Table 6: Rate of organisms isolated from different types of infections (n=102)

**Organisms isolated	Post operative wound infection (n=74)		Post Catheterized UTI (n=16)		Diabetic wound infection (n=12)		*Total (n=102)	
	No.	%	No.	%	No.	%	No.	%
Esch..coli	42	56.7	11	68.7	4	33.3	57	55.9
Pseudomonas sp.	20	27.0	3	18.7	11	91.7	34	33.3
Proteus sp.	10	13.5	1	6.2	2	16.7	13	12.7
Staph. aureus	6	8.0	0	00.0	0	00.0	6	5.9
Klebsiella sp.	4	5.4	1	6.2	0	00.0	5	4.9
Acinetobacter sp.	3	4.0	1	6.2	0	00.0	4	3.9

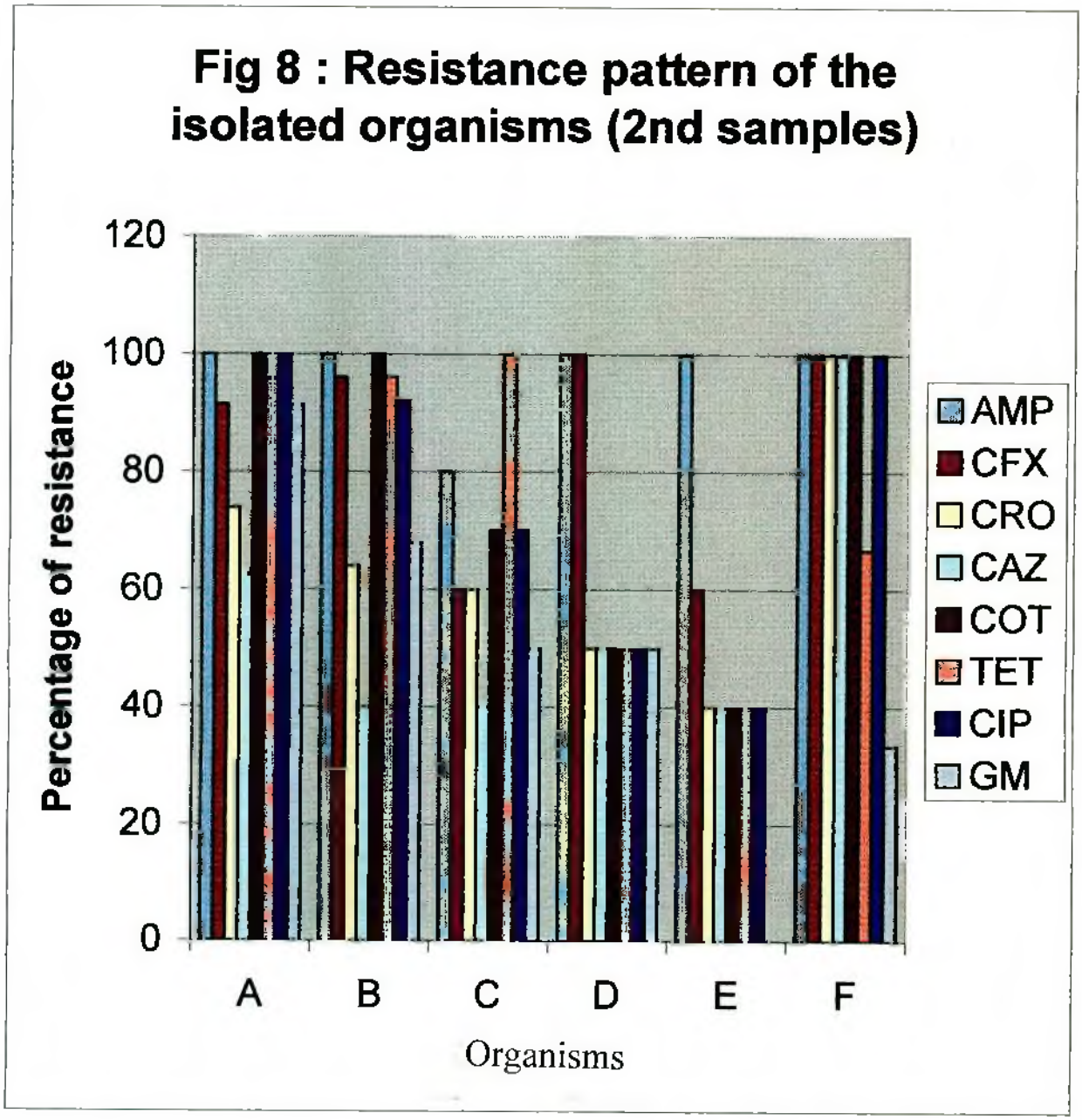
*Multiple organisms isolated from one sample

**Note , Various species of different organisms isolated were: *Pseudomonas aeruginosa* =30, *Pseudomonas flusrescens* =2, *Pseudomonas cepacia* =2, *Proteus mirabilis* =10, *Proteus vulgaris* =3, *Klebsiella pneumonae* =3, *Klebsiella oxytoca* =1. *Acinetobacter* sp=4

Fig 7 : Resistance pattern of the isolated organisms (1st samples)



A=*Esch.coli*
B=*Pseudomonas* sp.
C=*Proteus* sp.
D=Staph. aureus
E=*Klebsiella* sp.
F=*Acinetobacter* sp.



A=*Esch.coli*
B=*Pseudomonas* sp.
C=*Proteus* sp.
D=Staph. aureus sp.
E=*Klebsiella* sp.
F=*Acinetobacter* sp.

Table 7: Antibiogram pattern of *Esch. coli* isolated from the 1st and 2nd samples of the infected cases

Antibiotics	1st sample (n=57)			2nd Sample (n=23)			P value
	S	M	R	S	M	R	
Ampicillin	0 (0.0)	0 (0.0)	57 (100.0)	0 (0.0)	0 (0.0)	23 (100.0)	p>0.05
Cephalexin	8 (14.0)	6 (10.5)	43 (75.4)	0 (0.0)	2 (8.7)	21 (91.5)	p>0.05
Ceftriaxone	19 (33.3)	13 (22.8)	25 (43.9)	3 (13.0)	3 (13.0)	17 (73.9)	P<0.01
Ceftazidime	25 (43.9)	16 (28.1)	16 (28.1)	4 (17.4)	4 (17.4)	15 (65.2)	P<0.002
Tetracycline	6 (10.5)	4 (7.0)	47 (82.5)	0 (0.0)	1 (4.3)	22 (95.7)	p>0.05
Cotrimoxazole	6 (10.5)	4 (7.0)	47 (82.5)	0 (0.0)	0 (0.0)	23 (100.0)	p>0.05
Ciprofloxacin	13 (22.8)	4 (7.0)	40 (70.2)	0 (0.0)	0 (0.0)	23 (100.0)	P<0.005
Gentamicin	11 (19.2)	11 (19.2)	35 (61.4)	0 (0.0)	2 (8.7)	21 (91.3)	P<0.008

Figures in parenthesis indicate percentage.

Table 8: Antibiogram pattern of *Pseudomonas* sp. isolated from the 1st and 2nd samples of the infected cases

Antibiotics	1st sample (n=34)			2nd sample (n=25)			P value
	S	M	R	S	M	R	
Ampicillins	0 (0.0)	2 (5.9)	32 (94.1)	0 (0.0)	0 (0.0)	25 (100.0)	p>0.05
Cephalexin	1 (2.9)	1 (2.9)	32 (94.1)	0 (0.0)	1 (4.0)	24 (96.0)	p>0.05
Ceftriaxone	12 (35.3)	6 (17.6)	16 (47.1)	3 (12.0)	6 (24.0)	16 (64.0)	p>0.05
Ceftazidime	17 (50.0)	6 (17.6)	11 (32.4)	9 (36.0)	6 (24.0)	10 (40.0)	p>0.05
Tetracycline	3 (8.8)	2 (5.9)	29 (85.3)	0 (0.0)	1 (40.0)	24 (96.0)	p>0.05
Cotrimoxazole	3 (8.8)	1 (2.9)	30 (88.2)	0 (0.0)	0 (0.0)	25 (100.)	p>0.05
Ciprofloxacin	11 (32.4)	3 (8.8)	20 (58.8)	2 (8.0)	0 (0.0)	23 (92.0)	P<0.004
Gentamicin	12 (35.3)	4 (11.8)	18 (52.9)	3 (12.0)	5 (20.0)	17 (68.0)	p>0.05

Figures in parenthesis indicate percentage.

N.B. *Pseudomonas* species included all the species isolated as mentioned in table 6.

Table 9: Antibiogram pattern of *Proteus* sp. isolated from the 1st and 2nd samples of the infected cases

Antibiotics	1st sample (n=13)			2nd sample (n=10)			P value
	S	M	R	S	M	R	
Ampicillins	2 (15.4)	0 (0.0)	11 (84.6)	1 (10.0)	1 (10.0)	8 (80.0)	p>0.05
Cephalexin	3 (23.1)	2 (15.4)	8 (51.5)	2 (20.0)	2 (20.0)	6 (60.0)	p>0.05
Ceftriaxone	9 (69.2)	0 (0.0)	4 (30.8)	4 (40.0)	0 (0.0)	6 (60.0)	p>0.05
Ceftazidime	10 (76.9)	1 (7.7)	2 (15.4)	4 (40.0)	2 (20.0)	4 (40.0)	p>0.05
Tetracycline	1 (7.7)	0 (0.0)	12 (92.3)	0 (0.0)	0 (0.0)	10 (100.0)	p>0.05
Cotrimoxazole	3 (23.1)	0 (0.0)	10 (76.9)	2 (20.0)	1 (10.0)	7 (70.0)	p>0.05
Ciprofloxacin	6 (42.0)	0 (0.0)	7 (53.8)	3 (30.0)	0 (0.0)	7 (70.0)	p>0.05
Gentamicin	8 (61.5)	3 (23.1)	2 (15.4)	3 (30.0)	2 (20.0)	5 (50.0)	p>0.05

Figures in parenthesis indicate percentage.

N.B. *Proteus* species included all the species isolated as mentioned in table 6.

Table 10: Variation of antibiotic resistance pattern of *Esch. coli* isolated from different sites of infection

Sites of infection	No. of isolates	Antibiotic Resistance				
		Cephalexin	Ceftriaxone	Ceftazidime	Ciprofloxacin	Gentamicin
Post operative wound infection	42	29 (69.0)	14 (33.3)	7 (16.0)	28 (66.7)	24 (57.1)
Post catheterized UTI	11	10 (90.9)	8 (72.7)	7 (63.6)	9 (81.8)	9 (81.8)
Diabetic wound infections	4	4 (100.0)	3 (75.0)	2 (50.0)	3 (75.0)	2 (50.0)

Figures in parenthesis indicate percentage.

Table 11: Antibiotic resistance pattern of *Pseudomonas* sp. isolated from different sites of infection

Sites of infection	No. of isolates	Antibiotic Resistance				
		Cephalexin	Ceftriaxone	Ceftazidime	Ciprofloxacin	Gentamicin
Postoperative wound infection	20	17 (85.0)	6 (30.0)	7 (35.0)	9 (45.0)	8 (40.0)
Post catheterized UTI	3	3 (100.0)	2 (66.7)	1 (33.3)	2 (66.7)	1 (33.3)
Diabetic wound infection	11	10 (90.9)	5 (45.5)	1 (9.1)	7 (63.6)	5 (45.5)

*Figures in parenthesis indicate percentage.

N.B. *Pseudomonas* species included all the species isolated as mentioned in table 6.

Table 12: Pattern of antibiotics used in infected and non-infected cases

Infected cases			Non-infected cases		
Antibiotics	**#**	**%**	**Antibiotics**	**#**	**%**
Ciprofloxacin	31	30.4	Amoxicillin	29	58.0
Ampicillin+Gentamicin	13	12.74	Ceftriaxone	8	16.0
Ampicillin+ Cipro	11	10.8	Ciprofloxacin	4	8.0
Amoxicillin+ Cipro	8	7.8	Amoxicillin+Genta+Cipro	4	8.0
Amoxicillin	6	5.9	Amoxicillin+Cipro	2	4.0
Cephalexin	4	3.9	Amoxicillin+Ceftriaxone	2	4.0
Cotrimoxazole	4	3.9	Ampicillin+Cloxa+Cipro	1	2.0
Ampicillin+Cephalexin	4	3.9			
Cephalexine+Cipro	3	2.9			
Cipro+Cloxacillin	3	2.9			
Ceftriaxone	2	2.0			
Neo-floxacin	1	1.0			
Cipro+Tetra+Cotrim	1	1.0			
Penicillin	1	1.0			
Amoxicillin+Ceftriaxone	1	1.0			
Ampicillin	1	1.0			
Ampicillin+Cloxacillin	2	2.0			
Amoxicillin+Cloxacillin	1	1.0			
Ampicillin+Cotrimoxazole	1	1.0			
Tetracycline+Cipro	1	1.0			
Gentamicin+Cipro	1	1.0			
Amoxicillin+Gentamicin	1	1.0			
Ampicillin+Ceftriaxone	1	1.0			
Total	102	100.0		50	100.0

Table 13: Antibiogram of isolated organisms to the prescribed antibiotics

Isolated Organisms	Antibiotics prescribed											
	AM		TS		CFX		GM		CRO		CIP	
	S	R	S	R	S	R	S	R	S	R	S	R
Esch. coli	0	27 (100.0)	0	3 (100.0)	0	3 (100.0)	1 (8.3)	11 (91.7)	1 (33.3)	2 (66.9)	5 (14.3)	30 (85.7)
Pseudomonas sp.	0	8 (100.0)	0	2 (100.0)	0	3 (100.0)	0	3 (100.0)	0	1 (100.0)	1 (5.9)	16 (94.1)
Proteus sp.	1 (14.3)	6 (85.7)	--	--	--	--	--	--	--	--	3 (37.5)	5 (62.5)

AM= Ampicillin, TS= Cotrimoxazole, CFX= Cephalexin, GM= Gentamicin, CRO= Ceftriaxone, CIP= Ciprofloxacin.

Table 14: Rate of isolation of single and multiple organisms in different types of infection

Types of Infections	Single organism		Two organisms		Three organisms		Total cases	
	No.	%	No.	%	No.	%	No.	%
Postoperative wound infections	64	86.5	9	12.2	1	1.3	74	100.0
Post catheterized UTI	15	93.7	1	6.2	0	0.0	16	100.0
Diabetic wound Infections	8	66.7	3	25.0	1	8.3	12	100.0
Total	**87**	**85.3**	**13**	**12.7**	**2**	**2.0**	**102**	**100.0**

Table 15: Duration of hospital stay of infected and non-infected cases

Category of patients	No.	Mean duration of stay (days)	S.D	t value	p value
Infected cases	102	23.5	13.3	8.9	0.001
Non-infected cases	50	9.5	6.1		
Total	**152**	**18.9**	**13.2**		

Table 16: Relationship between type of infection and duration of hospital stay

Category of patients	No.	Mean duration of stay	S.D	F value	p value
Postoperative wound infection	74	23.9	14.3	17.6	0.001
Post catheterized UTI	16	19.9	10.1		
Diabetic wound infection	12	26.1	9.9		
Postoperative non-infected cases (control)	50	9.5	6.1		

Table 17: Relationship between duration of hospital stay with single and multiple orgaisms

No. of organisms	No. of patients	Mean	S.D.	t value	p value
Single	87	21.8	10.8	3.3	0.001
Multiple	15	33.5	20.6		
Total	102	18.91	13.2		

Table 18: Relationship between type of infecting organisms and duration of hospital stay

Types of Organisms	No.	Mean duration of stay (days)	S.D	F.value	P.value
Esch.coli	49	21.2	9.1	19.63	0.001
***Pseudomonas* sp.**	21	18.7	8.7		
Mixed organisms*	15	33.5	20.6		
Other Organisms**	17	27.4	14.1		
Total	**102**	**23.5**	**13.3**		
Non-infected Group	50	9.5	6.1		
Total	**152**	**23.5**	**13.3**		

**Esch. Coli, Pseudomonas* sp, *Proteus* sp.

***Staph. aureus, Klebsiella* sp., *Acinetobacter* sp.

Table 20: Organisms isolated from different objects in hospital environment

Organisms	Frequency of isolates	Sources
Esch. coli	7	OT air, table, floor, tap water, Suction tube, Ward floor
Pseudomonas sp.	2	OT air, boiled water
Klebsiella sp.	2	OT floor,
Proteus sp.	1	OT gloves
Strept. Viridans	6	Nasal cavity
Staphylococcus (Coagulase negative)	15	OT air, Nasal cavity, palm, nail bed and inter digital spaces of the OT and ward personnel,
B. Subtalis	12	OT floor, ward floor and bed sheet

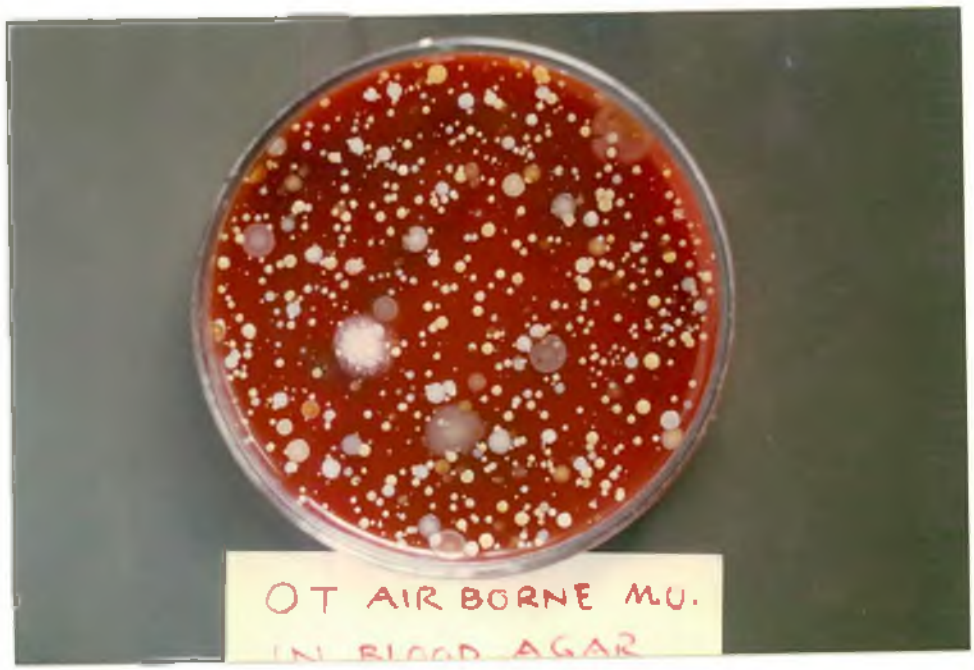


Fig 9:OT air borne microorganisms in blood agar media

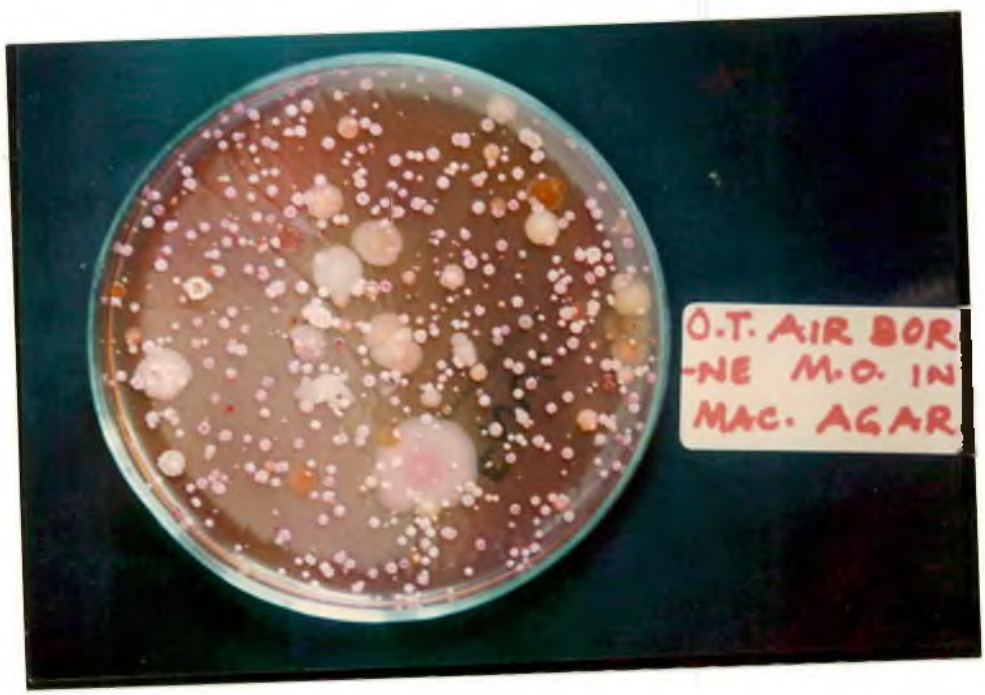


Fig 10:OT air borne microorganisms in MacConkey agar media

Fig 11: OT floor microorganisms in MacConkey agar media

Fig 12:OT table microorganisms in MacConkey agar media

Fig 13: OT gloves microorganisms in blood agar media

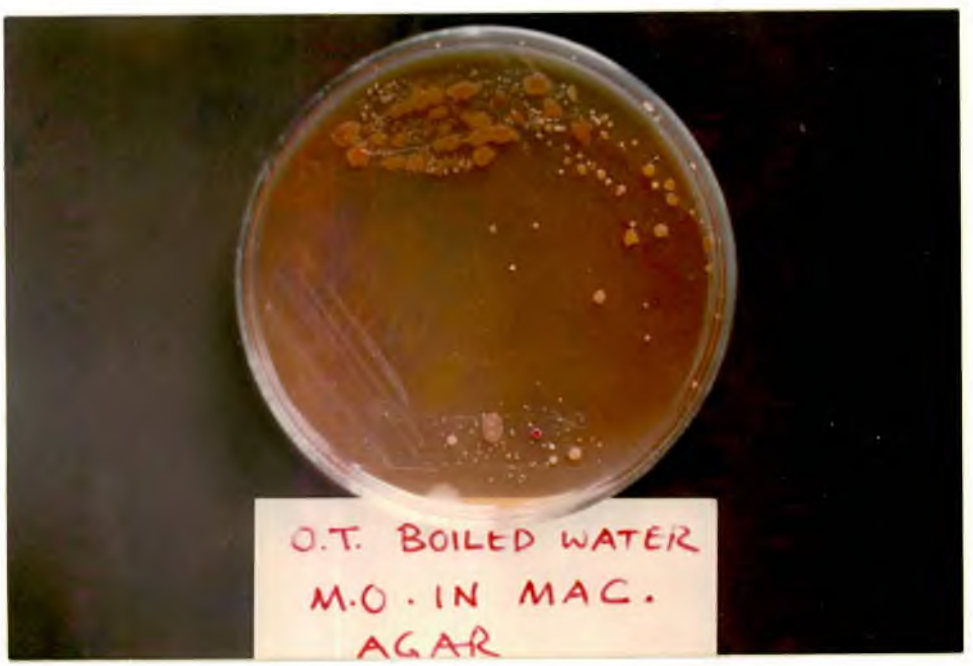


Fig 14: OT boiled water microorganisms in MacConkey agar media

Table 21(a): Antibiogram of *Esch. coli* isolated from different objects of hospital environment

Organisms	*AM*	CFX	CRO	CAZ	COT	TET	CIP	GM
Esch. Coli 1	R	R	S	M	M	R	S	S
Esch. Coli 2	R	M	S	M	R	R	S	S
Esch. Coli 3	R	R	S	S	R	R	S	S
Esch. Coli 4	M	S	S	S	M	R	S	S
Esch. Coli 5	R	R	M	S	R	R	M	M
Esch. Coli 6	R	R	R	M	M	S	R	S
Esch. Coli 7	R	R	S	M	R	R	S	S

Table 21(b):Antibiogram of *Pseudomonas* sp. isolated from different objects of hospital environment

Organisms	AM	CFX	CRO	CAZ	COT	TET	CIP	GM
Pseudomonas 1	*R*	R	R	R	R	R	R	R
Pseudomonas 2	R	R	S	S	R	R	R	R

AM-ampicillin, CFX-cephalexin, CRO-ceftriaxone, CAZ-ceftazidime,
COT-cotrimoxazole, TET-tetracycline, CIP-ciprofloxacin, GM-gentamicin
S-sensitive, M-moderately sensitive, R-resistant

Table 21(c):Antibiogram of *Klebsiella* sp. isolated from different objects of hospital environment

Organisms	AM	CFX	CRO	CAZ	COT	TET	CIP	GM
Klebsiella 1	*R*	R	M	M	M	S	S	S
Klebsiella 2	R	R	S	S	R	R	M	M

Table 21(d):Antibiogram of *Proteus* sp. isolated from different objects of hospital environment

Organisms	AM	CFX	CRO	CAZ	COT	TET	CIP	GM
Proteus	*R*	M	S	S	R	R	R	M

AM-ampicillin, CFX-cephalexin, CRO-ceftriaxone, CAZ-ceftazidime,
COT-cotrimoxazole, TET-tetracycline, CIP-ciprofloxacin, GM-gentamicin
S-sensitive, M-moderately sensitive, R-resistant

Table 21(e):Antibiogram of Coagulase negative *Staphylococcus* isolated from different objects of hospital environment

Organism (n=15)	AM			CFX			CRO			CAZ			COT			TET			CIP			GM		
	S	M	R	S		R	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R
Coagulase negative *Staphylococcus*	12	2	1	14	1	-	12	3	-	15	-	-	10	3	2	10	5	-	14	1	-	15	-	-

AM-ampicillin, CFX-cephalexin, CRO-ceftriaxone, CAZ-ceftazidime, COT-cotrimoxazole, TET-tetracycline, CIP-ciprofloxacin, GM-gentamicin
S-sensitive, M-moderately sensitive, R-resistant

Table 22: The molecular weight and the number of plasmid DNA present in the isolates

Organisms isolated from the patients					Organisms isolated from the sources				
SP No.	Name	Source	Plasmid		SP No.	Name	Source	Plasmid	
			No.	Mol. wt. (Mdasis)				No	Mol. wt. (Mdasis)
58	***Esch.coli***	Wound	—	—					
59	*Klebsiella* sp.	Wound	1	26.7	OT_2	***Klebsiella* sp.**	OT floor	3	18.7, 8, 6.7
63	*Esch.coli*	Wound	2	28, 16.7	OT_1	*Esch.coli*	OT table	4	28, 25.3, 16.7, 5.3
7R	*Pseudomonas* sp.	Wound	1	18.7	7A*	*Pseudomonas sp.*	Wound	2	20, 18.7
9R	*Pseudomonas* sp.	Wound	1	16.7	9A**	*Pseudomonas* sp.	Wound	1	16.7

* Sample 7A is a second sample corresponding to sample 7R (1st sample), and collected 7 days after the collection of 1st sample from a wound.

** Sample 9A is a second sample corresponding to sample 9R (1st sample), and collected 7 days after the collection of 1st sample from a wound.

Chapter V

Discussion

Nosocomial infection is an endemic problem affecting the hospitalized patients. It is a burning problem both in developed and developing countries. In developed countries many interventions were made to control nosocomial infection. But in developing countries like Bangladesh no emphasis has yet been given in this field. In different situations and perspective the pattern of nosocomial infections is different. In the present study, an attempt was made to explore the pattern of organisms responsible for hospital acquired infection in the large hospitals of Dhaka City. The antibiogram pattern of the offending organisms and the probable sources of infection was also investigated. In the present study, a total of 152 patients of 2 hospitals were studied. The patients were divided into 4 groups- a) Patients with post operative wound infection, b) Patients with post catheterized UTI, c) Patients with diabetic wound infection and d) Patients without postoperative wound infection. The group (d) was regarded as control group.

In the present study, it is appeared that though infection rate was higher in cases above 50 years of age (76.7%), the difference of infection rate in other age group was not statistically significant ($p>0.05$) which ranged between 58.3-69.7%. In an earlier study in Bangladesh, Hussain *et al.* (1991) reported that

higher infection rate occurred among the old age group. The absence of increased infection rate in older age group compared to younger age group in the present study could be due to small and non-random selection of samples and extensive use of antibiotics. In this study, the infection rate was relatively higher in female than male due to lack of uniformity of samples taken over the entire length of study period.

In the present study, 14 different types of operations were performed on 124 patients. Out of 124 operated cases 74 (59.7%) were post operative infected and 50 (40.2%) were post operative non-infected cases. Among the operative procedures laparotomy topped the list 42 (33.9%), followed by caesarean section operation 22 (17.7%), appendisectomy 18 (14.5%), inguinal herniorrhaphy 9 (7.2%) and cholecystectomy 7 (5.6%). In the same operative procedures post operative infected and postoperative non-infected cases were observed. It was observed that in laparotomy, postoperative infected cases were predominating (61.9%). But in appendisectomy, inguinal herniorrhaphy and cholecystectomy post operative non-infected cases were predominating and the rates were 88.9%, 88.9% and 85.7% respectively (Table-5).

In this study, it was observed that the most common infecting organism was *Esch.coli* (55.9%) followed by *Pseudomonas* sp.(33.3%) and *Proteus* sp.(12.7%). In an earlier study in Bangladesh, Ashraf *et al.* (1973) found that

in wound infection *Esch.coli* (37.5%) was the predominating organism followed by S*taph. aureus* (21.7%) and *Psendomonas* sp. (15.1%). In another study in Bangladesh, Zaman *et al.* (1992) showed that *Esch.coli* was the major pathogen in the postoperative wound infection (60.0%) followed by *Staph. aureus* (20.0%). In a study in Canada, Westwood (1974) found that majority of the organisms responsible for nosocomial infection was *Esch.coli* (25.3%), followed by *Klebsiella* sp.(16.8%) and S*taph. aur*eus (12.5%). However, Aman (1982) in Lahore found that the predominating causative organism of surgical wound infection was S*taph. aureus* (28.6%), followed by *Esch coli* (24.7%) and *Pseudomonas* sp.(23.7%). The isolation rate of organisms varied in different clinical samples. In the present study, *Esch.coli* was the predominating organism in postoperative wound infection and UTI, while in diabetic wound *Pseudomonas* sp. was the predominating organism. Similarly, many studies in Bangladesh and abroad showed different isolation rate in different clinical samples (*Ashraf et al.,* 1973, *Westwood* 1974, *Aman* 1982, *Zaman et al.,* 1992). But in the diabetic wounds, *Pseudomonas* sp. was the offending organism (91.7%), followed by *Esch.coli* (33.3%). In Bangladesh Jinnah *et al.* (1998) reported that *Pseudomonas* sp. was the major isolate from the patients with diabetic wound infection. However, their isolation rate of *Pseudomonas* sp. was much less (i.e. 36.9%) as compared to the present study. This difference rate might be due to small sample size or difference in sample selection procedure. The variation in the isolation of organisms by various

workers in the same or in the different localities over time might be explained by the fact that the use of different antibiotics has changed the organism in different localities eliminating the more susceptible ones by the antibiotic resistant organisms like *Pseudomonas aeruginosa* and *proteus* (*Baqui* ,1986).

The anitbiogram pattern of the organisms isolated from the 1st and 2nd samples of the infected cases were analyzed with commonly used antibiotics. It was observed that resistance of the isolated organisms to the prescribed antibiotics ranged from 32.4% to 100.0% in the first samples. In Spain, Bouza (1999) found that resistance rate of *Pseudomonas* sp. to ciprofloxacin was (23.0%), ceftazidime (15.0%), and gentamicin (30.0%). This higher rate of resistance of the organisms to the antibiotics in our study might be due to widespread use of antibiotics in our hospitals. The antibiogram pattern of the organisms isolated from 1st samples was also compared with that of 2nd samples (i.e. samples collected 5-7 days after the collection of the 1st samples). It was observed that (100.0%) *Esch.coli* was resistant to ampicillin in both 1st and 2nd samples. It was also observed that sensitivity of *Esch.coli* to the other antibiotics markedly reduced in 2nd sample where there was significant increase in resistance to ceftriaxone (from 43.9 to 73.9%; $p<0.01$), ceftazidime (from 28.1 to 65.2%; $p<0.002$), ciprofloxacin (from 70.2 to 100.0%; $p<0.005$) and gentamicin (from 61.4 to 91.3%; $p<0.008$). In case of *Pseudomonas* sp. and *Proteus* sp. a markedly increased level of resistance to antibiotics were also observed.

Resistance rate highly increased in 2nd samples in case of both the organisms. In case of *Pseudomonas* sp, resistance to ciprofloxacin increased significantly in 2nd sample (from 58.8 to 92.0%; $p<0.004$). Resistance rate of *Staph. aureus, Klebsiella* sp., *Acinetobacter* sp. also increased markedly in the 2nd samples. It was also observed that resistance rate of *Staph.aureus* was relatively lower than that of *Esch.coli, Pseudomonas* sp., *Proteus* sp., *Klebsiella* sp., and *Acinetobacter* sp.. This increased resistance might be due to the production of extended spectrum Beta Lactamases (ESBLs) by gram negative organisms (Pitout *et al.*, 1998). Jinnah *et al.* (1998), Coronado *et al.* (1995), Zaman *et al.* (1992), Sharafi *et al.* (1973) showed a markedly increased level of resistance of the organisms to the commonly used antibiotics.

Antibiotic resistance pattern of *Esch.coli* and *Pseudomonas* sp. isolated from the different sites were compared in the present study. It was observed that resistance pattern of *Esch.coli* and *Pseudomonas* sp. varied in different sites of infection. *Esch.coli* isolated from the diabetic wound infection showed the highest rate of resistance to cephalexin (100.0%) and ceftriaxone (75.0%) as compared to same isolated from post catheterized UTI, which showed the highest resistance to ceftazidime (63.6%), ciprofloxacin (81.8%) and gentamicin (81.8%). It was also observed that *Esch.coli* isolated from the postoperative wound infection showed lower rate of resistance to all the used antibiotics except gentamicin (57.1%). In case of *Pseudomonas* sp. it was

found that strains isolated from the post catheterized UTI showed the highest resistance to cephalexin (100.0%), ceftriaxone (66.7%) and ciprofloxacin (66.7%) as compared to same isolated from the diabetic wound infection which showed the higher rate of resistance to gentamicin (45.5%), *Pseudomonas* sp. isolated from the postoperative wound infection showed the lower rate of resistance to all the used antibiotics except ceftazidime (35.0%). This difference in resistance pattern of the organisms might be due to indiscriminate use of antibiotics (i.e. frequently use of certain antibiotics for treatment of infections at certain sites).

In this study, it was evident that antibiotics were prescribed to all the study patients. It was observed that rate of ciprofloxacin prescription was the highest (30.4%) in post operative infected cases, followed by ampicillin + gentamicin (12.7%), ampicillin + ciprofloxacin (10.8%). In case of postoperative non-infected cases rate of amoxicillin prescription was the highest (58.0%), followed by ceftriaxone (16.0%), and ciprofloxacin (8.0%). It was important to note that in the present study the resistance rate of commonly isolated organisms to the prescribed antibiotics ranged from 62.5% to 100.0%. In this study it was observed that single type of organism was the common cause of infection in (85.3%) of cases. The remaining (14.7%) was caused by multiple type of organisms. In an earlier study, Shaw *et al.* (1973) showed that out of 204 infected cases, (86.3%) was caused by single type of organisms and the

remaining 13.7% was caused by mixed organisms. Culberton (1961) found that out of 16 postoperative infection (43.8%) was monomicrobic and 56.2% was due to polymicrobic. This higher rate of monomicrobic infection might be due to difference in site of operation that is operations having entry into the organs of digestive system predisposed to polymicrobial infection.

Duration of hospital stay of infected and non-infected patients was compared in the present study. It was observed that hospital stay in case of infected cases was significantly increased than that of non-infected cases ($p<0.001$). A similar result was observed by Zaman *et al.* (1992). It was reported that mean postoperative stay in case of nosocomial infection was 25 days, about 3 times more than that of cases without nosocomial infection (8.6 days). Haley *et al.* (1981) found that hospital stay was prolonged by 3.1 to 4.5 days due to nosocomial infection. In the present study, it was found that mean duration of hospital stay in infected cases was 23.5 ±13.3 days and non-infected cases 9.5±6.1 days and the mean difference was statistically significant ($p<0.001$). A similar result was observed by Haley *et al.* (1980). It was reported that mean length of hospital stay in infected cases was 22.8±1.5 days and in non-infected cases it was 9.4±0.7 days. Westwood (1974) showed that patients with nosocomial infection spent on an average 22 excess days in hospital. Cruse (1970) reported that postoperative wound infection delayed patient's discharge by an average of 7.7 days. Relationship between type of infection and duration

of hospital stay was compared in this study. It was found that duration of hospital stay was the highest (26.1±9.9 days) in case of diabetic wound infection, followed by (23.9±14.3 days) in post operative infection and it was (19.9±10.1 days) in post catheterized UTI. But in postoperative non-infected cases it was (9.5±6.1 days). It was evident that duration of hospital stay was significantly higher in infected cases. It was observed that mean duration of hospital stay was significantly higher in case of infection caused by multiple type of organisms ($p<0.001$). This might be due to the fact that infection was severe in case of multiple type of infection. The duration of hospital stay was also compared with type of infecting organisms. It was found that the highest duration of hospital stay (33.5±20.6 days) was due to infection with multiple type of organisms followed by (27.4±14.1days) infection with other organisms (i,e *Staphylococcus aureus, Klebsiella* sp., *Acinetobacter* sp.). It was also observed that infection with *Esch.coli* caused more duration of stay than that of *Pseudomonas* sp. It was evident that hospital stay in case of infected cases increased significantly than that of non-infected cases ($p<0.001$).

Relation between length of operation time and causation of postoperative infection was compared in this study. It was found that length of operation time had no effect on causation of wound infection. Shaw *et al.* (1973) observed a similar result. But Garibaldi *et al.* (1991) found that duration of surgery more than two hours was associated with relative risk of 3 for surgical site infections.

Cruse and Foord (1980) found a direct relationship between duration surgery and the infection rate. It was reported that the infection rates for operations lasting 1, 2 and 3 hours were 1.3%, 2.7% and 3.6% respectively. The absence of influence of length of operation time on postoperative wound infection in this study might be due to the fact that all the operative patients received preoperative and postoperative antibiotics. Edwards (1976) and Valentine (1986) reported that microorganisms causing infection at the remote sites might gain access to the operative site by haematogenous or lymphogenous seeding.

In the study different organisms were isolated from different objects of the hospital environment. It was found that *Esch.coli* was the major isolates from the OT air, table, floor, Tap water, suction tube and Ward floor. *Pseudomonas* sp. was isolated from OT air and boiled water, *Klebsiella* sp. was isolated from OT floor, *Proteus* sp. was isolated from OT gloves. Besides these *Strept. Viridans*, *Staphyloccus* (coagulase negative) were isolated from the different objects. The isolated organisms from the possible sources showed a wide range of resistance to the used antibiotics in the study.

In the present study, an attempt was made to trace the sources of nosocomial infection. In this regards, Plasmid Profile Analysis (PPA) and RFLP assay was done with the representative organisms isolated from the different objects of the hospital environment and with those of wound samples of the study

population. Molecular weight of the individual plasmids of isolates were determined by comparing with the known molecular weight marker. It was observed that plasmid profile of the isolates of the probable sources was not of similar to those of wound samples.

In this study, RFLP was also done to detect the source of infection, but due to inadequate laboratory facilities we could not establish the procedure and therefore no fruitful conclusions could be drawn. Though sources of nosocomial infection could not be traced by PPA and RFLP with limited representative samples and inadequate laboratory facilities and constrains of time, it was evident that *Esch.coli*, *Pseudumonas* sp., *Proteus* sp., *Klebsiella* sp. were present and isolated from the different objects of hospital environment. These organisms might be the possible source of nosocomial infection.

Chapter VI

Conclusions and recommendations

Conclusions

Nosocomial infection is an alarming problem in developing countries like Bangladesh. By increasing our understanding of spectrum of nosocomial infections, the susceptible host, the reservoirs of infections and the modes of transmission, we should be able to control this growing problem. From the present study the following conclusions were made:

- The isolated organisms in the study according to rate of occurrence were *Esch. coli, Pseudomonas* sp., *Proteus* sp., *Staph. aureus. Klebsiella* sp.and *Acinetobacter* sp.

- The sensitivity pattern of the isolated organisms to the commonly used antibiotics ranged from 0 to 80%. The resistance pattern of the organisms isolated from the 2nd samples of the corresponding sites of infections at an interval of 5-7 days was markedly increased.

- Mean duration of hospital stay was significantly increased in patients with infection caused by mixed organisms ($p<0.001$).

- Duration of hospital stay of the postoperative infected cases increased significantly in comparison to the postoperative non-infected cases ($p<0.001$).

- Mean length of operation time was almost same in case of postoperative infected and non-infected cases. So, length of operation time did not influence in the occurrence of nosocomial infection ($p>0.05$).

- All of the study population received antibiotics but the resistance rate of the isolated organisms to the prescribed antibiotics ranged from 62.5% to 100.0%.

- Sources of nosocomial infections could not be detected by the methods done in the present study.

Recommendations

As nosocomial infection is an alarming problem in Bangladesh, the ultimate goal is to reduce the risk to the patients acquiring nosocomial infection. Taking this view, the following recommendations were drawn to control the nosocomial infections.

- As the study was smaller one, a large-scale study should be carried out to find out the overall magnitude of the problems.

- Sources of nosocomial infection should be detected by application of appropriate methods such as phage typing, Plasmid profile analysis, RFLP, RAPD for effective control measures to be taken for prevention of nosocomial infections in Bangladesh.

- A Surveillance System should be established to determine the pattern of nosocomial infection in hospital so that a long-term programme should be undertaken to control the problem

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APPENDIX-A

DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF DHAKA

Checklist

PART-I

Category of Patients:

a. Post operative wound infection b. Post catheterized UTI
c. Diabetic wound infection d. Postoperative non-infected wound

1. SL No.: .....Regn. No.: .....Institute: .....
2. Patient's Name: .....
3. Age: .....Years 4. Sex: Male/Female
5. Ward: ..... 6. Bed No.: .....
7. Father's Name/Husband's Name: .....
8. Address:.....
9. Diagnosis: .....
10. Date of Admission: .....
11. Date of Discharge: .....
12. Date of operation: .....
13. Duration of Preoperative stay: ..... (Days)
14. Duration of total hospital stay: ..... (Days)
15. Name of operation: .....
16. Duration of operation: .....(minutes)
17. Date of 1st sample collection: .....
18. Isolated organism from 1st sample: .....
19. Date of 2nd sample collection: .....

20. Isolated organism from 2nd sample: .....

Antibiogram pattern

1st Sample

Bacteria	AM	CFX	CRO	CAZ	TS	T	CIP	GM.
Bacteria	AM	CFX	CRO	CAZ	TS	T	CIP	GM.
Bacteria	AM	CFX	CRO	CAZ	TS	T	CIP	GM.

2nd Sample

Bacteria	AM	CFX	CRO	CAZ	TS	T	CIP	GM.
Bacteria	AM	CFX	CRO	CAZ	TS	T	CIP	GM.
Bacteria	AM	CFX	CRO	CAZ	TS	T	CIP	GM.

PART-II

Determination of source of infection

Date	Objects of hospital environment	Isolated organism
	1. OT Table Floor of OT and ward	1.
	2. Bed sheets of ward	2.
	3. Tools of OT	3.
	4. Dressing materials of ward	4.
	5. Nasal cavity of the OT and ward personnel	5.
	6. Nail folds, palm and interdigital spaces of OT & ward personnel	6.
	7. Air sampling from OT and ward	7.

Antibiogram pattern

Bacteria	AM	CFX	CRO	CAZ	TS	T	CIP	GM

APPENDIX-B

Media

Blood agar

Dehydrated Blood agar base (oxoid)	40.0 g
Distilled water	1.0 liter
pH	6.8

Autoclaved at 121°C for 15 minutes under 15 lbs pressure per sq. inch, when cooled down at 50°C, 7% sheep blood was added, mixed well, and poured into petridishes to a depth of 5 mm.

MacConkey Agar

Dehydrated MacConkey Agar (oxoid)	38.0 g
Distilled water	1000.0 ml
pH	7.4

Dispensed in flasks and autoclaved at 121°C for 15 minutes under 15 lbs per sq. inch pressure. When cooled, the medium was poured into sterile into sterile petridishes to a depth of 5 mm.

Muller-Hilton agar

Dehydrated Muller-Hilton agar base (oxoid)	38.0 g
Distilled water	1000.0 ml
pH	7.4

Dispensed in flask and autoclaved at 121° C for 15 lbs. pressure per sq. inch. When cooled, the medium was poured into the sterile petridishes to a depth of 5 mm.

Simmons Citrate Agar

Dehydrated citrate agar base (oxoid)	23 gm
Distilled water	100 ml
pH	7±0.2

Kligler Iron agar (KIA)

Dehydrated Kligler Iron agar base (oxoid)	55.0 g
Distilled water	1000.0 ml
pH	7.4±0.2

Luria Broth (100 ml)

Yeast Extract (GIBCOBRL)	0.5 gm
Peptone	1 gm
Sodium Chloride	1 gm
Distilled water	100 ml
pH	7.1±0.2

APPENDIX-C

CHEMICALS AND REAGENTS

Oxidase reagent: 1% solution of tetramethyl-p-phenylenediamine dihydrochloride soaked in a piece of Whatman filter paper no.1

Bromocresol purple: 0.2% Bromocresol purple solution was prepared in ethanol (1:1).

Ethyl alcohol: 95% Ethyl alcohol was used.

Kovac's reagent :

Amyl or Isoamyl alcohol	150 ml
P-dimethyl –aminobenzaldehyde	10 g
Conc. hydrochloric acid	50 ml

The aldehyde dissolved in alcohol and the acid is then added slowly. Prepared in small quantities and stored in refrigerator. Shaken gently before use.

Peptone water (Collee et al., 1996)

Peptone	10.0 g
Sodium	5.0 g
Distilled water	1000.0ml

Dissolved pH adjusted to 7.4-7.5 distributed in 3ml amounts in test tubes and autoclaved at 121°C for 15 minutes.

Reagents used for plasmid extraction procedures

Solution-I

Glucose	50 mM
Tris-Hcl (pH 8.0)	25 mM
EDTA (pH 8.0)	10 mM

Solution-II

(Freshly prepared and made weekly from 10N NaOH stock solution)

NaoH	0.2N
SDS	1%

Solution-III

K-acetate	3 M
Glacial acetic acid	11.5% (V/V)
Distilled water	100 ml
pH	8.0

TE Buffer (Tris EDTA Buffer)

Tris-Hcl (pH 8.0)	10 mM
EDTA (pH 8.0)	1 mM

TBE Electrophoresis Buffer (Tris-Borate-EDTA Buffer)

Tris-base	54 gm/L
Boric acid	27.5 gm/L
0.5M EDTA (pH 8.0)	20 ml/L

APPENDIX-D

MacFarland Standard Tube No. 1

Composition and preparation:

1% (v/v) solution of chemically pure (0.36N) sulphuric acid and 1.175% (w/v) solution of chemically pure (0.048m) barium chloride was prepared in nutrient broth in two separate sterile flasks. Then 9.9 ml of sulphuric acid and 0.1 ml of barium chloride were added to the clean screw capped test tube and sealed. The barium sulphate suspension corresponds approximately to Macfarland Standard tube No. 1 with corresponding cell density of $3x10^8$ organisms/ml of organisms.

To make the turbidity standard of cell density to one half of the Mac Farland Standard tube No.1 which corresponds to cell density of $1.5x10^8$ organisms/ml for determination of antibiotic sensitivity by Kirby-Bauer inoculated technique, 0.5 ml of 1.175% (w/v) barium chloride ($BaCl_2$, $2H_2O$) was added to 99.5 ml of 1% (v/v) sulphuric acid (0.36 N), mixed well and 5-10 ml was distributed in sterile screw capped test tubes and sealed.

APPENDIX-E

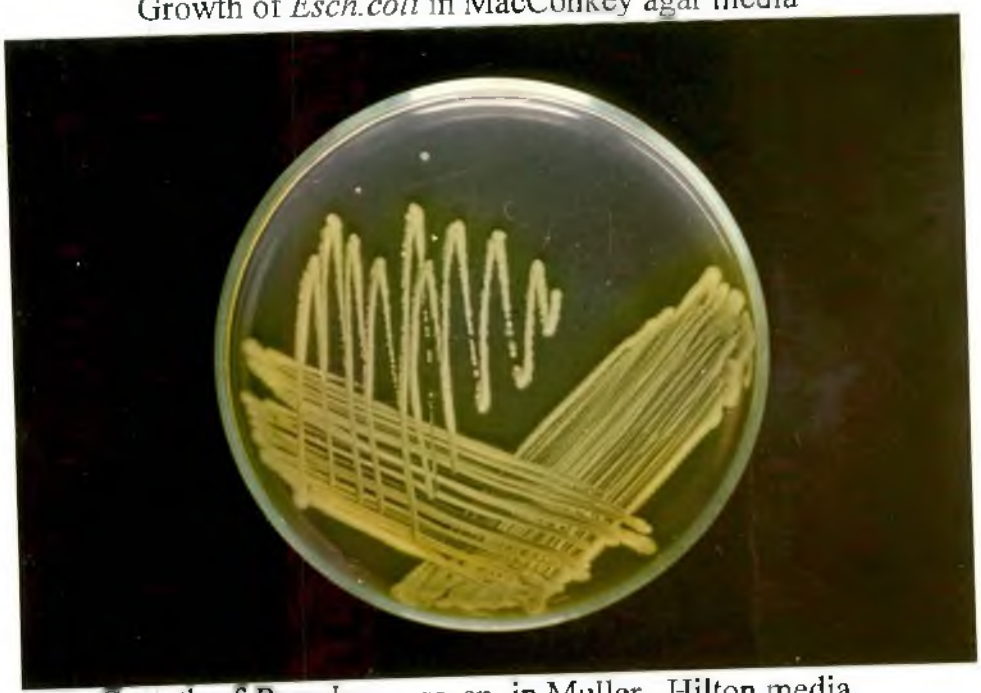
Organisms isolated from different objects of hospital environment

Date	Air sampling	O.T. Floor	O.T. table	OT Boiled water	Nasal cavity of OT Perso-nnel	Tap water of OT and ward	OT Suction tube	Ward Floor	Bed sheet	Palm and nail bed of OT pers-onnel	OT Gloves	Dressing metarials	OT instruments
6.7.98	*Coagulase negative Staph*	*B. Subt-allis*	-	-	*Streptovarid-ans*	-	-	*B. Subtall-is*	-	-	-	-	-
20.7.98	*Coagulase negative Staph*	*Esch. coli*	-	-	*Streptovarid-ans*	-	-	-	-	-	-	-	-
3.8.98	*Coagulase negative Staph*	-	*Kleb-siella*	-	-	-	-	*B. Subtalis*	-	-	-	-	-
17.8.98	*Pseudomo-nas*	-	-	-	-	-	-	*B. Subtalis*	-	-	-	-	-
7.9.98	*Coagulase negative Staph.*	-	-	-	*Coagu-lase negati-ve Staph.*	-	*Esch. Coli*	B. Subtalis	*B. Subtal-is.*	-	-	-	-
21.9.98	*Coagulase negative Staph.*	-	-	-	*Coagu-lase negati-ve Staph.*	*Esch. coli*	-	*B. Subtalis*	-	-	-	--	-
5.10.98	*Coagulase negative Staph.*	-	-	-	*Coagulase negati-ve Staph.*	-	-	*Esch. Coli*	-	-	*Proteus*	-	-
19.10.98	*Coagulase negative Staph.*	*Esch. coli.*	-	*Pseu-domo-nas*	*Strepto virida-ns*	-	-	-		-	-	-	-
2.11.98	*Coagulase negative Staph.*	*B. subt-alis*	-	-	*Strepto virida-ns*	-	-	-	-	*Coa-gula-se nega-tive staph*	-	-	-
16.11.98	*Coagulase negative Staph.*	-	*Esch. coli.*	-	-	-	-	*B. subt-alis*	-	*Coa-gula-se nega-tive staph*	-	-	-
7.12.98	*Esch. Coli*	-	-	-	*Strepto virida-ns*	-	-	-	-	-	-	-	-
21.12.98	*Coagulase negative Staph.*	*Kleb-siella*	-	-	*Strepto virida-ns*	-	-	*B. subt-alis*	*B. Subtal-is.*	-	-	--	-

APPENDIX –F

Colony morphology of isolated organisms

Growth of *Esch.coli* in MacConkey agar media



Growth of *Pseudomonas* sp. in Muller –Hilton media