Review of Literature
REVIEW OF LITERATURE

HISTORICAL REVIEW

The history of hospital acquired infection goes back to the period when the sick were housed together for treatment. The enormity of the problem of hospital acquired infection (HAI) during the pre-Listeriae era can be best stated by quoting John Bell who in 1801 wrote "there is no hospital, howsoever small, airy or well regulated, where this epidemic ulcer is not to be found at times; and then no operation be performed; every case stands still; every wound becomes sore, every sore is apt to run into gangrene; but in great hospitals, specially, it prevails at all times and is a real gangrene, it has been named the "Hospital gangrene" and such were the ravages at Hotel Dieu Paris (that great store house of corruption of diseases) that the surgeons did not dare to call it by its true name (Mukherjee, 1992). Prior to Lister's monumental impact on surgical practice, postoperative wound infection was the rule rather than the exception. Compound fractures of the femur requiring amputation, had an attended mortality of greater than 90% and mortality from gun shot wound of the abdomen approached 100%, both predominantly because of infection.

In 1550's pioneers like Theodoric of Bologna (Major, 1954) and Stromayr (1559) tried reforming hospital practice by bathing patients or shaving the site of operation with little success. Towards the end of the 18th century Madam Necker (Tenon, 1788) proposed "Nursing the Sick in a Single Bed". Earlier to this time up to 8 patients were nursed in a bed.

Semmelweis (1861) undertook the first hospital-based epidemiologic study. Semmelweis linked the increased rates of hospital infections to a lack of handwashing. After institution of handwashing as a control measure, the ward-specific excess rate of puerperal sepsis and mortality declined (Semmelweis, 1861).
Antiseptic surgery (1867) was introduced with extensive use of carbolic acid for packing wounds (especially compound fractures), sterilizing instruments and sutures, decontaminating the hands and finally as an air spray. The antiseptic era was followed by the practice of asepsis. Louis Pasteur in his celebrated lecture in Academie de Medicine on 30th April 1873 said “If I had the honour of being a surgeon, not only would I use absolutely clean instruments but after cleaning my hands with great care, I would only use sponges previously raised to a heat of 130-150°F. I would still have to fear germs suspended in the air surrounding the patient.” Antiseptic surgery was replaced by Burgman’s asepsis (Schimmelbusch, 1894) and by the end of the century surgical gloves were introduced in USA. In these years, during which many fundamental discoveries in bacteriology were being made, other principles of hospital infection control were established.

Flugge (1897, 1899) showed the importance of droplet and aerial spread of tuberculosis. Hutinel (1894) and others established basic isolation systems for diphtheria and other infectious diseases in children and fever hospitals. In early 20th century the discovery of pathogenic bacteria provided a new basis for the study of hospital infections and the importance of Streptococcus pyogenes was demonstrated during this period. The concept of cubicle and barrier nursing was introduced during this period in the United Kingdom. Dukes (1929) recognized the importance of indwelling catheters as a means of introducing infection into the bladder.

The arrival of penicillin in the later years of World War II led to a decrease in the prevalence of chronic sepsis, mainly caused by Staphylococcus aureus (Fletcher, 1984). In these years, standard methods of infection control, supply of clean air for operating theatres, procedures for wound dressings provision of isolation units (Williams et al, 1960), appointment of medical control of infection officers, infection control nurses and control of infection committees (Colebrook, 1955; Gardner et al, 1962) were established.
Kislak et al (1964) and Barrett et al (1968) conducted some of the first modern prevalence studies and reported that 15% of inpatients had nosocomial infections. By 1968, the Center for Disease Control was training ICPs in Surveillance, Prevention and Control of Nosocomial Infection. In 1969, the Joint Commission for Accreditation of Health Care Organisation mandated that all hospitals support an infection control nurse.

The NNIS system, created in 1970, composed of non-randomly selected hospitals in US conducted one of the first studies, the Study on Efficacy of Nosocomial Infection Control (SENIC) which was to determine whether infection surveillance and control programs reduce the rate of nosocomial infections (Haley et al, 1981).

For a period the importance of multidrug-resistant Staph. aureus appeared to fade and interest shifted to gram negative bacilli; antibiotic resistant enterobacteria, such as Klebsiella and later Serratia spp, which cause large outbreaks of colonization. The advent of HIV infection in early 1980's gave a new dimension to the problem of nosocomial infections ranging from newer, rarer and life-threatening opportunistic infections, to the risk of acquiring HIV infection by means like needle stick injuries. More recently, again as a result of the introduction of newer antibiotics, as well as of the extensive use of indwelling medical devices, gram positive cocci have again become the predominant cause of infection in many hospitals. In the past few years the gravity of the problem of nosocomial infections was felt world over and necessary steps are being taken to tackle the problem.

**NOSOCOMIAL INFECTIONS / HOSPITAL ACQUIRED INFECTIONS:**

Hospital acquired infections are an important cause of morbidity and mortality among hospitalized patients. Estimates in 1989 world over suggest that a total of more than 1.4 million people are suffering from HAI at any one time with a prevalence of about 9% (Chandrasekhar, 1992).
In the USA, it has been estimated that more than 2 million to 4 million patients are infected in hospitals each year, which results in about 2,500 deaths annually (Emori et al., 1993). The Center for Disease Control and Prevention (CDC) estimates that nosocomial infection contributes to 0.7% to 10% of deaths and are responsible for 0.1% to 4.4% of all deaths occurring in hospitals (Center for Disease Control, 1992).

In Great Britain and Ireland, a cross-sectional prevalence survey (Emmerson et al., 1996) suggested an overall nosocomial infection prevalence rate of 9.0% with significantly higher prevalence in teaching (11.2%) than in non-teaching (8.4%) hospitals. Nation wide prevalence survey in Spain (EPINE, 1992, 1995) has shown an overall nosocomial infection rate of 8.5% in 1990, falling to 7.2% in 1994. A similar investigation in Norway found a prevalence of 6.1% (Scheel et al, 1999). A prevalence survey in Australia (Murphy, 2000) found 5.5% of patients to have a hospital-acquired infection. A similar investigation in Hong Kong (Kam and Mak, 1993) showed a prevalence rate of 8.6%. A prevalence survey in Nigeria (Oni et al, 1997) found 4.9% of patients to have a hospital acquired infection. Similar studies in Brazil and France found a prevalence of 21.20% (Wagner et al, 1997) and 5 to 10% (Astagneau et al, 1998) respectively. Another New Zealand study found a prevalence of 4.1% (Helliaratchy et al, 1983).

Statistics on prevalence and incidence of HAI in India are limited. Nationwide surveillance data is not available. Ganguly et al (1995) from Aligarh, North India reported a HAI prevalence rate of 38.8% in a teaching hospital. Nosocomial infection rate was higher in males (41.6%) than in females (34.7%). Another study from New Delhi, India, found an overall nosocomial infection rate of 16.8/1000 patient days (Pawa et al, 1997). It was estimated that a nosocomial infection added an average of 4-6 extra hospital days and $2,100 to the patient costs (Emori, 1993). In total these infections added approximately $4.5 billion to the cost of the health care system (Perl et al, 1998).
Infections most commonly acquired in hospitals are surgical wound infections, infections of the urinary and respiratory tract and bacteraemia. Most common HAI were those of the urinary tract (42%) accounting for 2/5th of all nosocomial infections. Surgical wound infections (24%) account for 1/4th of all HAI, respiratory tract infection (11%) account for 1/8th, bacteraemia (5%), all others (18%) about 1/6th. These data are expected to vary from hospital to hospital (Haley et al, 1985). In another study, data submitted between 1986 and 1990 identified 54% of nosocomial infections to be among elderly patients. In this age group the most common infection was UTI (44%), followed by pneumonias (18%), surgical wound infection (11%) and blood stream infection (8.5%) (Emori et al, 1991).

According to the NNIS data 1996, UTI's account for 34.5% of nosocomial infections, surgical site infections (SSIs) for 17.4%, blood stream infections (BSIs) for 14.2%, lower respiratory tract infections (LRIs) for 13.2% and others for 20.8% (National Nosocomial Infections Surveillance System, 1996).

The overall incidence of nosocomial infection in ICUs may be up to 42% (Madhavan, 1992). The most frequently observed infections in ICUs are nosocomial pneumonia, urinary tract infections, postoperative wound infection and bacteraemia. Increasing age, severity of acute illness on admission (APACHE SCORE), the admission diagnosis, use of invasive devices, duration of stay in the ICU, recent major surgery, renal failure, high dose steroid and antineoplastic therapy are strongly implicated as risk factors for HAI (Madhavan, 1992). Nosocomial infection within ICU cause a three fold increase in mortality and considerable additional morbidity (Wenzel et al, 1983). Nosocomial blood stream infections carry a high fatality rate and together with ventilator-associated pneumonia, are the leading cause of deaths associated with nosocomial infections in ICUs (Valles et al, 1997). Among the pathogens, gram positive cocci, especially coagulase negative staphylococci. S.aureus and enterococci account for over 40% of blood stream infections (Valles et al, 1997). Candida is emerging as an important pathogen, accounting for 8% of infection. Lower respiratory tract infections are caused by P.aeruginosa, S.aureus, enterococci, and surgical wound
infections are due to Enterobacter spp, coagulase negative Staphylococci and Enterococci. Candida spp, E.coli, Enterococci P.aeruginosa, Enterobacter spp, are implicated in nosocomial ICU associated UTIs (Madhavan, 1992).

According to another recent study which was conducted among 2,267 children hospitalized in a nationwide network of 122 public hospitals caring for children (Cashat-Cruz et al, 1998). The most prevalent nosocomial infections among children were pneumonia (20%), sepsis/bacteremia (17.5%) and urinary tract infections (5.2%). Roghmann et al (1998) reported nosocomial infections among chronically ill patients in a House Health Care Setting 58% developed an infection of which 35% were urinary tract infection (UTI); 13% respiratory tract infection (RI), 23% soft tissue infections (STI) and 18% other sites. In a similar study by Mylottle et al (2000), most common infections were those of the urinary tract (30% of 94 infections) or a surgical site (17%), C.difficile diarrhoea (15%) and blood stream (12.8%).

URINARY TRACT INFECTIONS:

On the basis of hospital surveillance data, 2,50,000 cases of pyelonephritis per year occur in developed countries. Data is practically nil from developing countries, where it is not known whether UTIs are more frequent or severe than in developed countries. Catheter-associated UTIs account for 40% of all hospital acquired infections and are thus the most common type of nosocomial infection. Overall, about 10% patients with short-term catheterization develop infections in developed countries, which results in an estimated 1-1.5 million catheter associated UTIs in a country like the United States per year. Besides accounting for extra hospital costs (~$400 per episode) these infections are important reservoir for selection and transmission of multidrug resistant strains and are a frequent source of gram negative bacteremia in hospitalized patients.

It is estimated that 150 million UTIs occur yearly on a global basis, resulting in more than 6 billion dollars in direct health care expenditures. Urinary tract
infections account for up to 40% of nosocomial infections (Crook and Bowler, 1996). Most cases are initially asymptomatic and can be detected by quantitative cultures. Studies have attributed up to three-fold excess mortality from these infections resulting from occult bacteraemia leading to sepsis (Platt et al., 1992).

Emori et al (1993) and Stamm (1991) reported that intrinsic patient factors which increase the risk of UTIs include advanced age, female gender and underlying disease.

Aerobic gram negative bacteria account for the vast majority of catheter associated urinary tract infections. In recent data collected by the NNIS system (1996) Enterobacteriaceae and Pseudomonas accounted for more than 80% of all culture positive infections. Of the gram positive organisms, group D Streptococci accounted for approximately 14% and Staphylococci caused about 5%.

*E. coli* remains an important pathogen and it is identified to be the cause of 50-60% of infections. Other Enterobacteriaceae including Klebsiella, Enterobacter and Serratia spp *Proteus mirabilis, Providentia stuartii, P. aeruginosa, Candida albicans* and gram positive organisms, particularly enterococci and coagulase negative Staphylococci are also frequently isolated (Nicolle, 2001). *P. mirabilis, P. stuartii, P. aeruginosa* and Enterococcus are the most frequently isolated pathogens among patients.

Nosocomial pneumonia:

Nosocomial pneumonia is defined as a lower respiratory tract infection that develops in hospitalized patients in whom infection was neither present nor incubating at the time of admission and occurs beyond 48 hours of hospitalization (Louse et al, 1991). The incidence of nosocomial pneumonia is only 0.5 to 5% in patients treated outside critical care setting, the incidence of nosocomial pneumonia in mechanically ventilated patients varied from 11% to 21%. Despite the advances in antibiotic therapy, pneumonia continues to be a major cause of morbidity and
It is currently the 5th most common cause of death and one of the most frequently encountered infectious diseases in developed countries.

Pneumonia develops in 0.6 to 2% of hospitalized patients (Mandell and Campbell, 1997). A mortality rate of 47, 76 and 30% has been reported among hospitalized patients with community acquired pneumonia in various studies (Crouch et al, 1996; Mandell and Campbell, 1997). Pneumonia is the 6th leading cause of death in developed countries. In United States this infection accounts for 6,00,000 hospital admission and 64 million days of restricted activity annually. Pneumonia is the most frequent nosocomial infection, in European ICUs (Vencenti et al, 1995) and the 2nd most common nosocomial infection in the ICUs of the US. According to a recent study conducted in India (Trivedi et al, 2000) the incidence of nosocomial pneumonia was 9.38%, with a mortality rate of 21.3%.

According to a study conducted (Bishara, 2000) over a 7 year period, pneumonia was the source of bacteraemia in 319 of 4,548 (7%) episodes, occurring in 295 patients, 211 (66%) episodes were community acquired and 108 (34%) were nosocomial.

The most frequently isolated pathogens in nosocomial pneumonia in different studies were *P. aeruginosa* (26.8%), *S. aureus* (24%), Klebsiella spp (12.1%) and Acinetobacter spp (10.5%). According to another study, Pseudomonas spp (17%), Klebsiella spp (11%) and *Staph. aureus* (10%), oxacillin sensitive *S. aureus*, *H. influenzae* and *S. pneumoniae* were the predominant pathogens (Pingleton et al, 1992).

**Nosocomial blood stream infections:**

Nosocomial bacteraemia is common, costly and morbid. Approximately, 2,50,000 patients develop nosocomial blood stream infections annually in the United States (Pittet, 1995). The costs associated with these infections are enormous in critically ill patients, reaching $34,500 to $40,000 per survivor. The
attributable mortality averages 27% (Pittet et al, 1995) and rises to 35% in patients who develop blood stream infections in the intensive care unit (Pittet et al, 1994). The incidence of nosocomial blood stream infections has increased over the last two decades, primarily due to a rise in the incidence of infections caused by coagulase negative Staphylococci, Enterococci and Candida species (Weinstein et al, 1997). Another recent study reported 31.3% crude mortality rate among patients with nosocomial bacteremias (Lark et al, 2000).

Clinical manifestations of blood stream infections may range from benign transient bacteraemia to fulminant disease with septic shock, associated with an overall mortality as high as 46% (Seifert et al, 1995).

The leading pathogens causing nosocomial bacteremia in different studies reported were coagulase negative Staphylococci, S. aureus and Enterococci (Lark, 2000), E. coli and Klebsiella pneumoniae. Candida spp were implicated in 7% of all nosocomial bacteremic episodes representing the 5th most common etiology (Lark, 2000). Coagulase negative Staphylococci (32%), S. aureus (16%) and Enterococci (11%) were the leading pathogens in US hospitals (Edmond et al, 1999; NNIS data, 1999). Intravascular catheters were the most frequently identified source of nosocomial bacteremia (Lark et al, 2000). According to a study by Lark et al, 40% of all nosocomial bacteremias were intravascular catheter related infections. The institution of newer technology such as antimicrobial coated intravascular catheters play an important role in controlling nosocomial bacteremia (Veenstra et al, 1999). A significant proportion of hospital acquired bacteremias could be prevented through use of standardized infection control practices and adherence to published guidelines (Mermel, 2000).

OTHER NOSOCOMIAL INFECTIONS:

A prospective observational cohort study of nosocomial sinusitis was reported from 2 medical intensive care units (George et al, 1998). The cumulative incidence of nosocomial sinusitis reported was 7.7%. In a study, incidence of
endophthalmitis after eye surgery was <0.5%, but it varied in reported series from 0.08% to 1% (Hassen et al, 1992). Most of the episodes of endophthalmitis are due to *P.aeruginosa*, *Enterobacteria*, *S.aureus*, & *S.epidermidis* (Shrader et al, 1990; Cruciani et al, 1998; Hassen et al, 1994).

Nosocomial diarrhoea has been reported in 7.7% to 41% of patients admitted in ICUs (Kelly et al, 1983; Lima et al, 1986) and 1.2% to 2.1% of all patients admitted in paediatric teaching hospitals (Brady et al, 1989). In paediatric services and in high risk nurseries, rota viruses were responsible for 75% and 51% respectively of all instances of nosocomial diarrhoea. *C.difficile* accounted for 95% of nosocomial diarrhoea in general surgery and medical services (Dhawan et al, 1998). The overall incidence of central nervous system infections in hospitals is 0.56%/10,000 discharges. Rates are higher in paediatric service (3.3 per 10,000 discharges, HRNS (2.1 per 10,000 discharges) and neurosurgical services (1.7 per 10,000 discharges). The most common pathogens causing central nervous system infections include coagulase negative *Staphylococci* (31%), gram negative bacilli (27%) and *Streptococci* (18%) and *S.aureus* (11%).

**Postoperative wound infections: Prevalence and Incidence:**

**Global scenario:**

In the developed countries, postoperative wound infections are the third most frequent nosocomial infections responsible for 29% of all nosocomial infections and 14% of all nosocomial adverse events (Leape et al, 1991; Smyth et al, 2000). Postoperative wound infections cause significant morbidity and account for 55% of all extra hospital days attributed to nosocomial infections (Haley, 1985). Recent studies have demonstrated that the average postoperative wound infection prolongs the hospital stay by 7.4 days (Martone et al, 1992). These infections are estimated to cost $3,152 per infection and contribute to 42% of total extra charges attributed to nosocomial infections (Anonymous, 1992). Postoperative wound infections contribute to 2% of deaths in the developed countries (Mayhall et al, 1992).
In a nationwide prevalence survey postoperative wound infection accounted for 12.3% of all hospital acquired infections (Emmerson et al, 1996). In United Kingdom postoperative wound infection accounted for 14-16% of all nosocomial infections (Smyth et al, 2000). In the United States of America, surgical wound infection accounted for 24% of nosocomial infection. According to a study conducted by Center for Disease Control (CDC) Atlanta between 1986-1996, of the 5,93,344 operations performed, only 3% of the cases developed postoperative wound infections. In the same study, a mortality rate of 3% was reported. Mitka (2000) reported a PWI incidence rate of 6.82%. A six month prospective surveillance conducted at Rio de Janero University hospital, Brazil reported a significant PWI rate of 16.9%. The same study showed that 52.7% of the PWI were apparent only after the patient was discharged from the hospital (Santos et al, 1997).

Two different studies conducted in Australia reported a PWI rate of 5.9% and 10.1% respectively (Kune et al, 1983; Mitchell et al, 1999). A German prevalence study of nosocomial wound infections carried out in 72 representatively selected hospitals in 1998 showed a prevalence rate of 1.61% (Wischnewski et al, 1998).

In the developing countries postoperative wound infection rates are relatively higher than in developed countries. Vilar-Compte et al (1999) from Mexico reported postoperative wound infection rate of 9.28% in Mexican hospitals. The same study reported PWI rates for clean contaminated, contaminated and dirty surgeries were 10.5, 17.3 and 21.5% respectively. Ojiegbbe et al (1990) from Nigeria reported an incidence rate in clean wounds of 14%, in clean contaminated 50%, in contaminated 66.66% and in dirty wounds 80%. Kotisso et al (1998) from Ethiopia reported a wound infection rate of 21% and infection rate significantly higher in contaminated or dirty wounds with the highest rate being 61.4%. Prevalence rate of postoperative wound infection was 31.37% in Bangladesh (Saha et al, 1995).
Indian Scenario:

Although accurate data is not available regarding the prevalence and incidence of hospital-acquired infections in Indian hospitals, ample evidence exists to indicate the magnitude of the problem. Overall, reported incidence of postoperative wound infection alone in different hospitals in India has varied from 10 to 33% (Rattan et al, 1992).

In 1980, a study from Lucknow reported a significantly high postoperative wound infection rate of 44% (Agarwal et al, 1980). From Southern India, Nandy and Ramachandran in 1983 have reported an incidence of postoperative wound infection rate of 13.3%. In 1990, Thakur et al reported postoperative wound infection rate of 2.34% from a referral hospital, New Delhi. Another study from Bombay reported clean wound infection rate of 5.4% and contaminated wound infection rate of 43% (Manelkar, 1992). Korula (1992) reported postoperative wound infection rate of 3.4% from a referral hospital, Vellore, Southern India.

A study from New Delhi carried out an active surveillance program in a 350-bed referral hospital. The overall incidence rate before adopting the surveillance programme was 4% and it was reduced to less than 1.0% during this study period (Mandal and Mishra, 1997).

A postoperative infection rate of 22.7% and a mortality rate of 4.7%, were reported from a New Delhi referral hospital (Sood et al, 1998). Cyriac et al (1998) have reported wound infection rate of 3% from a super speciality hospital in Bombay. Nagarajan et al (1998) reported a postoperative wound infection rate of 2 to 3.5% from a tertiary care hospital in Delhi. The overall postoperative sepsis rate of 13% (clinical) and 12% (bacteriological) was reported from a referral hospital in Manipal, S.Karnataka (Murthy et al, 1998).
A postoperative wound infection rate of 6.09% was reported from Aurangabad. In the same study, the infection rate in wounds following clean surgeries was 4.04% while in those following clean contaminated surgeries was 10.06% (Anvikar et al, 1999).

SURVEILLANCE OF THE SURGICAL WOUND:

Direct surveillance of healing operative wounds was suggested by Gardner and colleagues (1962) as one means to obtain accurate data concerning the incidence of wound infection and other septic postoperative complications. In institutions where surveillance programmes have been instituted, improved reporting of infections has been noted (Mulholland et al, 1974) and a decline in the incidence of wound infection follows (Cruse, 1980, 1981).

Surveillance methods:

A number of methods for the surveillance of nosocomial infections have been developed and their sensitivity and specificity have been assessed. These include chart review (Olsen et al, 1990), Kardex (nursing care plan), review (Wenzel et al, 1976), laboratory-based ward surveillance (Glenister et al, 1993), laboratory-based telephone surveillance (Glenister et al, 1993), ward liaison surveillance (Scheckler et al, 1988; Glenister et al, 1993), treat chart surveillance (Wenzel et al, 1976; Glenister et al, 1993), temperature chart surveillance (Wenzel et al, 1976; Glenister et al, 1993), treatment and temperature chart surveillance (Wenzel et al, 1976; Glenister et al, 1993), risk factor surveillance (Wenzel et al, 1976; Glenister et al, 1993), antimicrobial use (Wenzel et al, 1976) and microbiology reports (Wenzel et al, 1976). Potential advantages and disadvantages of surveillance methods for detection of SSI's (Manian et al, 1997) are listed in the Table 1.

Surgical site infection (SSI) surveillance:

Surgical site infections have been identified by employing direct observation or by traditional infection control (indirect) methods. Surveillance has involved direct observation of the surgical site by the surgeon, trained nurse or infection
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<th>Method</th>
<th>Potential advantages</th>
<th>Potential disadvantages</th>
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<tr>
<td>Routine direct wound examination by trained professionals</td>
<td>High sensitivity and specificity</td>
<td>Labour intensive</td>
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<tr>
<td>Outpatient chart review by trained professionals</td>
<td>Acceptable sensitivity and specificity</td>
<td>Labour intensive, suboptimal documentation</td>
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<td>Surgeon reporting:</td>
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<tr>
<td>Self initiated</td>
<td>High specificity, resource efficient</td>
<td>Poor sensitivity</td>
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<td>By survey (mail)</td>
<td>Acceptable specificity, relatively resource efficient</td>
<td>Suboptimal sensitivity</td>
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<td>Patient reporting:</td>
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<tr>
<td>By mail</td>
<td>Relatively resource efficient</td>
<td>Unreliable sensitivity and specificity</td>
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<tr>
<td>By telephone</td>
<td>Good public relations</td>
<td>Labour intensive, unreliable sensitivity and specificity</td>
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<tr>
<td>Microbiology data</td>
<td>Relatively resource efficient may ‘flag’ potential SSIs</td>
<td>Unreliable sensitivity and specificity when used in isolation</td>
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control personnel (Olsen et al, 1990) Indirect detection of SSI's has been achieved by infection control personnel through a review of laboratory reports, patient records and discussions with primary care providers (Olsen et al, 1990, Condon et al, 1983) The surgical literature suggests that direct observation of surgical sites is the most accurate method of detecting SSIs, nevertheless sensitivity date are lacking (Mangram et al, 1999) Much of the SSI data reported in the infection control literature has been generated by indirect case finding methods (Mangram et al, 1999), but some studies of direct methods have been conducted (Mangram et al, 1999) Some studies have used both methods of detection (Mangram et al, 1999)

Traditional surveillance of the surgical wound, which was practiced widely into the 1970s, depended primarily on the infection control personnel's searching for positive cultures from the Microbiology Laboratory (Nichols, 1998)

Two major studies including one carried out in a Canadian teaching hospital by Cruse and Foord (1980) demonstrated reduction in surgical wound infection rates during a surveillance decade that involved 62,939 operations Another study, Haley et al (1985) from Center for Disease Control, Atlanta, demonstrated the benefits of properly designed wound infection surveillance, in a detailed analysis of 59,352 patients who underwent surgery This work suggested that an effective infection surveillance program could reduce a hospital's surgical wound infection rate by as much as 35%

In a later 10 year wound infection surveillance program, procedure-specific rates rather than surgeon-specific rates were calculated annually (Olson et al, 1990) The results of this study showed a significant reduction in wound infection rates in the last 9 years of surveillance in every class of surgical wound compared with the index year rates Estimated savings in hospital room costs alone reached $3 million during the 10 years The use of computer surveillance to improve the use of antibiotic administration in both the prevention and the treatment of nosocomial
Infections, including wound infections has also been stressed (Evans et al, 1986). Many different computer based programs have been developed for the monitoring of surgical wound infection and the identification of risk factors for the development of infection (Bremmelgaard et al, 1989, Kjaeldgaard et al, 1989).

Large studies have shown that about 50% of all infections can be identified after hospital discharge if adequate surveillance is carried out (Brown et al, 1987, Krukowski et al, 1988).

French et al (1989) reported that in a 1400 bed teaching hospital, where single day prevalence surveys of hospital infection were done every six months for a period of 3 years, all infections including postoperative wound infections fell linearly from 10.5% in the secondary survey to 5.6% in the last survey. The authors concluded that infection control policies can have a substantial impact on the prevalence of HAI and their effectiveness can readily be measured by repeated prevalence surveys.

A study conducted by Thakur et al (1990) found out the direct influence of environmental surveillance in reducing postoperative infection rates in a teaching-cum-research hospital. Bacteriological surveillance of air, disinfectants, solutions, hospital personnel and equipment were carried out. The results showed the air in various OTs and ICUs and postoperative ward to be unsatisfactory 60-80% of the time. 68.64% of air samples showed *Staphylococcus aureus*. *S aureus* was responsible for 29.46% of postoperative infection. From disinfectants and suction bottles pathogens recovered were 21.16% and 74.24% respectively. 64.7% people were found to be nasal carriers of *S aureus*. The authors reported that after implementing the effective control measures, the postoperative wound infection rate came down to 2.72%.

In another recent study, Mandal et al (1997) compared continuous and selective surveillance programs to reduce hospital acquired infection.
Bacteriological surveillance of the environment and patients with infections was carried out. Overall postoperative wound infection rates were reduced to less than 1%. The authors concluded that laboratory based ward liaison hospital infection surveillance program was effective and less time consuming. A recent study conducted by Cynac et al. (1998) examined the relationship between patient related factors and the development of postoperative wound infections. Use of 4% chlorhexidine and MRSA screening preoperatively showed drastic reduction in wound infection rate. The marked reduction of postoperative wound infection from 4% in December 1997 to 1.2% in May 1998 was mainly due to good preoperative preparation, continuous supervised on going education on postoperative care, strict adherence to hand washing and aseptic techniques and vigilant nursing care including recording and reporting if any signs and symptoms of infection were noted.

**Classification of Surgical Wounds:**

Heusinkveld et al. (1966) considered wound infection to be any purulent discharge from the primary surgical incision appearing within 60 days of the date of surgery other than that issuing from drainage site.

Various classifications have been adopted by different workers. Culbertson et al. (1961) classified wounds into four categories as Clean, Clean contaminated, Contaminated and Dirty depending upon certain criteria, such as the use of aseptic technique, transection of gastrointestinal, genitourinary or tracheobronchial systems, violence or gross spillage at the time of transection of hollow viscera and continuous contamination of the wound by faecal or tracheobronchial or genitourinary discharge. Wasek et al. (1965) classified wounds as clean and clean but contaminated. Srivastava et al. (1969) classified wounds as "clean" when aseptic technique was used and without any breach in the alimentary, genitourinary, biliary or tracheobronchial systems, "potentially contaminated" as those wounds wherein the above systems were transgressed.
A widely used classification of surgical wounds is based on an estimate of likelihood of bacterial contamination of operative site.

CDC (1974) classification is the most widely used criteria for defining the type of postoperative wound infection (Center for Disease Control and Prevention, 1974)

**CDC Criteria for defining the type of surgical wound (Centre for Disease Control, 1974)**

**Operative wound, clean - Class I:** Non-traumatic wound in which no inflammation is encountered, no break in technique occurred and respiratory, alimentary and genitourinary tracts are not entered.

**Operative wound, clean contaminated - Class II:** Non-traumatic wound in which minor break in technique occurred or in which gastrointestinal genitourinary or respiratory tracts were entered. Significant spillage includes transection of appendix or cholecystic duct in the absence of acute inflammation and entrance into genitourinary or biliary tracts in the absence of infected bile or urine. Hysterectomy is included in this category.

**Operative wound, contaminated - Class III:** Any fresh traumatic wound from a relatively clean source or an operative wound in which there is a major break in technique, gross spillage from the gastrointestinal tract, or entrance into genitourinary or biliary tracts in the presence of infected urine or bile. This includes incisions encountering acute, non-purulent inflammation.

**Operative wound, dirty - Class IV:** Traumatic wound from a dirty source or with delayed treatment, fecal contamination foreign body or retained devitalized tissue. Also includes operative wounds in which acute bacterial inflammation or a perforated viscus is encountered or in which clean tissue is transected to gain...
access to a collection of pus. Classification of postoperative wound infections described by different workers is given in the Table 2.

FACTORS INFLUENCING THE INCIDENCE OF SURGICAL WOUND INFECTION:

PREOPERATIVE STAY IN HOSPITAL:

Cruse (1988) reported that every hospital with preoperative assessment clinic (POAC) obtained a reduced clean wound infection rate which thereby reduced the cost of hospitalization. David Beatty et al (1983) reported that overall wound infection rate decreased when preoperative stay was less than 3 days among patients undergoing mastectomy. Another study reported that infection rate increased with increased length of preoperative hospitalization in both clean and clean contaminated categories after observing a continuous 10 year wound infection surveillance. Olson et al (1990) showed that preoperative stay did not have any effect on infection rate. A study conducted by Manian and Meyer (1988) reported that longer preoperative hospitalization and hospital admission on the same day of operation no longer significantly lowered the wound infection rates.

PREOPERATIVE SHAVE:

Seropian and Reynolds (1971) study showed that incidence of infection was 3.1% when shaving was done immediately before surgery, 7.1% when done less than 24 hours before surgery, and 20% when done more than 24 hours before surgery. Wesley Alexander et al (1983) reported that shaving on the morning of operation offered no advantage over shaving the night before. Balthazar et al (1982) proposed the method of hair clipping which has an acceptably low infection rate of 1.7% compared to other methods. Mishnki et al (1990) documented patients who did not have preoperative shave had lower wound infection rate compared to patients who had preoperative shave the day before the surgery. The influence of preoperative shaving vs clipping on infection rate was studied in 4013 patients and it was reported that preoperative shaving is deleterious and the practice should be abandoned.
<table>
<thead>
<tr>
<th>Author and Year</th>
<th>Classification</th>
</tr>
</thead>
</table>
| 1 Barnes et al (1961) | a) Minor sepsis  
b) Major sepsis |
| 2 Culbertson (1961) | a) Clean  
b) Clean contaminated  
c) Contaminated  
d) Dirty |
| 3 Wasek et al (1965) | a) Clean  
b) Clean but contaminated |
| 4 Srivastava et al (1969) | a) Clean  
b) Potentially contaminated |
| 5 NRC/NAS Study (1964) | a) Refined - Clean  
b) Other clean  
c) Clean contaminated  
d) Contaminated  
e) Dirty |
| (Ad-hoc Committee on Trauma, 1964) | |
| 6 American College of Surgeons Committee on Control of Surgical Infections (Meakins, 1989) | a) Clean non-traumatic surgical wounds  
b) Clean contaminated non-traumatic surgical wounds  
c) Contaminated fresh wounds of trauma |
| 7 Traditional method (Nichols, 1991) | a) Clean surgical wounds  
b) Clean contaminated wounds  
c) Contaminated wounds  
d) Dirty-infected wounds |
PREOPERATIVE CLEANSING:

A recently published, large multihospital study of clean operative procedures showed no fewer wound infections when patients bathed thrice preoperatively with chlorhexidine and detergent than when they used detergent alone (Rotter et al, 1988). Ayliffe et al (1980) found that preoperative washing with an antiseptic did not reduce the infection rate in Birmingham, England. Lowbury and Lily et al (1964) showed that 1% iodine in 70% alcohol and 0.5% chlorhexidine in 70% alcohol are the most effective skin antiseptics and reported that clean wound infection rate decreased to 0.7%.

PRESENCE OF REMOTE INFECTIONS:

Edwards (1976) and Valentine et al (1986) reported the presence of an active remote infection at the time of elective operation has been shown to greatly influence the development of subsequent postoperative wound infection. In order of frequency these infections occur in the urinary tract, skin and respiratory tract. Valentine et al (1986) reported preoperative treatment (more than 2 hours before surgery) has been shown to reduce postoperative wound infections significantly.

LENGTH OF OPERATION:

There is a direct relationship between the operating time and the succeeding infection rate. The clean infection rate roughly doubles with every hour. Other studies have also shown an increase in the infection rate associated with prolongation of operating time. Miles et al (1980) in their study proposed four possible reasons. The dose of exogenous contamination increases with time and wounded cells are damaged by drying and retraction. Increased amounts of stenilization and electrocoagulation may further reduce the local resistance of the wound and longer procedures are more likely to be associated with blood loss and shock which reduce the general resistance of the patient. Shapiro and coworkers (1982) reported that an increasing duration of hysterectomy was associated with a decreasing effect of antibiotic prophylaxis in preventing infection at the operative.
In another study of postoperative wound infection, in 676 paediatric patients an increased rate of wound infection was associated with operative procedures of longer than 1 hour duration (Bhattacharyya et al, 1990) Nicholas (1984) and Culver et al (1992) reported that exact risk associated with the duration of operation differs from procedure to procedure.

**USE OF PROPHYLACTIC ABDOMINAL DRAINAGE:**

Nora and coworkers (1972) reported both clinical and experimental studies of dangers of using prophylactic drain in abdominal surgery on the basis of their frequent finding of skin bacteria in the interior of the abdominal drains. Cense and associates (1970) reported increased infection rates after splenectomy when drains were employed. Magee (1976) demonstrated that the presence of either elastic or latex penrose drains in experimental wounds dramatically enhanced the wound infection rate even in the presence of subinfective doses of bacteria. Alexander and coworkers (1976) indicated closed suction drainage as the method of choice when abdominal drainage is indicated.

**DURATION OF HAND SCRUB:**

Dineen (1969) made colony counts of surgeon's hands at the end of 2 hour operation and found no difference between 5 and 10 minute scrubs, provided an antiseptic was used. Galle and colleagues (1978) reported that a 10 minute scrub under running water used 50 gallons of water.

**ANTIMICROBIAL PROPHYLAXIS:**

**Choice of antibiotics:**

Kernodle et al (1990) reported that up to date analysis of the antimicrobial susceptibilities of wound isolates in local hospitals are most essential to detect important shifts in patterns of resistance. Dipiro et al (1984) reported the routine use of second or third generation cephalosporins have not improved the clinical results over those achieved with first generation cephalosporins. Several studies (Kaiser et al, 1986, Gorbach, 1989, ASHP Commission on therapeutics, 1992)
suggested that procedures involving ileum, colon or appendix, the drugs used should be active against both Enterobacteraceae and common enteric anaerobic species, especially B fragilis group. Tuomala et al (1985) and Hemsell et al (1991) showed that cefazolin alone was superior to other drug combination used for anaerobic species in gynaecologic infections. ASHP Commission on Therapeutics (1992) reported that newer generation agents have not been proved to be more effective than cefazolin, cefoxitin or ceftetan for prophylactic purposes.

**Timing:**

Several studies proved that single preoperative dose of antibiotic is as efficacious as multiple doses of prophylactic antibiotics given during the perioperative course (Dipiro et al, 1986). Shapiro et al (1979) showed that no additional benefit is derived from longer course of antibiotic prophylaxis (>24 hours) even for immunosuppressed patients. Nichols (1990) showed that in oral preoperative antibiotic preparations for elective colon resection, the agents were given during the 24 hours before operation in order to attain significant intraluminal (local) and serum (systemic) levels. Kaiser (1986) reported that longer periods of preoperative preparation are unnecessary and have been associated with the isolation of resistant organisms within the colon lumen at the time of resection.

Nichols (1989) and Kaiser (1989) indicated antibiotic prophylaxis in clean surgical procedures that utilized a foreign material, grafts or prosthetic devices and in many vascular, cardiac and orthopedic operations. According to a study, Gorbach et al (1989), prophylactic drug regimen in the above setting is continued for 24 hours to 48 hours postoperatively despite lack of knowledge about their effectiveness. Moleski (1986) and Canon et al (1988) reported that continuing the prophylactic antibiotic regimen beyond perioperative period increases the cost and associated with the development of *C difficile* colitis and also with a high level of colonization with methicillin-resistant Staphylococcus.
Lindsey et al (1990) and Smith et al (1990) reported that both or minimal and a mucosal related microflora are qualitatively and quantitatively similar in humans as well as animals. Before 1970s majority of surgeons utilized mechanical cleansing alone before elective colon surgery (Nichols, 1971).

Nichols (1990) documented that all patients undergoing elective colon resections are at significant risk of developing postoperative infection because of the greater number of bacteria in the colon microflora which increases in protracted operations and those done on the extraperitoneal rectum. Kaiser and colleagues (1977) studied different approaches to preoperative antibiotic prophylaxis for elective colon resection and showed a direct correlation between the duration of operation and postoperative infection rate. Nichols (1990) and Solla et al (1990) documented that majority of surgeons employ both oral and parenteral antibiotics and mechanical cleansing as preparation for elective colon resections. Three regimens of oral antibiotics, neomycin with either erythromycin base, metronidazole or tetracycline are usually followed (Nichols, 1990). Many studies support the advantage of oral neomycin-erythromycin base in preventing infections following a elective colon resection (Kaiser, 1989, Nichols, 1990). Most investigators recommend perioperative administration of one to five doses of parenteral agent during a 24 hour period starting shortly before operation. Jewesson et al (1997) documented that cefotizoxime was superior to cefoxitin and metronidazole gentamicin in prevention of colorectal surgical wound infections.

In placebo-controlled trials, wound infection rates without antibiotics range from 4% to 9% for simple appendicitis (Gottrup, 1980). Perioperative antibiotics reduce the rate of wound infection to 1% to 5%. Cefoxitin has been the subject of several studies and is effective in a short course regimen (Bauer et al, 1989, Winslow et al, 1983, Browden et al, 1989). Metronidazole, although deficient in its spectrum has been successfully used as a single agent (Gottrup, 1980). Andersen et al (1972) documented that topical agents have also proved effective prophylaxis for simple acute appendicitis. Haddock et al (1988) in their nationwide study.
reported that 84% of surgeons used metronidazole alone and 13% metronidazole in combination with cephalosporins either by rectal route or intravenous route as antibiotic prophylaxis for appendicectomy. Cann et al (1988) concluded that mezlocillin may be effective as the sole prophylactic agent in appendicectomy.

THE PATIENT’S RESISTANCE:

Culbertson and colleagues (1961) stated that the risk of wound infection is determined according to the following equation:

\[
\text{Dose of bacterial contamination} \times \text{Virulence} \div \text{Resistance of the host}
\]

This equation explains the risk of healing without complications of heavily contaminated wounds in patients with normal host defenses. Host resistance can be classified into general and local factors.

GENERAL FACTORS:

Age: All studies have shown an increase in wound infection rates with advancing years. High morbidity and death rates are frequently reported for surgery in the elderly (Cruse, 1980). Co-existing disease and delay of elective surgery complicate evaluation of operative risk in that group.

Healthy old and young persons are immunologically similar before surgery. Some aspects of the immune responses are more depressed by surgery in older person and that some immune responses correlate with surgical outcome. Cruse (1980) observed an increased risk of infection with age noting a clean wound infection rate of 2.3% for patients 66 years and older. A slight preponderance of wound infections in older patients when compared with age distribution of all types of patients admitted in their hospital was noted. In 1988, Sramova et al reported higher infection rates among children under 3 years age, person over 60 (prevalence 27-36%) and general surgical patients over 50 (prevalence 14-21%).
Mead et al (1986) reported highest infection rates in the very young (<1 year) and in the older population (>50 years)

   Sex: The infection rate in clean wound is the same in male and female patients

DIABETES, OBESITY AND MALNUTRITION:

   Ganguly et al (1995) reported some underlying diseases in the patients with anaemia (53.7%), diabetes mellitus (85.2%), hypertension (82.0%) and obesity (51.5%) influenced the rate of infection to a great extent compared to patients with no such underlying disease (17.5%) Risk factors associated with SSI are given in the Table 3

AETIOLOGY OF SURGICAL WOUND INFECTIONS:

   Emon et al (1993) reported that gram positive organisms cause 56% of surgical wound infections, gram negative organisms cause 35% of SSI's and Candida spp cause 4% A study from Nigerian University College hospital reported that the ratio of gram negative to gram positive organisms in postoperative wound infection was 3.1 (Oni et al, 1997) Mayhall et al (1992) reported that 45% of surgical wound infections (SWI) are caused by gram positive cocci (15% each of S aureus, Enterococci and coagulase negative Staphylococci) and the remainder of SWI are due to gram negative organisms and yeasts The predominant microorganisms isolated from surgical wound infections are S aureus, coagulase negative Staphylococci, Enterococci spp, Streptococcus spp, E coli, Klebsiella spp, Proteus spp, Enterobacter spp, Citrobacter spp, P aeruginosa and Acinetobacter spp (Namias et al, 2000, Groot et al, 1998, Giacometti et al, 2000, Anvikar et al, 1999)

S aureus:

   Recently published data from NNIS system reported that S aureus accounted for 19% of 11,724 surgical wound infections In the developing
<table>
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<tr>
<th>Host-related risk factors</th>
<th>Procedure-related risk factors</th>
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<td><strong>Definite</strong></td>
<td><strong>Definite</strong></td>
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<td>Age</td>
<td>Preoperative hair removal</td>
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<td>Obesity</td>
<td>Type of procedure</td>
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<td>Disease severity</td>
<td>Antibiotic prophylaxis</td>
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<tr>
<td>ASA Score</td>
<td>Duration of surgery</td>
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<td>Remote infection</td>
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<td>Duration of preoperative hospitalization</td>
<td></td>
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<tr>
<td><strong>Likely</strong></td>
<td><strong>Likely</strong></td>
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<tr>
<td>Malnutrition and low serum albumin</td>
<td>Multiple procedures</td>
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<tr>
<td>Diabetes mellitus</td>
<td>Tissue trauma</td>
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<td>Foreign material</td>
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<td>Blood transfusion</td>
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<td><strong>Possible</strong></td>
<td><strong>Possible</strong></td>
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<td>Malignancy</td>
<td>Preoperative showers</td>
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<td>Immunosuppressive therapy</td>
<td>Emergency surgery</td>
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<td>Breast size in women</td>
<td>Drains</td>
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</table>
countries, the rates are even higher, i.e., *S. aureus* causes almost half of the surgical wound infections (Prabhakar et al., 1983). In nosocomial surgical wound infections, data are always affected by delayed infections often due to *S. aureus* that are manifested months or even years after surgery (Simmons, 1982). In a continuous 10 year wound infection surveillance from Minneapolis *S. aureus* was the leading pathogen in Class I wounds and one of the pathogens among Class II and Class III wounds (Olson et al., 1990). In a study conducted from New Delhi, India, on environmental surveillance and its role in postoperative hospital acquired infection, 64.7% were found to be nasal carriers of *S. aureus* (Thakur, 1990). In a study, *S. aureus* alone was found to be the pathogen in 73.9% of PWI cases from Government Medical College, Lucknow (Agarwal et al., 1980). In several other studies, *S. aureus* is the single most important leading pathogen and most frequently isolated agent among postoperative wound infections in teaching tertiary care hospital (Kotisso et al., 1998), in Russian General Hospital (Filipenko et al., 1989) in University College Hospital, Nigeria (Oni et al., 1997), in Government Medical College Gopal Ganj, Bangladesh (Saha et al., 1995), in Miami School of Medicine, USA (Namias et al., 2000), in 33 Dutch Hospital in Netherlands (Groot et al., 1998). In various studies, *S. aureus* accounted for 9.4% to 73.9% of total pathogens isolated from postoperative wound infections (Agrawal et al., 1980, Mahmood, 2000, Emele, 1999, Vilari Compte, 1999). Frequency of *S. aureus* isolated from postoperative wound infections from different studies listed in the Table 4. Yoshida et al (1995) from Japan reported that an isolation policy was initiated in the surgical ward to arrest the endemic multiresistant *S. aureus*. In National Shimonoseki Hospital, biotyping and antibiotyping of environmental isolates showed that isolation policy led to interward transfer of strains and stopped intraward transfer of MRSA strains. Among the *S. aureus* strains isolated from wound infection, methicillin resistance was reported in 20% to 74.2% of isolates in various studies (Giacometti et al., 2000, Choojitr et al., 1995, Wang et al., 2001, Eveillard et al., 2001). An outbreak of nosocomial infections occurred in a postoperative intensive care unit due to oxacillin-resistant *S. aureus* was traced to a staff member who was a nasal carrier by using antibiogram, bacteriophage type,
capsular polysacchande type and esterase electrophoretic type involved (Bouvet et al, 1990)

According to other studies, S aureus wound infection sources were patients themselves (44.1%), medical staff (56.2%), paramedical staff (62.5%), environmental sources in the operation theatre and surgical wards (52.6%) (Agrawal et al, 1980, Krasilnikov et al, 1985, Ako-Nai et al, 1992, Filipenko et al, 1989, Bouvet et al, 1990)

Coagulase negative Staphylococcus (CNS):

Coagulase negative Staphylococci are increasingly being reported as one of the predominant pathogens associated with post-surgical wound infections (Giacometti et al, 2000, Santos et al, 1997) Santos et al (1997) from Brazil University teaching hospital reported that coagulate negative Staphylococci (CNS) are the 3rd most important pathogen causing postoperative wound infections and multidrug-resistance was found in 66% of CNS strains. Few studies from India have reported multidrug resistance in CNS isolates (Cynac, 1998) From tertiary health care centres and University hospitals of Italy, Mexico, Turkey, Minneapolis CNS accounted for 71, 13.6, 21.7 and 8% respectively of total pathogens isolated from postoperative wound infections (Giacometti et al, 2000, Vilar Compte et al, 1999, Yalcin et al, 1995, Olson et al, 1990)

Enterococci:

Enterococci are one of the commonest nosocomial pathogens associated with postoperative wound infections (Cynac, 1998, Vilar Compte et al, 1999) They are often found in association with other more virulent organisms (French, 1998) E faecalis accounts for 90% of Enterococcal isolates, in recent years E faecalis is also increasingly being reported (Murray, 1990, Moellereng, 1992) The increased isolation rate for Enterococci constituted multiple endogenous strains that spread from patient to patients on hands of hospital staff was reported (Rhinehart et al, 1990) In different studies, Enterococcus spp was the causative organism in 14.6,
7.7 and 5.6 percentage of postoperative wound infection in various centers and university hospitals (Giacometti et al., 2000, Vilar Compte et al., 1999, Olson et al., 1990). Vancomycin resistance reported among wound infection isolates of Enterococci was 2.7 to 4.5% (Giacometti, 2000, Vilar Compte, 1999).

**Streptococcus spp:**

In different studies, the association of Streptococcus spp in postoperative wound infection was reported to be 1.3, 2.3 and 2.5% (Mahmood, 2000, Anvikar et al., 1999 and Olson et al., 1990). Drug resistance reported in these isolates is 0 to 4% to different antibiotics (Anvikar et al., 1999, Giacometti et al., 2000). *Streptococcus pyogenes* is the predominant Streptococci causing postoperative wound infection accounting for 1.3% and 2.3% respectively (Mahmood et al., 2000, Anvikar et al., 1999).

**Enterobacteriaceae:**

In different studies, *E. coli* was reported as 1st, 2nd, 3rd most leading pathogen causing surgical wound infections in association with *S. aureus* and *P. aeruginosa* (Giacometti et al., 2000, Vilar Compte et al., 1999, Colizza et al., 1999, Abussaud, 1996). The frequency of *E. coli* association among postoperative wound infections ranged from 7% to 31% (Abussaud, 1996, Olson, 1990, Santos et al., 1997, Kotisso et al., 1998). Drug resistance among these isolates varied between 0 to 100% to different antibiotics (Mahmood, 2000, Anvikar et al., 1999, Giacometti et al., 2000).

From past decade *Klebsiella pneumoniae* and other Klebsiella spp were the most common and predominant pathogens causing surgical wound infections (Abussaud, 1996, Mahmood, 2000, Sood et al., 1998, Thakur et al., 1990, Anvikar et al., 1999). Frequency of *K. pneumoniae* associated with wound infections ranged from 10 to 26.80% in different studies (Abussaud, 1996, Anvikar et al., 1999, Sood et al., 1998). Drug resistance to different antibiotics varying from 40 to 100% has been reported in different studies (Anvikar et al., 1999, Giacometti et al., 2000).
Proteus vulgans and Proteus mirabilis were the other Enterobactenaceae members causing postoperative wound infections (Saha et al, 1995, Mary et al, 1996, Giacometti et al, 2000). Susceptibility to different antibiotics like ampicillin, gentamicin, ciprofloxacin, ceftriaxone, imipenem, netilmicin, cefazolin, piperacillin varied from 100% to 32.3% (Giacometti et al, 2000, Saha et al, 1995). Mary et al (1996) reported that Proteus mirabilis and P. vulgans were the predominant pathogens associated with postoperative wound infections in surgical wards and none of the environmental samples yielded Proteus strains. In the above study, autoinfection was found to be the commonest mode of infection, the most common source of infection was rectum (85.2%) followed by the perineum, groin and urethra (Mary et al, 1996).

Frequency of Enterobacter spp causing wound infections reported is 17.6, 7 and 2.90% in different hospitals like University Hospital, Turkey, Veteran Administration Medical center, Minneapolis, Government Medical College, Aurangabad, India respectively (Yalcın et al, 1995, Olson et al, 1990, Anvikar et al, 1999). Resistance to different antimicrobials varied from 3 to 80% (Giacometti et al, 2000). Citrobacter spp and Serratia spp were also reported as bacteria causing infection in few studies (Olson et al, 1990, Giacometti et al, 2000). Giacometti et al (2000) reported that drug resistance among Serratia spp varied between 100 to 18% to antibiotics like ampicillin, amx-CLV, piperacillin, cefazolin, ceftriaxone, imipenem, ciprofloxacin, netilmicin and SXT-TMP. Olson et al (1990) reported that Serratia spp was isolated in 4.8% of 1,032 postoperative wound infection cases. Bennet et al (1995) reported that Moraxella osloensis was one of the etiological agents causing wound infection. Frequency of isolation of different Enterobacteraceae members has been given in the Table 4. Chabbra et al (1998) have reported Mycobacterium abscessus as sole pathogen causing postoperative wound in 9 patients in a private nursing home in New Delhi, India.
<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Name of the organism</th>
<th>% of Isolation</th>
<th>Name of the Hospital / Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S. aureus</td>
<td>28 20</td>
<td>Institute of Infectious Disease and Public Health, Ancona, Italy</td>
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<td></td>
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<td>28 80</td>
<td>Gondar College of Medical Sciences, Ethiopia</td>
<td>Kottso et al (1998)</td>
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<td></td>
<td></td>
<td>65 60</td>
<td>Russian Surgical Clinic, Russia</td>
<td>Filippenko et al (1989)</td>
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<td></td>
<td></td>
<td>33 90</td>
<td>Rio de Janeiro University Hospital, Brazil</td>
<td>Santos et al (1997)</td>
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<td>21 92</td>
<td>Voghera General Hospital, North Italy</td>
<td>Cestan et al (1996)</td>
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<td></td>
<td></td>
<td>50 32</td>
<td>PNS Shifa Hospital, Karachi</td>
<td>Mahmood (2000)</td>
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<td></td>
<td></td>
<td>39 00</td>
<td>Ekpoma College of Medicine, Nigeria</td>
<td>Emele et al (1999)</td>
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<td></td>
<td></td>
<td>19 70</td>
<td>Cumhuriyet University Medicine Faculty Hospital, Turkey</td>
<td>Yalon et al (1995)</td>
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<td></td>
<td></td>
<td>35 00</td>
<td>Yarmouk University Hospital, Jordan</td>
<td>Abussaud (1996)</td>
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<td></td>
<td></td>
<td>56 00</td>
<td>Among 10 Public Hospitals, France</td>
<td>Holzapfel et al (1999)</td>
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<td></td>
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<td>73 80</td>
<td>K.G Medical College, Lucknow, India</td>
<td>Agrawal et al (1980)</td>
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<td></td>
<td></td>
<td>20 00</td>
<td>P.D Hinduja National Hospital, Mumbai, India</td>
<td>Renuka Cynac et al (1998)</td>
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<td>25 00</td>
<td>Government Medical College, Aurangabad, India</td>
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<td>Giacometti et al (2000)</td>
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<td></td>
<td></td>
<td>12 60</td>
<td>Minneapolis Veterans Administration Medical center, Minneapolis</td>
<td>Olson et al (1990)</td>
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<td></td>
<td></td>
<td>13 00</td>
<td>National Cancer Institute, Mexico</td>
<td>Vilar Compte et al (1999)</td>
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<td>20 37</td>
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<td>3</td>
<td>E. coli</td>
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<td>University Hospital, Sarajevo</td>
<td>Aganovic et al (1994)</td>
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<td>24 00</td>
<td>Ekpoma University Hospital, Nigeria</td>
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<td></td>
<td></td>
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<td>Olson et al (1990)</td>
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<td>Government Medical College, Aurangabad, India</td>
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**P. aeruginosa:**

*Pseudomonas aeruginosa* was detected in 2037, 3127, 252, 163 and 107% respectively of all postoperative wound infections in different studies from different countries (Aganovic et al, 1994, Cestan et al, 1999, Giacometti et al, 2000, Mahmood, 2000, Yalcin et al, 1995). It is the 1st or 2nd most predominant organism causing nosocomial wound infections (Thakur et al, 1990, Agnovic, 1994, Cestan et al, 1999).

Anvikar et al (1999) and Mahmood (2000) reported that 76% of *P aeruginosa* isolates were resistant to gentamicin and ciprofloxacin and 3rd generation cephalosporins. Giacometti et al (2000) reported 18% to 100% drug resistance to different antibiotics like AMX-CLV, Piperacillin, Cefazolin, Ceftazidime, Ceftriaxone, imipenem, Ciprofloxacin, Netilmicin and SXT-TMP. Pitten et al (2001) from German University hospital reported transmission of a multiresistant *Pseudomonas aeruginosa* strain among 60 postoperative patients. Also the same strain was isolated from tracheal secretions, blood, urine, venous catheters, ascites and several wounds. Environmental sampling revealed several wash basins in the intensive care units were contaminated with same strain. The handling of respirators, resuscitation tubes, urine bottles and bedpans resulted in the contamination of the patient's environment. The genetic relationship of the isolates was investigated by PFGE. The isolates were resistant to β-lactam antibiotics including carbapenems and aztreonam, to aminoglycosides and quinolones. Susceptibility to polymyxin B was observed. Frequency of *P aeruginosa* isolated from wound infections is given in the Table 4.

**Acinetobacter spp:**

Nosocomial Acinetobacter infections may involve any site, but they predominate in the postoperative wound infections along with respiratory tract and urinary tract. Many isolates from the skin and respiratory tract were considered to be colonizing rather than infecting organisms. A steady increase in the proportion of Acinetobacter isolates from wound infections has been recovered over the past.
ten years (Joly-Guillou and Bergogue-Berezin, 1990) Skin, respiratory tract and wounds were considered as potential reservoirs for infection caused by Acinetobacter spp during outbreak periods. A recent study conducted by Giacometti et al (2000) reported Acinetobacter spp as important causative agents associated with postoperative wound infections. Susceptibility pattern to ampicillin and cefazolin reported as 0%, piperacillin and ceftriaxone was 59.5% and 57.1%, sulphamethoxazole was 52.3%, ciprofloxacin, imipenem and netilmicin was 92.8%, 95.2% and 92.8% respectively (Giacometti et al, 2000)

ANAEROBES IN SURGICAL WOUND INFECTION:

Anaerobic bacteria have been increasingly implicated in a number of human infections in the recent years, among which intra-abdominal, genitourinary and pulmonary infections are the most important. Both microbiologists and clinicians have taken interest in anaerobic micro-organisms which were neglected in the past. The major factor in this change of attitude towards anaerobic bacteria is the simplification of anaerobic bacteriologic techniques to some extent.

Leshchenko et al (1996) from Russia reported that of the 145 patients with surgical infections, aerobic agents were responsible in 16.6% and anaerobic nonclostridial agents in 33.1% of all cases. Mixed flora contributed to 44.1%. The most important anaerobic nonclostridial agents were Bacteroides (38.3%), Peptostreptococcus (27.3%) and the Peptococcus (17.2%). In a study conducted at Calcutta, India, Chatterjee and Chakraborti (1995) showed the magnitude of nonsporing anaerobic (NSA) infections in postoperative wounds of colorectum in children (57.1%), general surgery (0%), abdominopelvic and uterocervical operations (11-45%) in gynaecologic and obstetrical cases and perforative peritonitis (25.8% to 32.3%). Out of the 22 species of NSA isolated, Peptostreptococcus anaerobius, Acidaminococcus fermentans and Peptococcus prevoti are the commonest. Others were Peptostreptococcus niger, Gaffky anaerobia, Lactobacillus bulganceus, Actinomycyes bovis, Bacteroids oralis, Fusobactenum gonidiaformans and the different species of peptococcus,
peptostreptococcus, Eubactenum, Propionibactenum and Fusobactenum. In another study from Moscow, blood from 110 patients suspected of anaerobic bacteremia in the suppurative septic complications of trauma and surgery patients was examined. In this study there were 37 blood cultures in a group and 24 strains of them were anaerobic (Okropiridze et al, 1996). Aldridge (1994) from New Orleans, USA reported that piperacillin / tazobactam was the beta lactam, beta lactamase combination that was most active against the Bacteroides fragilis group. In the same study, Bacteroides fragilis, Prevotella, Porphyromonas and Fusobacterium were the predominant anaerobes in polymicrobial surgical infections. Anaerobic bactena were present in 29% of the cases (5% pure anaerobic and 24% mixed aerobic and anaerobic) predominant anaerobic bacteria isolated were Propionibactenum spp (18%) (Hartog et al, 1995) Brook and Frazier (1998) from Bethesda studied the aerobic and anaerobic microbiologic characteristics of 584 wounds and correlated with the infection site. Aerobic or facultative bacteria were present in 223 specimens, anaerobes in 177 specimens and mixed flora in 184 specimens. The predominant anaerobic organisms were Bacteroides spp, Peptostreptococcus spp, Clostridium spp and Fusobactenum spp.

In a recent study, Deng et al (1997) from Changsha analysed the anaerobic infection of maxillofacial surgery and Bacillus melaninogenicus, Veillonella Peptococcus and Peptostreptococcus were the predominant bacteria isolated.

Sources of agents associated with postoperative wound infection:

Hasselgren and Holm (1981) reported that cultures from patients with PWI, other patients in ICU, health care personnel environment of the intensive care unit and ward were compared to trace out the sources and routes of postoperative wound infections. The results showed patients themselves were a source of the bacteria in all cases of wound infections. Filipenko et al (1989) reported that the sources of pathogens for postoperative wound infections in their hospitals were carriers among medical staff, medical students and patients.
The mechanism of heterogeneity of bacterial populations of pathological foci in patients with wound infections were studied (Krasinikov et al, 1985). The results showed that the first source of intrapopulation variability of wound populations of microbes and presence of varying strains and variants in the infecting dose of the causative agent. The 2nd source consists of repeated superinfection of pathological processes by new, usually nosocomial strains and variants of the same species with their subsequent selection during patients stay in the health service establishment. Other sources of minor importance include mutation and recombination. Ako Nai et al (1992) reported that patient’s microflora and operating room air were the major sources of intraoperative bacterial colonization of postoperative wounds in their University hospital.

Andenaes et al (1996) did not find any statistically significant relationship between preoperative bacterial colonization and postoperative wound infections. CDC (1995) reported the results of an investigation conducted at 7 hospitals because of an unusual outbreak of surgical wound infections after surgical procedures. The source of the outbreak was traced to patients exposure to propofol, a lipid based anaesthetic agent, was significantly associated with postoperative complications at all hospitals (Bennett et al, 1995).

Delogu et al (1997) conducted a study to estimate phagocytic killing by neutrophils (PMNS) of Pseudomonas aeruginosa pre-exposed to subinhibitory concentration of amikacin and imipenem among patients with critical postoperative complications. Results suggest a possible impairment of PMNS due to the critical disease and in some way responsible for the host adverse interaction between granulocytes, antibiotics and pathogens.
ANTIBIOTIC RESISTANCE IN NOSOCOMIAL PATHOGENS:

Mechanism of Antibiotic Resistance:

Clinical antibiotic resistance:

Rapid emergence and dissemination of antimicrobial resistant microorganisms in hospitals worldwide is a problem of crisis dimensions. High rates of antibiotic resistance found in nosocomial bacteria have been documented in numerous reports (Fass et al, 1995, Itokazu et al, 1996, Manian et al, 1996, Lester et al, 1990, Schiappa et al, 1996, Medecins, 1993). The main concern has been the frequent multiple antibiotic resistance shown by nosocomial pathogens like MRSA and E. coli, P. aeruginosa, K. pneumoniae, Acinetobacter spp, which results in greater therapeutic problem while treating the patients with nosocomial bacterial infection in hospitals.

Isolates of S. aureus, whether community or hospital acquired produce beta-lactamase and are resistant to penicillins such as penicillin G, ampicillin, amoxicillin and piperacillin (Dekker et al, 1987). In India, the prevalence of beta-lactamase producing Staphylococci is reported to be about 60% (a Multicenter study, Expert Group on Antibiotic Susceptibility Tests, 1995). According to this study, pooled data from various hospitals in India indicates that 60% to 80% of isolates of S. aureus are resistant to sulbactam/ampicillin and amoxicillin/clavulanic acid. In vitro resistance to cephalosporins in India has been reported to be as high as 20% to 50%. However, this is not reflected in actual clinical outcome (cefoperazone post-marketing surveillance group, 1996). In a study from India, over 30% isolates were reported to be resistant to gentamicin and 10-20% to amikacin (a Multicenter study, 1995). Staphylococcal resistance to erythromycin varied between 5 and 20% (Eykyri, 1996).

In a study conducted in France, Eveillard et al (2001) reported that over the years, the global multidrug-resistant bacterial isolates incidence was 1.26 per 1000 patient days (PD) the MRSA incidence was 0.89 per 1000 patient days (PD) and the ESBL incidence was 0.38 per 1000 PD.
MRSA are resistant not only to all isoxazolyl and other penicillins but also to all other beta-lactam antibiotics as well as macrolides, aminoglycosides and often to quinolones. The incidence of methicillin resistance is about 15% among \textit{S. aureus} strains in the USA and may be as high as 80% among hospital acquired strains of coagulase-negative Staphylococci (Froggatt et al, 1989) Methicillin resistant Staphylococcus strains are endemic in numerous hospitals and chronic care institution (Goldmann, 1997)

Enterococci

Vancomycin resistance in Enterococci had not been reported before 1989 According to a study conducted by CDC (1993) more than 10% of hospital-acquired Enterococci isolated from patients in intensive care units are resistant to vancomycin Treatment of infections due to vancomycin-resistant Enterococci has become extraordinarily difficult because many of the strains are also resistant to most other available agents, including beta-lactams and aminoglycosides (Murray, 1990) Intensive use of broad spectrum antibiotics has facilitated the emergence of resistance among gram negative bacteria (Goldman, 1997)

\textit{P. aeruginosa}:

The greatest range in resistance rates has been documented for \textit{P. aeruginosa} in numerous reports (Acar et al, 1993, Dalhoff, 1994, Wolff et al, 1985) They vary from 5% to greater than 50% depending on the origin of the strains (Kresken et al, 1994, Goldstein et al, 1995, Prosser et al, 1995) Strains of \textit{P. aeruginosa} that elaborate extended spectrum beta-lactamases capable of inactivating carbapenems have recently been recovered in Japan (Osano et al, 1994, Watanabe et al, 1991) Reiley et al (1984) have documented that hospitals as reservoirs for the dissemination of antimicrobial resistant pathogens in the community In their study, Reiley et al identified a series of small outbreaks of infection caused by this antimicrobial resistant nosocomial organisms
A recent multicenter study conducted in Europe (Fluit et al, 2000) reported that *P. aeruginosa* strains isolated from PWI showed higher susceptibility to amikacin (83%), meropenem (86.7%) and piperacillin/tazobactam (86.8%). Susceptibility to ciprofloxacin was 68.4%, levofloxacin 65.3% and ofloxacin 61.0%. Evangelos et al (1997) reported that all tested nosocomial *P. aeruginosa* strains (n=27) were found to be resistant to nine antimicrobial agents with known antipseudomonal activity, i.e. ticarcillin, piperacillin, ceftazidime, imipenem, meropenem, ciprofloxacin, gentamicin, tobramycin and amikacin. MIC₅₀-MIC₉₀ to ceftazidime, meropenem, imipenem and amikacin were 64 to >256, 16 to 128, 32 to >256 and 128 to >256 μg/ml respectively. Mahmoud et al (1996) reported that the most effective antibiotic against *P. aeruginosa* isolated from PWI in descending order were cefotaxime (56%), gentamicin (33%), chloramphenicol (29%), cefoxitin (25%), cephalothin (25%) and ampicillin (8%) respectively. Ojha et al (1997) from India reported that *P. aeruginosa* strain showed highest susceptibility to piperacillin 77.5%, amikacin 74%, ceftazidime 66%, ciprofloxacin 53%, cefotaxime 46%, ceftizoxime 42%, norfloxacin 36% and carbenicillin 36% respectively. Wattal et al (1998) from India reported that in paediatric and adult population, the percentage of resistance to aminoglycosides, third generation cephalosporins and quinolones was ranging from 48-68%, 38-75% and 30-68% respectively in gram-negative organisms alone. And among *P. aeruginosa* isolates the percentage of resistance varied between 38 and 78 (for cefotaxime 61%, ceftazidime 60%, norfloxacin 62%, netilmicin 60%, amikacin 60%, carbenicillin 78% and piperacillin 38%).

**Enterobacteriaceae:**

*E. coli* isolates from patients with HAI still display more than 98% susceptibility to fluoroquinolones in most developed countries. In France and Spain, increase in the percentage of resistant strains has been documented more frequently up to 10% (Soussy et al, 1991, Goldstein, 1995). Resistance is at worrisome levels in some developing countries, up to 14% in some South African countries, 25% in Korea and Philippines and up to 52.9% in China (Turnidge, 1995, Casellas et al, 1994). More than 40% of fluoroquinolone resistant strains of
*K. pneumoniae* are reported from France and a few other countries (Goldstein et al, 1995) A high percentage of resistance to fluoroquinolones was observed among other nosocomial bacteria such as *Serratia marcescens*, *Enterobacter* spp, *Citrobacter freundii* and indole positive *Proteus* spp

A study done by Rolinski et al (1994) showed a very high level of resistance of *E. coli* isolates (61-97%) to ampicillin and oxytetracycline. Abussaud et al (1996) reported that 88%, 57%, 51%, 47%, 38%, 33% and 18% of the *E. coli* isolates from PWIs were resistant to ampicillin, tetracycline, gentamicin, chloramphenicol, cephalothin, cefoxitin and cefotaxime respectively. Kotisso et al (1998) reported that *E. coli* strains isolated from PWI showed 94% of susceptibility to gentamicin, where as 81.2% and 56.2% were resistant to ampicillin and chloramphenicol respectively. Tsaknis et al (1997) showed that 10% of *E. coli* isolates were resistant to piperacillin / tazobactam, and 44.6% to ampicillin, 38.7% to mezlocillin, 22.4% to cefazolin, 10.6% to cefuroxime, 7.3% to ceftazidime and 32.1% to ampicillin / sulbactam respectively. Wattal (1998) from India reported high level of resistance of *E. coli* isolates to ampicillin (85%), ampicillin / sulbactam (71%), cepahlexin (68%), cefuroxime (56%), cefotaxime (57%), ceftriaxone (56%), cotrimaxazole (80%), norfloxacin (68%), ofloxacin (64%), gentamicin (58%) and netilmicin (55%) respectively. Ojha et al (1997) from India reported that 100%, 81%, 75%, 74%, 63%, 48% and 37% of nosocomial *E. coli* isolates were sensitive to amikacin, ampicillin / sulbactam, cefotaxime, ceftriaxone, netilmicin, ceftazidime and ciprofloxacin respectively.

A study conducted by Center for Disease Control and Prevention (Archibald et al, 1997) reported that the percentage of resistant isolates from hospitalized patients was higher than that from outpatients for the following combinations of antimicrobials and organisms: methicillin/coagulase-negative *Staphylococci* (49.0% vs 36.0% respectively, p < 0.01), methicillin/*S. aureus* (33.0% vs 14.5% respectively, p < 0.01), ceftazidime/Enterobacter cloacae (26.0% vs 12.0% respectively, p < 0.01), imipenem / *P. aeruginosa* (12.0% vs 6.5%, respectively, p < 0.01),
Ceftazidime/P aeruginosa (7.8% vs 4.0, respectively, p < 0.01) and vancomycin/Enterococcus spp (6.3 vs 1.4%, respectively, p < 0.01)

Acinetobacter spp:

Currently, high proportions of Acinetobacter spp, have become resistant to most commonly used antimicrobial agents, such as aminopenicillins, ureidopenicillins, narrow and broad spectrum cephalosporins (Joly-Guillou et al, 1985) and most aminoglycosides, aminocycloittols, chloramphenicol and tetracyclines (Goldstein et al, 1983) For relatively new antibiotics, such as broad spectrum cephalosporins, imipenem, tobramycin, amikacin and fluoroquinolones, partial susceptibility remains Recent analysis of hospital outbreaks have documented the spread of a imipenem-resistant strains (Go et al, 1994, Manikal et al, 2000)

Giacometti et al (2000) from Italy reported that more than 50% of the Enterobacteriaceae isolated from PWI were resistant to ampicillin and less than 20% were resistant to the combination of amoxicillin and clavulanate Eighty percent isolates were susceptible to ceftnaxone, but more than 50% were resistant to cefazolin Most P aeruginosa isolates were susceptible to piperacillin, ceftazidine, imipenem and netilmicin and majority of Pseudomonas isolates were resistant to ciprofloxacin In this study, methicillin resistance was documented in 54.4% of S aureus isolates

Mahmood et al (2000) from Karachi, Pakistan reported that 50% of the S aureus strains isolated from PWI cases were methicillin resistant and and more than 60% of the P aeruginosa and E coli strains were gentamicin resistant Resistance to third generation cephalosporins and ciprofloxacin was also reported to be high

A recent study from Aurangabad, India reported multidrug resistance among bacterial strains isolated from postoperative wound infections Gram positive cocci,
members of Enterobacteriaceae, *P. aeruginosa* and other non-fermenters showed 100% resistance to ampicillin, 60 to 98% resistance to gentamicin, 71 to 100% resistance to tetracycline and 89 to 100% resistance to cotrimoxazole, *S. aureus* strains showed 96% and 100% resistance to erythromycin and penicillin respectively (Anvikar et al, 1999)

**MECHANISMS OF ANTIBIOTIC RESISTANCE:**

It is the nosocomial pathogens' ability to respond rapidly to challenge with antibiotics, combined with indiscriminate use of antibiotics in the hospital which is responsible for their success as nosocomial pathogens

Both plasmids and transposons play a vital role in the biology of all the nosocomial pathogens. Numerous studies have documented that more than 80% of these bacteria carry multiple indigenous plasmids (Seifert et al, 1994) Although many clinical isolates are multidrug resistant, only a few studies have been able to demonstrate the plasmid-mediated transfer of resistance genes (Paton et al, 1993, Scaife et al, 1995) This may simply reflect the absence of a suitable test system for detecting such transfer as postulated by Towner (1991) Complex and varied transfer frequencies of standard plasmids belonging to different incompatibility groups have been observed between *Acinetobacter* strains EBF 65/65 and *E. coli* K-12 However, most of these transfers need an additional mobilizing plasmid for transfer to occur (Chopade et al, 1985)

In most reported cases plasmids are responsible for the development of antibiotic resistance. In addition, transposons may play a significant role for the spread of multiple antibiotic resistance in some clinical isolates Other nongenetic mechanisms include a decrease in membrane permeability and active efflux system which confers intrinsic resistance in these bacteria In addition, the selective pressure exerted by the antibiotics in these reservoirs may select resistant mutants Thus nosocomial bacteria have all necessary conditions to acquire multidrug-resistance
Beta-lactams:

All the members of Enterobactenaceae produce β-lactamases (usually chromosomal) which are able to hydrolyze β-lactam antibiotics and affect the clinical use of these compounds (Moosden, 1997). In addition these bacteria also carry R plasmids specific for β-lactamases. Since the introduction of cephalosporins, plasmid-mediated β-lactamases have been produced by Enterobactenaceae, Pseudomonas etc. The early β-lactamases like TEM and SHV types have further evolved into the so-called extended spectrum β-lactamases (ESBLs) which are capable of inactivating extended spectrum cephalosporins. SHV-derived enzymes confer much higher resistance than do TEM-derived enzymes. CEP-1 was reported in Proteus mirabilis even before introduction of new cephalosporins in 1976 (Bobrowski et al., 1976). Enzymes such as MIR-1, CMY-1 and CMY-2 found in *E. coli* and *K. pneumoniae* are able to confer high level resistance to the cephemycins (Papanicolaou et al., 1990).

BiL-1 and FEC-1 which are found in *E. coli* a predominantly cephalosporinases (Payne et al., 1992), the FUR enzyme found in *E. coli* and *K. pneumoniae* confers resistance to ceftazidime (Vuye et al., 1989). Investigations from Japan revealed plasmid-mediated enzyme responsible for resistance to β-lactams (including imipenem) in *P. aeruginosa* (Watanabe et al., 1991). More recently, outbreaks of strains of *Serratia marcescens* that are highly resistant to broad spectrum β-lactams and carbapenems have been reported from Japan and found to be caused by plasmid-mediated metallo β-lactamase (Ito et al., 1995).

In the recent past TEM-1, TEM-2, SHIV-1, OX-1, PSE-1 and PSE-4 had little activity against most of the newer cephalosporins (O’colagham, 1979). These enzymes confer resistance to many of the newer cephalosporins, monobactams and penicillins that are used to treat gram negative bacterial infection (Jacoby et al., 1991).
Recent studies indicate that in addition to resistance to cephapemycins, imipenem resistance occurs in *K. pneumoniae* when a high level of a plasmid-mediated Amp C-like β-lactamase or an extended spectrum SHV derivative is present in combination with the loss of a major outer-membrane protein (Bradford et al., 1997, Mac Kenzie et al., 1997).

The phenomenon of hetero-resistance in *S. aureus* is not understood. Strains are heteroresistant when they carry the complete regulatory region for MecA or just MecA. There is no correlation between the type of resistance and the amount of PBP 2a/2’ produced except when PBP 2a/2’ is strongly repressed or slowly induced. It has been shown that heteroresistance is due to a chromosomal mutation (Chr°) independent of MecA and Fem AB and unrelated to the other fem/aux genes (Ryffel et al., 1994).

Methicillin resistant *S. aureus* have acquired the MecA gene which confers clinical resistance to all β-lactam antibiotics (Grubb, 1998). The MecA gene is located on the bacterial chromosome in a sequence of a DNA (Meco region), which contains non-Staphylococcal DNA such as sequences similar to an *E. coli* gene contained in a series of directly related units (drug) on the 3’ side of MecA (Hiramatsu, 1995). Noble, Virani and Cree (1992) have demonstrated in the laboratory that *S. aureus* can acquire high levels of vancomycin resistance (VanA) from Enterococci and the recent finding of low level vancomycin resistance in Japanese MRSA is of great concern (Hiramatsu et al., 1997).

TEM-1 and TEM-2 enzymes are the common β-lactamases in Acinetobacter (Donald et al., 2000). Few studies strongly suggest the possible role of plasmid encoded extended spectrum β-lactamases in these bacteria for the development of resistance (Scaife et al., 1995). The plasmid-encoded penicillinases do not seem to play a significant role in acquisition of long term resistance in Acinetobacter spp. The identification of novel β-lactamase ARI-1 in an imipenem resistant strain of *A. baumannii* has recently been reported (Paton et al., 1993).
Aminoglycosides:

Aminoglycosides are widely being used for the treatment of nosocomial infections. For the last two decades there have been an increasing number of studies reporting highly resistant nosocomial bacteria for aminoglycosides and their number is also increasing in an exponential manner. All three types of aminoglycosides modifying enzymes have been identified in these strains. Recently, the aminoglycoside resistance mechanism in aminoglycoside-resistant isolates collected in a series of surveys between 1988 and 1993 were published. These studies showed a much greater incidence of complexity of aminoglycoside resistance mechanisms in all bacterial groups and also showed more than one aminoglycoside resistance mechanisms in these isolates. The complexity of aminoglycoside resistance varied with geographic region. There are geographic variations even in the incidence of resistance genes. The most complex strains were found in two regions of Latin America, Greece and Turkey. Fifty three different mechanisms were observed in the 2080 isolates of Escherichia, Morganella, Proteus, Salmonella and Shigella spp from Europe and of these 18 mechanisms accounted for 95.5% of the isolates. This increasing complexity of aminoglycoside resistance mechanism was suggested to be due to increasing complexity of aminoglycoside usage (Miller et al, 1997). In other Enterobacteriaceae, the increasing complexity of mechanisms was most often due to combinations of gentamicin-modifying enzyme with AAC(6')-I, which acetylates amikacin but not gentamicin (The Aminoglycoside Resistance Study Groups, 1995 and Miller et al, 1995). The occurrence of these combinations varied with geographical region and hospitals. The frequency of these combinations correlated with aminoglycoside usage in either the geographical region or in individual hospitals.

Unlike those in Pseudomonas spp, aminoglycoside mechanism in Acinetobacter spp were very complex. 67 aminoglycoside resistance mechanisms were noted in 1,189 isolates. The novel gene Aac(6')-I was identified in A. haemolyticus, where it concur resistance to amikacin.
Unlike those in Acinetobacter spp, aminoglycoside mechanisms in Staphylococcus spp (The Aminoglycoside Resistance study Groups, 1995) were quite simple, three single aminoglycoside resistance mechanisms were found alone and in all possible combinations 49.1% of the isolates had multiple aminoglycoside resistance mechanisms. The bifunctional aminoglycoside resistance mechanism APH(2") + AAC(6') (gentamicin, tobramycin, netilmicin, amikacin, isepamicin, dibekacin, kanamycin, fortimicin and arbekacin) was the most common (90.8%) and occurred at similar frequencies in all the surveys.

Quinolones:

The development of 4-quinolone resistance is often quite difficult to demonstrate in the clinical laboratory as in the clinical situation. Many a time it gives contradictory results. This is true with E. coli but not with non-fermentive gram negative bacteria. Resistance to fluoroquinolone is chromosomally mediated. Acquired resistance to fluoroquinolones is due to different mechanisms (Vila et al., 1996, Hooper, 1995, Piddock, 1995, Poole, 1994) involving mutations either in the structural genes governing the target of fluoroquinolones-DNA gyrase (gyrA or gyrB) or topoisomerase-IV or in the regulatory genes governing bacterial permeability or the efflux capacity of the bacteria. Very recently, a mutational alteration of a new topoisomerase, (grl A), has been noted in S. aureus, these mutants are associated with very small increase in MIC's (Kaatz et al., 1995). Permeability mutants present only in gram negative bacteria may give rise to increased MIC's of fluoroquinolones and of some unrelated antibiotics such as β-lactam drugs, tetracyclines, chloramphenicol (as in mutants of Enterobactenaceae spp or RS mutants of P. aeruginosa (Piddock, 1995, Poole, 1994).

Active efflux has been noted in S. aureus (Nor A) and many gram negative bacteria, including species of Enterobactenaceae (Mar A) and Pseudomonas spp.

Acinetobacter spp can develop quinolone resistance rapidly. However, the precise mechanism for this resistance has not been elucidated usually, gyrA
mutations that cause changes in DNA gyrase subunits in bacteria were implicated for 4-quinolone resistance development in many bacteria. PCR has been used to amplify DNA surrounding the active site region of the gyrA gene from 13 clinical isolates of A baumannii having MIC's range 0.25 to 64 mg/L for ciprofloxacin (Vila et al, 1996)

ANTIBIOTIC RESISTANCE IN ANAEROBES: For members of genus Bacteroides (Rasmussen et al, 1993, Bourgault et al 1992, Bandon et al, 1993), data shows that rates of resistance to several agents including tetracycline, erythromycin, penicillin, ampicillin and amoxicillin were already high in 1977 (Rasmussen et al, 1993). Resistance to clindamycin has increased in most countries (Bandon et al, 1993, Horn et al, 1992, Betnu et al, 1992, Goldstein et al, 1993), with particularly pronounced changes in Brazil (Cuchural et al, 1992), Japan (Aldndge et al, 1994) and Spain (Betnu et al, 1992). Resistance to cephalosporins (Rasmussen et al, 1993, Bourgault et al, 1992, Horn et al, 1992) has risen in the same countries and in Canada (Hill, 1991) and France (Mastrantonio et al, 1994). An increase in resistance to piperacillin has been noted in France (from no resistance in 1985 to 13% in 1992) (Mastrantonio et al, 1994), moreover a relatively high rate of resistance to ticarcillin and piperacillin has been documented in several other countries. The frequency of resistance to ticarcillin was reported to be high among all Bacteroides species in Canada (Hill et al, 1991) and resistance to piperacillin was highly prevalent among strains of Bacteroides thetaiotaomicron in Japan and among strains of Bacteroides ovatus and Bacteroides uniformis in the United States (Goldstein et al, 1993)

**TYPING METHODS:**

Various typing methods have been reported for establishing the sources and modes of transmission of epidemic strains in the outbreaks. The reproducibility, sensitivity, stability, availability, cost and applicability for wide range of organisms have been compared and contradicted. There appears to be no ideal typing system as each method has its strength and weaknesses.

**BIOTYPING:**

Biotyping is the characterisation of strain by biochemical and physiologic characteristics (Brenner DJ, 1978, Farmer JJ, 1978). Biochemical profiles comprise binary characteristics for comparative typing of strains. Biotyping refers to establishing the pattern of activity of ≤ 20 cellular metabolic enzymes. A commercial API TD32 Staphylococcus system, (conventional biotyping) system consisting of 16 tests, Staphylococcus-Zym and a rapid UZA method were compared using 444 consecutive strains of coagulase-negative Staphylococci (Leven et al, 1995). The API-ID 32 method, Staph-Zym and Staph-UZA method, correctly identified 419(95.2%), 429(97.5%) and 430(97.7%) and misidentified 8(1.8%), 4(0.9%) and 1(0.2%) respectively. Tenover et al, 1994 used commercial API Staphylococci identification system (Biomeneux Vitek). Differences in single enzymes have been used in biotyping Staphylococcus aureus but the discriminatory power was poor when used alone (Geyid et al, 1991, Hanifah et al, 1992). The commercial API-20NE system has been used to distinguish 31 different biotypes among 122 different Acinetobacter strains (Towne and Chopade, 1987), but this system sometimes has problems with sensitivity and reproducibility (Kropec et al, 1993). Cluster analysis of carbon source growth assays has been used to identify major clusters of isolates related to the epidemiological origin of the strains (Dijkstra et al, 1993). This has been used to identify *E coli* 0157 H7 strain that was not detected among other D-Sorbitrate negative strains (Abbott et al, 1994). Biochemical methods used in identification of the family Enterobactenaceae were those of Farmer et al, 1985 and Brenner, 1984.
A biotyping system consisting of 5 tests (Bouvet et al., 1990; Gerner-Smidt, 1993) devised originally for dividing isolates of *A. baumannii* into 19 biotypes, and has been used to type closely related genomic species of *A. baumannii* such as genospecies 3 and 13 TU in various hospital outbreaks of infection (Bouvet et al., 1990; Gerner-Smidt and Tjernberg, 1993).

**ANTIBIOTYPING:**

Numerous studies have used antibiotic susceptibility pattern (antibiograms) to detect emerging resistance patterns and to group similar isolates (Tenover et al., 1994; Biendo et al., 1997; Go et al., 1994). Actual diameter of inhibition zones in disk diffusion tests for cluster analysis have been used. Such groupings have been shown to correlate well with other typing and epidemiological data (Dijkshoorn et al., 1993). However, it must be emphasized that antibiogram typing results should be interpreted with caution, since unrelated strains may exhibit the same antibiogram and changes in susceptibility may occur during episodes of infection (Joly-Guillou and Bergogne-Berezin, 1990).

**SEROTYPING:**

Serotyping has been applied to numerous nosocomial species including *S. aureus, E. coli, Proteus spp, Serratia spp, Acinetobacter spp* (Maslow et al., 1993). Serotyping was first described for *P. vulgaris* and *P. mirabilis* with 49 O antigens and 19 H antigens.

*E. coli* comprises many serotypes based on combinations of O (cell-wall-lipopolysaccharide), H (flagellar protein) and K (capsular polysaccharide or envelope) antigens. Antisera raised against 173 O antigens, 56 H antigens and more than 100 K antigens are currently in use (Ewing, 1986). Identification of group specific heat stable lipopolysaccharide antigens by agglutination form the basis of O serotyping in *P. aeruginosa* (Pitt, 1988).
Serotyping differentiates epidemiological strains of *K. pneumoniae* (Gaston et al., 1987). An analysis of *K. pneumoniae* isolated from blood showed that K2 (8.9% of isolates), K21 (7.8%) and K55 (4.8%) were the commonest serotypes and that 25 serotypes accounted for 70% of the isolates (Cryz et al., 1986). In a UK survey, K21 isolates (42% of isolates and K2 (16.5%) were commonest isolates from nosocomial infections (Casewell and Talshafter, 1979).

The international antigenic typing scheme (IATS) uses 12 O groups for *P. aeruginosa*. Serotyping is a reliable marker of Staphylococcal strains because of the stability of the antigens. Three major serotypes I, II, and III of *S. aureus* are distinguished by slide agglutination in different epidemiological studies (Cowan, 1939).

There have been various attempts to type Acinetobacter strains by serological methods (Singh et al., 1983), but only limited success was obtained in early work. Recent study has successfully used serotyping for typing 1 genomic spp, 13 TU they were able to differentiate these isolates from that of *A. baumannii* strains (Pantophlet et al., 1999).

**PHAGE TYPING:**

Phage typing based on geographical distribution have been used to type *S. aureus*. Phage group III was the predominant phage group identified in different studies (Agarwal et al., 1980, Archer et al., 1983, Struelens et al., 1992, Tenover et al., 1994, Udayashankar et al., 1997). A substantial proportion of strains from different geographical areas have been shown to be untypeable. In a recent multicentre study reported from 6 countries in Europe, 42 experimental phages were used against 744 isolates of MRSA with the intention of defining a phage set to augment the international set. The use of these experimental phages increased the percentage typeability from 75% with the international set to 93% and the number of identifiable lytic patterns from 192 to 424 (Richardson et al., 1999).
number of systems of bacteriophage typing are available for Proteus spp (Schmid and Jeffnes, 1974)

Xiaoqing and Ruonanpan (1992) developed bacteriophage lytic patterns for identification of Salmonellae, Shigellae, *E. coli*, *Citrobacter freundii* and *Enterobacter cloacae* for routine use in public hygiene and clinical laboratories. In their study, after 20,280 cultures of 27 spp and 9 biogroups of 15 genera of the family Enterobacteraeaceae and 276 cultures of 8 spp of 6 genera outside Enterobacteraeaceae were tested, it was shown that most strains of Salmonellae, *E. coli*, *C. freundii* and *E. cloacae* can be identified accurately. The sensitivities of identification were 83.6% for *E. cloacae*, 88.8% for *C. freundii*, 90.3% for *E. coli* and 95.76% for Salmonellae. The specificities were 99.78% for Salmonella, 99.84% for *E. cloacae*, 99.89% for *E. coli* and 99.97% for *C. freundii* (Xiaoqing and Ruonan, 1992). For *E. coli* the phage typing scheme of Ahmed et al. (1987) and extended by Khakhria et al. (1984) is being used in different studies (Ratnam et al., 1988, Griff et al., 1998). The scheme comprises 16 bacteriophages and recognizes 88 phage types (Khakhria, 1984).

Two complementary sets of bacteriophages have been used in a number of studies of *Acinetobacter* strains from France and other European countries (Bouvet et al., 1990, Buisson et al., 1990). One comprises 25 phages, allowing the identification of 125 phage types and the other where in 14 phages were used allowing the identification of 25 phage types. Phage type numbers 17 and 124 were the predominant phage types identified in some outbreaks.

**BACTERIOCIN TYPING:**

Typing of *P. aeruginosa* based on pyocin production is the most popular method (Pitt, 1988). Pyocin typing offers greater discriminating power than serotyping whilst retaining simplicity and reliability (Pitt, 1988). A number of methods have been described (Lanyi and Bergan, 1978, Brokopp and Farmer,
1979) of which the method described by Govan and Gilles (1969) has been found useful (Govan, 1978)

Proteocin typing for Proteus spp was done by Al-Jumaili (1975) employing 12 standard proteocine producing strains. In a study conducted from Pondicherry, India, Mary et al (1996) typed nosocomial Proteus isolates obtained from postoperative surgical wounds. Fifty one (63.8%) of the strains could be divided into 22 different proteocine sensitivity patterns.

Limited bactenocin typing of Acinetobacter isolates has been published. In a study, 176 strains were typed by means of ten indicator strains that were susceptible to bactenocins. Overall typeability in a study was 65%, of which 56% belonged to 2 groups (Andrews, 1986).

MULTILOCUS ENZYME ELECTROPHORESIS TYPING:

Multilocus enzyme electrophoresis investigates the relative electrophoretic mobilities of a large number of cellular enzymes (Selander et al, 1986). Selander and co-workers in particular have used this technique to characterize clonal populations in numerous pathogenic micro-organisms including E. coli (Selander et al, 1986). Among E. coli, isolates cultured from patients with pyelonephritis represented a limited number of closely related electrophoretic types (Arthur et al, 1990).

Tenover et al (1994) used multilocus enzyme electrophoresis (MLEE) as one of phenotypic tests to compare strains of S. aureus isolated from an outbreak. In this study, 59 isolates of S. aureus were typed by 72 methods, 12 enzymes were used for performing MLEE. MLEE correctly identified 26 of 29 outbreak related isolates, but included 4 additional isolates in the clusters.

In another study, investigations of 27 esterases and 2 dehydrogenases in 81 Acinetobacter isolates identified between 9 and 17 variants for each enzyme.
(Picard et al, 1989) A separate study of 13 enzymes in 65 Acinetobacter clinical isolates identified 14 different types, of which one was found in 41 multiresistant isolates with common whole cell protein profiles (Thurm and Ritters, 1993)

**Plasmid profiles:**

Numerous studies have shown plasmid typing as a rapid and simple method for typing nosocomial bacteria (Melo Cnistine et al, 1989, Garcia et al, 1989, Hebert et al, 1988, Khatib et al, 1995)

The plasmids found in gram positive and gram negative bacilli vary considerably in both size and number. In a study, all 43 isolates of MRSA, which were tested had definable plasmids varying in size from 15 to 36 megadaltons (Mda). An average of 1.77 plasmids per isolate was found in the study (Licitra et al, 1989). In another study, Archer et al (1983) have compared different epidemiological markers to investigate an outbreak of MRSA infection. In this study, all the tested epidemic multi-drug resistant S aureus cells contained 3 plasmids approximately 34, 18 and 18 megadaltons in molecular size. Plasmid pattern analysis yielded a unique fingerprint which distinguished the epidemic strains. From all indigenous isolates plasmid pattern analysis is a promising epidemiological tool for nosocomial outbreaks. Numerous studies have shown that plasmid profile analysis is a promising technique in identifying the source of epidemic P aeruginosa and E coli, coagulase negative Staphylococci and S aureus strains in the hospital (Zuccarelli et al, 1990, Melo Cnistine et al, 1989, Garcia et al, 1989, Ratnam et al, 1988, Khatib et al, 1995)

Analysis of plasmid profiles has been useful in delineating several outbreaks of Acinetobacter infections (Kropec et al, 1993) strains with similar plasmid profiles have been differentiated further either by restriction endonuclease digestion of plasmid DNA to generate plasmid fingerprints (Kropec et al, 1993) or by hybridization of plasmids (a labeled probe from one of the strains (Gemer-Smidt et al, 1993)
Restriction endonuclease analysis (REA):

Examination of chromosomal DNA is useful for those organisms that do not contain plasmids or for organisms in which the plasmid content is unstable (Hobranska et al., 1990). Numerous studies have shown REA as a rapid and reliable method for typing nosocomial bacteria (Harstein et al., 1990, Zuccarelli et al., 1990, Meher et al., 1993, Blanc et al., 1993, Ratnam et al., 1988, Khatib et al., 1995). Zuccarelli et al. (1990) evaluated restriction enzyme analysis of plasmid DNA for use as an epidemiological marker of MRSA strains. 120 clinical and environmental MRSA isolates showed 37 distinct EcoR1 digestion patterns. 42% strains lacked plasmids, 12.5% isolates contained 2 or more plasmids.

Harstein et al. (1990) have used REAP for typing paired S. aureus blood culture isolates and out of 50 pairs of S. aureus blood isolates, 17 pairs did not have detectable plasmids. Isolates from 33 pairs had plasmids classified into 17 distinct REAP DNA profiles. Paired isolates from 31 of these episodes were identical to one another. Struelens et al. (1992) evaluated the usefulness of serotypic analysis for epidemiologic typing of MRSA. In their study REA of genomic DNA showed restriction fragments of 2.5 to 6 Kb and 7 restriction patterns were found. The reproducibility of REA was 100% and its discriminative index was 0.95%.

REA of genomic DNA has been used for characterization of 17 strains of E. coli serotype O157 H7 representing human isolates obtained from outbreaks and sporadic cases of hemorrhagic colitis, hemolytic uremic syndrome and non bloody diarrhoeal illnesses as well as from asymptomatic carriers.

Takahashi et al. (1984) have identified a rapid procedure for isolation of purified plasmid DNA which was used directly for restriction endonuclease analysis for isolates of E. coli, K. pneumoniae, Citrobacter spp., Klebsiella oxytoca, Proteus vulgaris, Providentia fetigiers, Morganella morgagnii, P. aeruginosa, H. influenzae and S. aureus.
Analysis by pulsed-field gel electrophoresis:

Pulsed field gel electrophoresis (PFGE) is often considered the "gold standard" of molecular typing methods. PFGE has proven to be superior to most other methods of biochemical and molecular typing. It is highly discriminatory and superior to most methods for analysis of E. coli, vancomycin-resistant Enterococci, S. aureus, Acinetobacter spp, P. aeruginosa and M. avium, Enterobacter spp, Klebsiella spp (Barbeer et al, 1996, Grundmann et al, 1995, Maslow et al, 1993).

Analysis by pulsed field gel electrophoresis (PFGE) of restriction fragment length polymorphisms generated from intact chromosomal DNA has been used to compare fingerprints obtained from P. aeruginosa, S. aureus, E. coli, Enterobacter cloacae and Acinetobacter spp strains following restriction with Smal (Tenover et al, 1994), XbaI (Gnff et al, 1998), NotI, XbaI (Haertl et al, 1993) and ApaI (Seifert et al, 1994, Tankovic et al, 1994). Smal (Allardet-Servent et al, 1989), ApaI, Smal (Gouby et al, 1992) and NheI and Smal (Struelens et al, 1992). These studies have indicated considerable DNA polymorphism in the clinically important genomic species even with good discriminatory power.

Ribotyping:

Ribotyping, a variation of RFLP Southern blotting in which the probes are derived from the 16S and 23S rRNA genes has been applied successfully in many studies in differentiating bacterial strains (Dijkshoorn et al, 1993, Gerner-Smidt, 1992, Koopman et al, 1993).

In a study, restriction fragment length polymorphism of ribosomal DNA regions (ribotyping of P. aeruginosa was evaluated as a tool for epidemiological purposes. Purified chromosomal DNA is digested with restriction enzymes, electrophoresed, blotted and then hybridized with a plasmid pKK3535 containing an rDNA operator of E. coli. Patterns generated by restriction with BamHI, ClaI, EcoRI.
and PsT I have been used to investigate 55 strains which were classified into 33
ribotypes (Blanc et al, 1993)

Tenover et al (1994) have compared 12 typing techniques for S aureus
strains using ribotyping patterns generated by restriction with Cla I and Hind III have
been used to investigate 29 outbreak related strains In a recent study, Gniff et al
(1998) have used automated ribotyping technique for epidemiological typing of
E coli strains

Gerner-Smidt (1992) have used ribotyping for identifying Acinetobacter spp
from 70 strains Excellent reproducibility was observed with combined use of 3
enzymes which generated 55 different types among 70 unrelated strains studied
Ribotype patterns within outbreaks have been shown to be stable and correlated
with the results obtained by other typing methods (Dijkshoorn et al, 1993) Diversity
of ribotypes within the A calcoaceticus, A baumanii complex is considerable
Common pattern in apparently unrelated strains have also been observed
(Dijkshoorn et al, 1993, Gerner-Smidt, 1992)

Polymerase chain reaction-based methods;

Finger printing on the basis of PCR amplification of DNA sequences by
specific and random primers is increasingly used now Two sets of PCR primers,
namely REP and ERIC are used for typing purposes REP sequences have been
described for numerous enteric bacteria (Grundman et al, 1995, Hulton et al, 1991)

ERIC sequences have been defined primarily based on sequence data
obtained from E coli (Hulton et al, 1991) REP-PCR is fast becoming the most
widely used method of DNA typing REP-PCR with primers based on REP and
ERIC sequences has been successfully used to differentiate strains of Citrobacter
diversus (Harvey et al, 1995), methicillin resistant S aureus (Delveechio et al, 1995),
A baumanii (Dijkshoorn et al, 1996), the core region of bacteriophage M13 has
been used as single primer to determine the relatedness of A baumanii strains
Repetitive elements such as REP-1 and REP-2 combined generated PCR fingerprints that demonstrated four discrete clusters that were unique epidemiologically (Reboli et al., 1994) Arbitrarily primed PCR (AP-PCR) and ribotyping were compared in an investigation to detect outbreaks (Vila et al., 1996) It was concurred that ribotyping and AP-PCR exhibited similar discriminatory power, however, AP-PCR had an additional advantage of speed and simplicity

Studies have compared arbitrarily primed PCR (AP-PCR) and FIGE, MCEE, PFGE, Ribotyping for epidemiological studies of *S. aureus*, *P. aeruginosa*, *E. coli* isolates (Olmos et al., 1998, Tenover et al., 1994, Elaichouni et al., 1994, Stacy-Phipps et al., 1995, Griff et al., 1998) The optimization of an arbitrarily primed PCR method for typing 96 MRSA isolates was compared with PFGE Identical results in the differentiation of MRSA clones and identification of the mass cluster that included 82 strains (88% of patients) were obtained by both techniques (Olmos et al., 1998) Tenover et al. (1994) typed 59 outbreak strains of *S. aureus* by using either antiobigrams, bacterophage typing, biotyping, immunoblotting, insertion sequence typing with IS257/431, multilocus enzyme electrophoresis, restriction analysis of plasmid DNA, pulsed field or field inversion gel electrophoresis, restriction analysis of PCR-amplified coagulase gene sequences restriction fragment length polymorphism typing by using four staphylococcal genes as probes or ribotyping Overall the DNA based techniques and immunoblotting were most effective in grouping outbreak related strains recognizing 27 to 29 of the 29 outbreak-related strains Elaichouni et al. (1994) typed a total of 18 outbreak strains of *P. aeruginosa* by Arbitrary primer (AP) PCR (performed independently with 3 different primers) and compared with serotyping, phage typing and antibiotic susceptibility testing The different AP PCR typing systems yielded almost identical patterns for the epidemic strains and enabled them to differentiate most of the nonrelated strains from each other and from the outbreak strains and typeability of AP PCR was greater than those of phage typing and serotyping Griff et al. (1998) reported that a set of 47 Australian human, food and veterinary *E. coli* 0157, H7 isolates were evaluated by using five different epidemiological typing methods
(Ribotyping, phage typing, RAPD-PCR, RAPD-ALFA and PFGE) Ribotyping, RAPD PCR methods recognized only 2 clusters. Both dendograms grouped most of the EHEC 0157 isolates into epidemiologically related subgroups. And authors concluded major differences can exist between results of multiple subtyping methods. *E. coli* 0157 isolates should not be classified as epidemiologically related or nonrelated on the basis of a single typing method alone. A multicentric study recently evaluated the reproducibility of PCR-based fingerprinting of Acinetobacter spp. Using standard protocols and reagents (Grundmann et al., 1997) in 7 laboratories of six European countries (96.4% of total isolate grouping was observed).