Discussion
DISCUSSION

INTRODUCTION

One of the most daunting concerns in modern medical practice is the problem of nosocomial infections and associated efforts to monitor and control it. Over the decades, the epidemiological analysis of hospital acquired infections (HAI) has undergone remarkable changes. While previously, scientists relied purely on phenotypic characterization, recent years have seen a variety of approaches to epidemiological analysis, including developments in molecular biology culminating in the advent of what is termed as "molecular epidemiology". In the present investigation, an attempt was made to study some of the bacterial agents associated with HAI with particular reference to postoperative wound infection.

Prevalence of hospital acquired infection in JIPMER:

In the first phase of the study, patients who developed HAI after their admission in medical and surgical specialities of JIPMER hospital were screened. This was done for a period of one year with the specific objective of finding out the prevalence and predominant types of HAI in the different specialities of the hospital and to monitor the common pathogens associated with it. A survey of 27,872 patients yielded 1,596 cases of HAI giving a prevalence rate of 5.73%.

This was within the range of 1.7 to 9% reported in different hospital prevalence surveys from India, Hongkong, Europe, Australia, USA, Spain, Italy (Mandal et al, 1997; Kam and Mak, 1993; Emmerson, 1996; Aavitsland et al, 1992; Murphy et al, 2000; EPINE, 1995; Pavia et al, 2000). However, higher prevalence rates compared to the present study have been reported from Great Britain and Ireland (9.0%) (Emmerson et al, 1996), Denmark (12%) (Jepsen et al, 1982), Italy (6.8%) (Moro et al, 1986), Czechoslovakia (6.1%) (Sramova et al, 1988), Spain (7.2%) (EPINE, 1995) and Hong Kong (8.6%) (Kam and Mak, 1993). The low prevalence of HAI (5.73%) in JIPMER hospital was explained by strict monitoring of
sterilization and disinfectant efficacy, close monitoring and isolation of infected patients, regular evaluation of infection control measures, active participation of infection control committee, regulation of antimicrobial usage, recognition of the emergence of resistant strains, compounding an isolation policy and bringing in improvement in sanitation. These are the measures undertaken to keep the overall prevalence of HAI in the hospital low.

Many prevalence surveys show that HAI is more frequent in surgical wards than medical wards (Britt et al, 1986; Sramova et al, 1988). The present study also showed that the prevalence of HAI among surgical specialities was high (6.2%) as compared to the medical specialities (4.8%). But the prevalence rates varied among surgical specialities and were influenced by age, sex and time of hospitalization. In the present study, the prevalence rate of HAI was high in orthopaedic surgery (12.4%), general surgery (10.2%) and urology (7.98%). Similar results were observed by Moro et al (1986). In the present study, the HAI prevalence was seen to be high in the younger age group (< 1 yr, 11.5%) and the elderly (65 yrs, 8.4%). These observations were similar to a survey reported from Italy (Moro et al, 1986).

Among the medical specialities, the present study showed a high prevalence of HAI among the patients admitted in the intensive care unit (ICU). However, the number of patients surveyed in the ICU were fewer. The high infection rate in ICU could be due to the fact that it was mainly patients who developed complications that were monitored in this unit.

Consistent with other studies [National Nosocomial Infections and Surveillance (NNIS, 1996; Sramova et al, 1988; Scheel et al, 1999)] the present study found that the most frequent site of HAI was the urinary tract 30.1% followed by surgical wounds 27.6%, respiratory tract 15.8%, bacteremia 12.1% and 14.4% constituted others.
Consistent with data from Europe and USA (Fluit et al., 2001; NNIS data, 1996), the most common pathogen isolated from any nosocomial infection in this study was *E. coli* (20.5%) followed by *S. aureus* (17.2%) and *P. aeruginosa* (15.8%).

The sentry data from North America (Gales et al., 2000) and Latin America (Doern et al., 1999) showed that *S. aureus* was the most important cause of blood stream infections (23% and 20.5% of isolates respectively), whereas this organism ranked 2nd in the present study. In contrast to the studies from Europe and Latin America, in the present study, *S. epidermidis*, *S. aureus* and *E. coli* were the most important bacteria causing blood stream infections in that order. These results were similar to the most recent studies from U.S. hospitals (Edmond et al., 1999), in which these organisms have been the leading pathogens causing blood stream infections. *E. coli* was the most important cause of blood stream infections in Europe (Fluit et al., 2001), but it ranked second with 18.7% of isolates in North America (Doern et al., 1999) and third in Latin America (Gales et al., 2000). Consistent with Latin American study, in the present study, *E. coli* ranked 3rd with 12.1% of isolates as causative agent for BSI (Gales et al., 2000). In the present study, *P. aeruginosa* and *K. pneumoniae* were the next most common isolates causing blood stream infections with 9.2% and 8.1% of isolates respectively. This was in concordance with the findings of the study from Europe (Fluit, 2001).

In the present study, *S. aureus* (24.1%), *P. aeruginosa* (16.5%) and *K. pneumoniae* (10.5%) ranked as the top 3 causative agents of bacterial nosocomial pneumonia, similar to the rates seen in the NNIS systems Report 1999 (CDC, 1999).

Comparison of the frequency of isolation of bacterial pathogens involved in postoperative wound infections in the Latin America with the data presented here showed a similar ranking and frequency of isolation for the top 4 organisms (*S. aureus* 26%, *E. coli* 24%, *P. aeruginosa* 17.3%, *E. faecalis* 8.5%) and some minor differences for the next four genera.
*E. coli* was the most important cause of UTI in the present study followed by Klebsiella pneumoniae, *P. aeruginosa* and *E. faecalis*. This data was consistent with the Latin American data (Gales et al, 2000).

**Antimicrobial susceptibility testing:**

Bacteria present in hospital environment are more resistant to antibiotics and infections caused by them are difficult to treat. Their resistance development is influenced by the frequency of antibiotic in use. In the present study, high levels of resistance was observed among postoperative wound and UTI isolates compared to pneumonia and blood stream isolates. Similar resistance patterns were observed by Fluit et al (2000). In the present study, high levels of resistance was recorded against ampicillin (91% to 100%) by *E. coli*, *P. mirabilis*, *K. oxytoca*, *Enterobacter cloacae*, and *S. aureus*. *S. epidermidis* and *A. baumannii* too showed relatively high resistance of 50% and 84.4% to ampicillin respectively.

Percentage of susceptibility of different pathogens to gentamicin in the present study was low: *E. coli* 55%, *K. pneumoniae* 20%, *P. mirabilis* 10%, *K. oxytoca* (29%), *Enterobacter cloacae* 15.3% and *A. baumannii* (37.5%), *P. aeruginosa* (57%) and *S. aureus* (54%). Overall percentage of resistance to ampicillin and gentamicin in this hospital is high. This finding is explained by the fact that ampicillin and gentamicin are the most extensively used antibiotics in this hospital (both in surgical and medical specialities). Similar resistance patterns were also observed in earlier surveys (Anvikar et al, 1999) highlighting that all hospital pathogens are increasingly becoming resistant to commonly used antibiotics.

A study done by Rolinski et al (1994) showed a very high level of resistance by *E. coli* (61 and 97%) to ampicillin and oxytetracycline. Also a high level of resistance of *P. aeruginosa* to chloramphenicol and among their *S. aureus* isolates, 81% were resistant to erythromycin and 64% to chloramphenicol. Similar to study by Rolinski et al (1994), in the present study, resistance to chloramphenicol was
high, viz. \textit{E.coli} 56.6\% \textit{K.pneumoniae} 77.2\%, \textit{P.mirabilis} (40\%), \textit{K.oxytoca} (47\%), Enterobacter 74.6\%, \textit{A.baumanni} 57.8\% and \textit{P.aeruginosa} 71.3\% respectively.

The percentage of resistance to cefotaxime in the present study was higher among non-fermenters like \textit{A.baumanni} 56.2\% and \textit{P.aeruginosa} 86.5\% compared to Enterobacteriaceae members ranging between 15-45\%. A higher percentage of resistance was shown by \textit{E.cloaceae} 45\% to cefotaxime, as compared to other Enterobacteriaceae members. Gram positive cocci too showed higher percentage of resistance to cefotaxime (41.4\% and 51.1\%).

In this study, results of susceptibility testing of cefotaxime reveal that the resistance of bacteria to this drug is on the rise in the hospital. The reason is that cefotaxime has become one of the most widely used cephalosporin in JIPMER hospital in recent times. These findings were similar to the findings of Shulman et al (1971). The authors of this study have reported that excessive use of the antimicrobial had dramatically increased isolation rates of resistant microorganisms and vice versa.

In this study, low percentage of resistance was observed to ciprofloxacin by all Enterobacteriaceae members (9\% to 28\%) and gram positive cocci (26.2\% to 48.6\%) as compared to non-fermenters \textit{A.baumannii} and \textit{P.aeruginosa} (resistance of 48.6\% and 51.4\%). These findings were similar to the findings of Ojha et al (1997).

In concordance with earlier studies (Ojha et al, 1997), the present study showed a 100\% susceptibility of gram positive cocci to vancomycin. In contrast to the earlier studies (Eykyn SII, 1996), the authors found that penicillin resistance of \textit{S.aureus} was 100\% and that of coagulase negative Staphylococci was 48.4\%. Erythromycin susceptibility to \textit{S.aureus} was 54\%. Consistent with earlier studies (Archibald et al, 1997), in the present study methicillin resistance in \textit{S.aureus} and coagulase negative Staphylococci was 32.9\% and 34\%. In the present study, all the nosocomial isolates showed a higher resistance to all the commonly used
antibiotics which is of much concern as infections caused by them pose great therapeutic problem. It is surprising that the level of resistance developed to ciprofloxacin and cefotaxime was moderate even though they were the commonest antibiotics used in this hospital.

Environmental surveillance:

Environmental surveillance conducted in the most important areas in this hospital OTs, ICUs, postoperative wards and other wards revealed that the air in these areas was unsatisfactory and S.aureus and coagulase negative Staphylococci were the aerobic bacteria isolated. Disinfectants and solutions used in the above mentioned areas were found to yield E.coli, P.aeruginosa, Klebsiella and Proteus species. Sixty one percent of the staff working in these areas were nasal carriers of S.aureus. All these findings were similar to those of Thakur et al (1990).

A knowledge of the predominant pathogen, site and source of infection and high risk factors along with regular patient oriented surveillance will help in setting up general or specific infections control policies (Moore, 1974; Shoji et al, 1974). In concordance with earlier studies (Mandal et al, 1997; Murphy et al, 2000) the present study also found that the continuous method of surveillance consumed more time (11 hours / 100 bed/week) than a laboratory based selective surveillance (6 hours / 100 bed/week). The latter method was found to be less time consuming and more suitable to this setup.

Phase II:

Based on the preliminary results obtained from phase I study, the second phase of the study was confined to one unit of general surgery department. Aerobic and anaerobic bacteria causing the wound infection in all types of surgeries were characterized. Environmental surveillance was carried out in the OTs where these patients were operated and the wards in which they were admitted. A study of the normal flora of the patients who developed postoperative wound infection,
surgeons and others health care personnel who came in contact with these patients was also carried out. Predominant bacteria isolated from patients, environment and health care personnel were correlated and compared using typing methods to find out the source of infection in these cases.

In the present study, during a 20 month period, 1282 operations were performed, of which 116 were complicated by surgical wound infections giving a wound infection rate of 9.04%. The wound infection rate varies from hospital to hospital and from department to department in the same hospital. Values between 2 and 20% are commonly reported (Nichols, 1990; Oni et al, 1997; Santos et al, 1997; Cestari et al, 1999; Smyth, 2000; Choojitr et al, 1995; Kotisso et al, 1998). Differences in the infection rate could be due to a number of factors including type and duration of the operation, therapeutic factors, number and type of microorganisms present in the wound, age and health condition of the patient as well as the hygienic condition. The infection rate reported in this unit of surgery was within the range reported in earlier studies. Based on the findings of an earlier study (Mangram et al, 1999; Weigelt et al, 1992), the majority of surgical wound infections are detected after patients were discharged from hospital. In the present study, followup study was not done for those postoperative patients who were discharged from this hospital.

Earlier studies (Cruse et al, 1981) showed that advanced age of the patients, prolonged postoperative hospitalization were associated with an increase in the infection rate of surgical wounds. In this study, the mean age of the patients was 57.95 ± 14.1 years, length of postoperative stay in the hospital ranged from 0 to >21 days (Median 9 days) which could have influenced the results. Swaltz et al (1980) at Virginia University Hospital (unpublished data) found that the patients with postoperative wound infection stayed in the hospital for an average of 23.87 extra days. Similar findings were observed in the present study. Mean length of stay in the hospital for postoperative wound infected patients was 29.7 ± 2.6 days as compared to 6.4 ± 1.0 days for uninfected patients.
Current rates for wound infections classified by the risk of contamination in the NNIS series (Culver et al, 1992) were 2.1%, 3.3%, 6.4% and 7.1% respectively for clean surgery, clean-contaminated, contaminated and dirty surgeries. In contrast, in the present study, the wound infection rates obtained for the risk of contamination of surgery were 6.7%, 5.5%, 12.1% and 15.5% for clean, clean-contaminated, contaminated and dirty surgeries respectively.

The type of operation was one of the major factors influencing the frequency of postoperative wound infection in the reports by Jepsen et al (1969), whereby an abdominal surgery was associated with an increased risk of wound infection. Data from 58,498 patients randomly selected from patients undergoing operations in 338 hospitals in USA (Haley, 1980) also revealed that abdominal site of operation was a stronger predictor of wound infection.

One explanation for the overall high rate of wound infection in this study may be that it included a large number of abdominal surgical wounds. The profound influence of endogenous contamination was evident from the analysis of wound categories, in which the rate of contaminated and dirty surgical wounds is enormously high as compared to clean and clean-contaminated surgical wounds. Endogenous contamination remains the main risk factor for development of surgical wound infection because of the large dose of the organism available from the bowel and other hollow muscular organs. Another reason could be the poor socioeconomic status and hygiene of patients in developing countries like India. In addition, the quality of the patient care can also influence the rates of wound infection.

In the present study, though all wound infected patients were healthier than those of earlier studies, one or more of the following factors may be the reason for high percentage of wound infection. Whether or not a wound gets infected following a surgical procedure depends on the interaction between the host, microbes, the
operation environment related factors and surgical techniques (Emmerson et al, 1998). Care exercise in tissue handling, removal of devitalized tissue, hemostasis and wound closure without tension are also most important factors (Emmerson et al, 1998; Altemier et al, 1984). The surgeon may be an important modulator for the patient (Holzheimer et al, 1997). Local and systemic host defences can be enhanced or suppressed by the surgeon (Windsor et al, 1995). Inappropriate timing (>2 hours before operation) of antimicrobial prophylaxis and alcohol abuse were shown to be a risk factor for the development of surgical wound infection (Classen, 1992; Lizan-Garcia et al, 1997; Rantala et al, 1997).

The rate of surgical wound infection is directly related to the duration of surgery (Cruse, 1992; Garibaldi et al, 1991). Similar results were observed in the present study (p < 0.01). In the study conducted by Cruse and Foord (1973), the infection rate in emergency surgeries was double the rate in elective surgeries. Similar findings were observed in the present study (p < 0.01).

Surgical wound infections cause almost 2% of all deaths in the United States (Mayhall et al, 1992). In the present study, a mortality rate of 2.5% was observed.

Aerobic bacterial isolation:

In contrast to earlier studies (Oni et al, 1997) in this study, the ratio of gram negative organisms to gram positive organisms in wound infection isolates was around 2:1. A total of 170 strains were isolated from 116 surgical wounds in this study. The leading isolates were E.coli (20.6%), S.aureus (17.7%) and P.aeruginosa (14.7%). This was in accordance with the findings of earlier studies (Kotisso et al, 1998). S.aureus was the main isolate from clean wounds while E.coli was most frequently isolated from contaminated and dirty wounds. This was again in agreement with earlier reports (Rabaa et al, 1987; Kotisso et al, 1998).
Anaerobic culture results:

Findings from the study of anaerobic bacteria in 24 of 116 (20.7%) postoperative wound infections in the present study revealed that no single case was found to be having anaerobic bacteria as the sole causative agent of postoperative wound infection. Similar findings were reported in earlier studies (Chatterjee et al, 1995; Okropiridze et al, 1996). In contrast, Brook (1989) and Brook and Frazier et al (1990) have reported anaerobic bacteria as the sole causative agent of 12% and 36% of postoperative wound infections respectively. In the present study, all 24 cases (20.69%) were found to contain mixture of aerobes and anaerobes.

A total of 30 anaerobic isolates were obtained from contaminated and dirty surgeries (duodenal ulcer with perforation, acute appendicitis, hepatic abscess and carcinoma of the rectum) in this study. Also, gram positive anaerobic isolates were common than gram negative isolates. No anaerobic bacteria were isolated from clean surgeries.

In the present study, microaerophilic Streptococci were the predominant anaerobic bacteria isolated followed by Bacteroides fragilis group, Peptostreptococci, Clostridium perfringens, Bacteroides melaninogenicus and Peptococcus. Earlier studies have shown Bacteroides spp, Clostridium spp, Fusobacterium spp, Peptostreptococcus and Peptococcus as the cause of surgical infections (Okropiridze et al, 1996; Brook, 1990). In contrast, none of the above studies showed microaerophilic streptococci as the predominant anaerobic bacteria isolated from postoperative wound infection. However, it was the predominant anaerobic bacteria isolated in the present study.

Antibiotic susceptibility of anaerobic bacteria:

Consistent with data from earlier studies (Giacometti et al, 2000) in the present study all anaerobic cocci were 100% susceptible to vancomycin. The present study found that 22% and 8% of microaerophilic streptococci, 20% and 5%
of peptostreptococci and 2% and 8% of *Bacteroides fragilis* group were resistant to metronidazole and clindamycin respectively in agreement with earlier studies (Chen et al, 1992; Watanabe et al, 1992). Two percent of the *Bacteroides fragilis* isolates were found to be resistant to metronidazole. Clindamycin and metronidazole are both quite active against *B. fragilis* group, although a frequency of resistance to clindamycin of up to 25% has been reported from Europe and Japan (Betriu et al, 1992; Watanabe et al, 1992). In contrast in the present study only 8% of the *B. fragilis* group were resistant to clindamycin. In this hospital metronidazole is the most widely used antimicrobial agent for anaerobic infections and a common prophylactic agent for abdominal surgeries. In spite of increase in metronidazole consumption there is no emergence of drug resistant anaerobic bacteria in this setup.

**Antimicrobial susceptibility testing of aerobic bacteria:**

In the present study almost all the strains of *S. aureus* tested were resistant to ampicillin and penicillin. Methicillin resistance was documented in 41.3% of *S. aureus* isolates. Although ciprofloxacin, cefotaxime and gentamicin were shown to be active in vitro against 56% of the isolates, according to National Committee for Clinical Laboratory Standards recommendations the methicillin resistant *Staphylococci* were considered resistant to all β lactams including all penicillins, cephalosporins, β-lactam-β-lactamase inhibitor combinations and carbapenems. Since these agents may be clinically ineffective against such organisms. Methicillin resistance was reported among as many as 55% of the *S. aureus* strains isolated from postoperative wound infections (Giacometti et al, 2000) in contrast to earlier studies. In the present study, 41.3% of *S. aureus* isolates tested were resistant to methicillin. In consistent with earlier studies (Giacometti et al, 2000) no *S. aureus* isolates in the present study showed resistance to vancomycin. In agreement with earlier studies (Rolinski et al, 1994; Abussaud, 1996) in the present study 62.5% of *S. aureus* isolates were resistant to erythromycin and 55% to chloramphenicol.
In consistent with the earlier studies (Abussaud, 1996; Rolinski et al, 1994) in the present study more than 60% of the *E.coli* strains were resistant to chloramphenicol. However, in this study resistance rates to β-lactams (Ceftazidime, Cefuroxime and Cefotaxime) was high and ranged from 12 to 32.4%. These resistance frequencies exceeded those reported earlier for broad spectrum cephalosporins. Only a few *E.coli* strains (<10%) were resistant to the quinolones. Ofloxacin showed 100% susceptibility and ciprofloxacin 88%. This finding is in contrast with the findings of earlier studies (Ojha et al, 1997) which showed 36% susceptibility to ciprofloxacin. Ofloxacin was the most active quinolone tested inhibiting 100% of *E.coli* isolates. In this study the *E.coli* isolated showed overall high susceptibility to amikacin (90%) of all aminoglycosides tested. This is in contrast to the earlier studies (Ojha et al, 1997) in which *E.coli* isolates showed 100% susceptibility to amikacin. For *E.coli*, aminoglycosides and cephalosporins still remain the drugs of choice but resistance to drugs is on the rise.

In agreement with earlier studies (Fluit et al, 2000) this study shows that *P.aeruginosa* isolates were highly resistant to cephalosporins, 100%, 92.1% and 30.3% were the percentages of resistance shown by cefuroxime, cefotaxime and ceftazidime respectively. In the present study, overall susceptibility was highest to amikacin (88%) of all aminoglycosides tested and that of gentamicin was 47%. Fluit et al (2000) have reported similar results with high susceptibility to amikacin (87.5%) and gentamicin (60%) respectively. Susceptibility to fluoroquinolones was relatively poor. Ofloxacin was the most active quinolone tested inhibiting 45% of all *P.aeruginosa* isolates. In earlier studies, Giacometti et al (2000) and Ojha et al (1997) have reported 35% to 60% susceptibility to quinolones. *P.aeruginosa* isolates from postoperative wounds showed a high level of resistance (70%) to chloramphenicol (Rolinski et al, 1994). In the present study chloramphenicol resistance (74.5%) was high and almost all the *P.aeruginosa* strains (98.7%) were resistant to cotrimoxazole. Similar results were observed by Giacometti et al (2000). *P.aeruginosa* isolates resistant to one of the antibiotic classes tested were often resistant to atleast more than one another class of antibiotics. Among all the
antibiotics tested for \textit{P. aeruginosa} comparatively low resistance was shown by amikacin and ceftazidime. While a severe decrease in ciprofloxacin activity has been noted in the recent past, therapy with amikacin or ceftazidime seems to be a possible empirical alternative.

Among all postoperative wound, environmental and carrier isolates tested overall percentage of resistance was high among wound isolates followed by environment and carrier isolates. The susceptibility data collected in this study suggest that some antibiotics would have very limited use for the prophylaxis or empirical treatment of the wound infections because most of the gram negative isolates were found to be resistant to ampicillin and gentamicin, while majority of Staphylococcal strains were resistant to methicillin. This is remarkable data, since virtually all the patients received 1\textsuperscript{st} or 2\textsuperscript{nd} generation cephalosporins as antibiotic prophylaxis.

Overall, a progressive variation in causative pathogens and resistance patterns has been observed throughout the study. In fact, the susceptibility to antibiotics constantly decreased while multiresistant \textit{P. aeruginosa} and \textit{S. aureus} strains were isolated with increasing frequency. Ampicillin and gentamicin are the most used agents for surgical prophylaxis in this hospital, but can be ineffective against the increasingly common wound pathogens like methicillin resistant \textit{S. aureus}, \textit{P. aeruginosa}, \textit{E. coli} and other species of Gram negative rods because of their indiscriminate use. The inappropriate usage of antimicrobials in surgical perioperative prophylaxis is still a problem and a close collaboration between surgeons and microbiologists is needed (Goreckie et al, 1999; Sawyer et al, 1994). On the basis of our results, antimicrobial agents or drug combinations with wider spectra of activity and stronger resistance to enzymatic degradation are desirable for perioperative prophylaxis or treatment of surgical infection.
Phenotyping:

Identification and taxonomic classification of microorganisms have been accomplished by phenotypic analysis in the past, and with modification, have served for decades as a basis for epidemiological evaluation (Bergey and Holt, 1994). Phenotyping deserves special attention since this is the only method readily available in all clinical microbiology laboratories. Over the years, biotyping, antibiotyping, serotyping, phage typing, multilocus enzyme and cell envelope protein electrophoresis have been the most common phenotyping approaches for assessing epidemiological relatedness. Not all methods mentioned above have good discriminatory power nor they are ideal for studying the epidemiology of nosocomial pathogens in the present day. However, if used correctly with caution, they may yield useful information (Dijkshoorn et al., 1993). In the present study we have used some of these methods cautiously and have been able to correlate certain findings.

Biochemical profile analysis: This scheme yielded 18, 21 and 24 phenotypes for 45 E.coli, 45 P.aeruginosa, 80 S.aureus isolates respectively. It recognised many subgroups within the species of E.coli, P.aeruginosa and S.aureus. This finding was in contrast to earlier studies (Hebert et al., 1988) which shows that this phenotyping method yielded only few profiles, per species and found to be very useful in epidemiological studies. Therefore, in the present study the biochemical profile was used with other tests to define subtypes for greater discrimination. Biochemical profile correctly identified the related species but did not discriminate within this related group. Similar results were observed by Goering et al. (1992). In the present study the results of this phenotyping method did not correlate well with either the epidemiologic data or the results of the other typing method. Tenover et al. (1994) reported similar findings and in their study biotyping was useful only to subtype isolates within clusters into smaller groups. It was not clear whether this would have any epidemiologic significance.
Phage typing:

In the present study, out of 80 *S. aureus* strains tested, 13 (16.25%) and 20 (25%) could be typed by conventional set of phages and set of MRSA phages respectively. The percentage typability of our strains is 16.25% by conventional set and 25% by the MRSA set which is very low as compared to the study of Richardson et al (1999) which reported 75% and 91% of typability respectively. However, our results were similar to the findings of Mathur and Mehndiratta (2000) who reported 16.25% and 45.6% of typability respectively. In contrast to the study of Mathur et al (2000) present study found that among the typical strains there was an increase in frequency of phage group II strains as compared to phage groups I and III. Overall typability was increased from 17.6% (by conventional set) to 25% (MRSA set). Our results were comparable to those of Mathur et al (2000) where 8.75% of increase in typability and discrimination within groups of typable isolates was reported. Consistent with findings of Mathur et al (2000) in the present study a larger number of strains were typable with phage 622. In the present study, phage types of *S. aureus* from postoperative wound sepsis were associated with phage types of *S. aureus* isolated from the carriers among the patients and staff and also from environmental sources. These findings are consistent with the reports of Agarwal et al (1980). It was found that the commonest *S. aureus* strains responsible for postoperative wound sepsis were untypable (73.3%) which were also predominant among the carriers and environment. Amongst the typable strains from postoperative wound sepsis, 10% were similar in their phage pattern to patient carriers, 3.8% to staff carriers and 4.16% to the environment sources. Similar observations have been reported by other workers (Dutta et al, 1976; Wasek et al, 1965; Agarwal et al, 1980).

Bacteriophage typing has been the reference epidemiologic typing method for *S. aureus* despite problems in reproducibility (Mulligan et al, 1991). However, in the present study strains are poorly typeable, with the standard set of phages and typeable strains tending to react with similar group of phages irrespective of their epidemiologic origins. Similar results were observed in many earlier studies.
(Mulligan et al, 1991; Cookson et al, 1988). Nevertheless, in this study, we found that phage typing at 100 times the routine test dilution (RTD) was a sensitive method that allowed discrimination between unrelated strains and confirmed the dissemination of predominant phage types among patients, carriers, and environment in our institution. Similar findings were reported earlier (Struelens et al, 1992).

Serotyping:

In the present study, out of 45 E.coli strains subjected to 'O' typing, 43 strains (95.6%) were typable 2 (4.4%) were untypable and 2 strains (4.4%) were of rough type. Similar results were reported by Ikaheimo et al (1993) who reported 80 typable, 59 nontypable and 9 rough types out of a total 148 E.coli isolates tested. In the present study, serotypes frequently encountered were 0101 (13 strains), 01 (5 strains), 0147 and 025 (3 strains each) respectively and 098, 0106, 06, 08, 020 (2 strains each) and 068, 015, 08, 013, 0162, 0153, 050, 0167, 081 (1 strain each) which was in contrast to most earlier reports (Fule et al, 1990; Bhalla et al, 1989). Geographical difference in the prevalence of E.coli serotype reported is possibly because of regional variation in serotype distribution of faecal E.coli. 0101 was the most common group of O antigens (28.8%) closely followed by 01 (11.1%) which was in contrast to earlier reports (Ikaheimo et al, 1993). In the present study common groups of strains prevalent in the environment and carriers were 01, 0101, 098 and 01, 0101, 068, 015, 025. In the present study most common groups of strains (7) causing nosocomial wound infections were 0101, 0147, 01, 0106, 06, 08 and 025, which were also included among the eight most common groups in several earlier studies reviewed by Johnson et al (1991). Of the strains causing nosocomial wound infections, 65.7% belonged to these seven serogroups which is significantly more ($p < 0.001$) than their prevalence in faecal flora (Sitonen et al, 1992).
Plasmid profile analysis:

Of the genotypic typing methods, plasmid profiling is favored as a simple and inexpensive technique (Wang et al, 1991). Plasmid profile analysis has been shown to be a useful technique for characterizing isolates from a variety of bacterial species (Mayer, 1988). In the present study, plasmid profile analysis showed that all 45 *E.coli* isolates and 45 *P.aeruginosa* strains had definite plasmids and only 5 (6.25%) of 80 *S.aureus* isolates lacked plasmids entirely.

On comparative analysis, the number of plasmids especially in low molecular weight range was more in patient carriers than in patients. Another interesting finding that emerged on the preponderant distribution of plasmids in *E.coli* strains used in the present study was that all the 9 isolates exhibited a plasmid size corresponding to molecular weight of 21.23 kb. No other plasmid of any molecular weight was uniformly discernible in the agarose gel in all the 9 *E.coli* isolates. Overall, barring a few isolates, majority of isolates contained fewer plasmid bands. These findings are consistent with earlier studies (Simor et al, 1990; Seifert et al, 1994). These findings assures the wide applicability of plasmid profiling for epidemiological investigation. An average of 2.47 to 4.02 plasmids per isolate was found with a range of 1-9 plasmids among *S.aureus*, *P.aeruginosa* and *E.coli* isolates. Among the nosocomial bacterial isolates as high as 5-6 plasmids per isolate with a range of 2-12 plasmids per isolate were reported in earlier studies (Hobrzanska et al, 1990). It was shown in this study that chromosomally different strains share up to 4 plasmids with the same molecular size (Table 2.19, 2.20, 2.21). Others have also reported that chromosomally different strains share plasmids of same molecular size (Hobrzanska et al, 1990). Interestingly, in the present study it was observed that in almost all strains of *S.aureus*, *E.coli* and *P.aeruginosa* one plasmid of 21.2 kb was always present. Whether this common plasmid contributes to a higher pathogenicity and persistence of strains of *E.coli*, *P.aeruginosa* and *S.aureus* in wounds awaits further investigation.
In the present study, 1-9 different plasmid profiles and 1-33 different sizes of plasmids were found in isolates of *E.coli*, *P.aeruginosa* and *S.aureus*. Similar findings were observed by Ratnam et al (1988) and Hobrazanska et al (1990). These observations suggest that the number of different plasmid and plasmid combinations that occur in nosocomial bacterial isolates is vast and much greater in number that the present study had identified. Such diversity endows this technique with the exceptional resolving power needed for epidemiological studies.

The rich diversity of plasmids among these strains suggest that there are mechanisms at work, perhaps involving conjugation, phage mediated contact, transduction, transposition and site specific or homologous recombination (Locksley et al, 1982; Lyon et al, 1983; Schaberg et al, 1986) that are continuously generating new plasmid forms. The rate at which these DNA reorganisations occur has a bearing upon the usefulness of plasmid profiling in epidemiological studies.

In the present study, amongst the typable *E.coli*, *P.aeruginosa* and *S.aureus* strains from postoperative wound sepsis 20%, 50%, 7.7% were similar in their plasmid patterns to patient carriers, 60%, 50% and 12.5% to environment respectively. The presence of common plasmid bands in the patient, carrier and environmental plasmid profiles of these isolates, supports their potential relatedness.

The observation that the 4 *E.coli* isolates 110(lane b), 115(lane c), 124(lane d) and 131(lane e) had near identical number and molecular weight of plasmids with that of isolate number 139(lane f) obtained from bathroom floor indicates (a) a possible genetic recombination between *E.coli* isolates present in post operative wound of the patients and that of the infecting *E.coli* originating from bathroom floor and (b) that consequent to administration of antibiotics and other related drugs, under selective pressure, a rearrangement on the size and number of plasmids had occurred, in the isolates 110(lane b), 115(lane c), 124(lane d) and 131(lane e). The fact that there existed a variation in the molecular weight of the plasmids in the
range of 3.2 to 3.8 kb in these isolates bears testimony to this. Since there existed no difference in the molecular weight distribution of other plasmids, it is not known whether the plasmids in the intermediate range of 3.2 to 3.8 kb play an important role which hitherto has not been discovered in offering pathogenicity to patients. E.coli isolates 143(lane g), 145(lane i) and 141(lane k) from patient wounds and the isolates 104(lane h), 106(lane j) and 119(lane l) from corresponding patient carriers showed no significant difference in respect to number of plasmids borne on them.

The near similarity in the number and molecular weight distribution of plasmids in patient and patient carrier is clear cut proof that extraneous P.aeruginosa isolated either from environment and patient carriers had not caused any infection. However, it is surprising that the P.aeruginosa isolated from patient carriers and bathroom floor had one extra band of 5.14 kb which was not encountered in other patients and corresponding carriers. The fact that the plasmid of molecular weight 3.7 kb prevalent in P.aeruginosa isolates 173 (lane m), 178 (lane n), 180 (lane o) and 176 (lane p) from dressing materials and IV fluids were also uniformly present in patient and patient carrier is confirmatory evidence that P.aeruginosa from these materials ought to have been positively transferred on to the subjects. Since plasmids are known to offer resistance to different antibiotics administered on the patients, the predominant presence or absence of various plasmid DNAs could be directly correlated with development of resistance for the antibiotics.

Under uniform conditions where preoperative and postoperative patients are housed, the infecting S.aureus isolates present in the microenvironment would be expected to colonize the wounds without any bias. The plasmid profile, which is an index of identifying and grouping similar isolates, would hopefully reveal the source of infection. Majority of the S.aureus isolates obtained from operated patients showed plasmid band corresponding to the S.aureus isolates obtained from O.T. Difference in plasmid profile of a few S.aureus strains obtained from patients wounds did not match with that of S.aureus isolates collected from O.T. The reason
for this observation remains a mystery and needs further investigation. However, 
*S. aureus* isolates obtained from patients wounds (191(lane b), 192(lane d) and 
193(lane k)), and carrier (247(Lane c), 248(lane e) and 249(lane l)) showed identical 
plasmid bands indicating no new introduction of *S. aureus* isolates from the 
environment or the staff and the pre and post surgical dressing materials.

Similar findings were reported by Hebert et al (1988) and Goering et al 
(1992) which describes the different but potentially related plasmid profiles that 
supports the inter-relationship of the isolates. Thus diversity and stability of plasmid 
profiles provide an effective means for discriminating between strains. Rapid 
methods for plasmid DNA extraction and data handling may make it a suitable and 
found plasmid analysis is superior, as most of the investigated isolates which were 
not typable by using conventional typing methods were found to contain distinct 
plasmid profile. In the present study, the specificity of this technique was found to 
be high, none of the epidemiologically unrelated isolates were misclassified.

**Restriction endonuclease analysis:**

The reliability of markers for epidemiological investigations of nosocomial 
infections depends on diversity and reproducibility. Genetic techniques are 
currently used to compare isolates. Digestion of total cellular DNA with restriction 
enzymes is also reported to be useful (Martin et al, 1990). However, under 
conventional electrophoretic conditions, the results may be difficult to analyse, 
although the differences in restriction endonuclease patterns are striking and 
convincing. Our study demonstrated that restriction endonuclease analysis of 
chromosomal DNAs of *E.coli, P.aeruginosa* and *S.aureus* allows a specific 
discrimination among species and strains within the species. The optimal 
conditions for REA analysis of *S.aureus, E.coli* and *P.aeruginosa* were determined 
in this study. The procedure had to be designed in such a way that sufficient yields 
of chromosomal DNA were reproducibly recovered. Initial problems are associated 
with frequent bacterial lysis during propagation in a liquid medium and inefficient
cell lysis during the DNA extraction. Additional measures to overcome this problems are as follows: Good yield of viable cells were obtained by growing all the isolates on LB agar plates and efficient lysis of isolates was ensured by including 0.3% glycine in a solid medium.

Restriction endonuclease analysis of chromosomal DNA was performed with Hind III, Sal I, BamH I / EcoR I that produced well resolved patterns of bands. The near identical pattern of distribution of DNA bands in all the E.coli stains after BamH1 restriction digestion of total DNA suggests that the ward environment was contaminated and ward environment could have become the source of infection.

It was observed in the present study that EcoR I restriction endonuclease was unsuccessful in digesting some E.coli strains. This finding indicates the genetic variability between strains within E.coli species. Some of these variations may be explained on the basis of a high frequency of DNA rearrangement or a high mutation rate (Reznikoff, 1983). Our observation of considerable genomic diversity among E.coli, S.aureus, P.aeruginosa isolates confirms the results previously reported by Majeuski and Goodwin (1988) and by Langenberg et al (1988). In the present study, isolates obtained from different patients invariably demonstrated unique DNA restriction patterns, although, Rauws et al (1989) used bacterial restriction endonuclease analysis to determine the source and route of infection caused by single H.pylori infected eight members of one family. Similarly, Majeuski and Goodwin (1988) recovered different strains causing infections among different members of the same family.

Restriction digestion of total DNA from any microorganism compared to plasmid DNA is known to yield rich information on the number and preponderance of different molecular weight DNA. The observation that there was no difference in migration of DNA bands in the molecular weight range of 19.4 to 48.5 kb in the isolates {146(lane b), 147(lane c), 148(lane d), 149(lane e)} obtained from patient wounds and {181(lane j), 182(lane k), 183(lane l), 184(lane m)} from patient carriers
is a clear indication that the *P. aeruginosa* strains were obviously absent in the microenvironment when the study was undertaken. That no well defined differences could be observed in the DNA bands of less than 12.2 kb molecular weight further confirms that the *P. aeruginosa* isolates present in patient wounds and patient carriers are the same and no new introduction of *P. aeruginosa* occurred. The near likeness of the distribution of DNA bands less than 12.2 kb molecular weight need not be interpreted as nil differences in the *P. aeruginosa* isolates obtained from different sources. It is probable that when a hitherto different restriction enzyme is used, the DNA profile might be different yielding more meaningful information. In the present study, REA was used to find out the route of spread and source of nosocomial wound infection. In general surgery ward, REA patterns demonstrated considerable diversity among these patients, environment and carrier isolates.

In the present study, *E. coli* strains from 1 environment and 4 patients wounds had identical REA patterns. *E. coli* strains from 3 patients wounds and 3 corresponding patient carriers had identical restriction endonuclease patterns. The conspicuous presence of a doublet band of 10.1 kb in the *E. coli* isolate 139 (lane f) isolated from bathroom floor and its absence in the *E. coli* isolates 104 (lane g), 106 (lane i), 119 (lane k) and 143 (lane h), 145 (lane j), 141 (lane l) from patients wounds and carriers respectively suggest that the contaminating *E. coli* isolates do not infect the wounds of postoperative patients or that the drugs administered keep the infection at bay.

The uniform distribution of a doublet DNA of molecular weight of 10.1 kb in the isolates 110 (lane b), 115 (lane c), 124 (lane d), 131 (lane e) obtained from wound corresponding to the *E. coli* isolated from the bathroom floor (139 Lane f) indicates the possibility of introduction of *E. coli* isolate 139 and its subsequent proliferation in the wounds of the patient.

Langenberg et al (1988) and Simor et al (1990) reported that the first and subsequent isolates from six patients had identical restriction endonuclease
patterns. Pseudomonas aeruginosa isolates obtained from four patients' and 4 corresponding patient carriers had identical restriction patterns in our study. S.aureus isolates obtained from four patients wounds and 1 environment had identical REA pattern. S.aureus strains isolated from 3 patients wounds and 3 corresponding patient carriers had identical REA patterns. The identical distribution of DNA bands in S.aureus isolates obtained from patient wounds {194(lane b), 200(lane c), 205(lane d), 210(lane e)} with that of S.aureus isolate 223(lane f) from OT air indicates that the microenvironment of O.T., loaded with S.aureus was responsible in causing infection in the patients. The DNA banding pattern of S.aureus isolates obtained from patient wounds and carriers were analogous to each other confirming our earlier observation that no new introduction did occur. However more detailed studies employing PCR amplification technique may yield wealth of information.

Ichiyama et al (1991) have correlated S.aureus strains from 18 patients and from doctor carriers using antibiotic pattern, plasmid profile pattern and restriction chromosomal pattern to find out the source, transmission and spread of nosocomial S.aureus infections. Different findings were observed by Blanc et al (1993) who correlated 51 clinical and 4 environmental P.aeruginosa strains and found out that clinical and environmental strains had different restriction patterns.

Comparison and correlation of the different typing methods:

Identification and typing of microorganisms are extremely important in efforts to monitor the nosocomial infections and their control. Many typing methods have been used by different investigators for elucidating the hospital outbreaks. However, a number of comparative studies on different typing methods have been reported (Blanc et al, 1993; Ichiyama et al, 1991; Renaud et al, 1988; Struelens et al, 1992). A comparative study was attempted in our hospital by using phenotyping, phage typing, serotyping, plasmid profile analysis and restriction endonuclease analysis for typing the isolates of E.coli, P.aeruginosa and S.aureus to investigate the source, transmission and spread of nosocomial wound infections.
The present comparison of epidemiologic typing systems for *E.coli*, *P.aeruginosa* and *S.aureus* indicated a good correlation between phenotypic and genotypic methods with 75% concordance in typing of strains. Phage type and serotype variables were observed among few *E.coli* and *S.aureus* strains belonging to a single genotype, as defined by both plasmid profile analysis and DNA restriction analysis. Although the phage typing was unable to type almost 75% of strains in our study.

The biochemical profile gives a good correlation with conventional methods (Kloos et al, 1985) for species identification (Kloos et al, 1982; Hebert et al, 1988). This rapid 18-21 test system, although designed to identify species of *E.coli*, *P.aeruginosa* and *S.aureus* has also been used to biotype strains within the species (Christensen et al, 1983). In the present study, the biochemical characteristics of these organisms were diverse enough to create several profiles and also to define the same species correctly. But too many strains belong to the same biotype and these biotypes were not always in agreement with the other typing methods such as pp analysis and REA. Although serotyping is a very stable marker, it was not sufficiently discriminatary. Phage typing is a useful method, but unfortunately many strains remains nontypable by this typing method. However, these nontypable strains were correctly characterized by REA in the present study.

Thus, molecular techniques have proved to be additional useful tools in epidemiologic studies of *S.aureus*, *E.coli* and *P.aeruginosa*, in the observations that is in concordance with earlier studies (Blanc et al, 1993; Bingen et al, 1996). Plasmid profile typing and restriction endonuclease analysis of chromosomal DNA proved to be useful methods of epidemiological typing of various bacterial species (Mayer, 1988). Plasmid analysis has enabled tracing of hospital transmission of nosocomial bacteria, although problems related to plasmid instability or to poor discrimination among the few conserved plasmids commonly found in nosocomial pathogens are significant limitation of this marker system. Similar findings were
observed by earlier reports (Ichiyama et al., 1991). However, in the present study the plasmid profiles were more stable as they reproduced same profile after probing period.

In the present study, plasmid analysis was found to be superior to phage typing as most of the investigated isolates were nontypable by phage. Archer and Mayhall (1983) reported similar findings in the investigation of an outbreak of MRSA infections. The present study observed that isolates of same phage types had different plasmid patterns. Collins et al. (1984) had findings similar to present study, while tracing the source of postoperative wound infections caused by S. aureus colonization and environment. The present study found testing of 80 isolates by plasmid analysis to be highly reproducible (95%) and found repetitive phage typing of these isolates poorly reproducible (40%). A finding similar to ours was found by Rhinehart et al. (1987). Restriction endonuclease digestions of plasmid DNA may increase the discriminatory power of plasmid analysis (Harstein et al., 1990), in particular for those strains that contain only a few plasmids with similar sizes. Digestion of plasmids was not considered necessary in our study, since the high number of plasmid bands in the strains investigated had facilitated interpretation and comparison of plasmid profiles and contributed to the discriminative power of this typing method. Similar findings were observed by Seifert et al. (1994). Reproducibility of plasmid profiles was excellent, even after prolonged storage of strains at room temperature. In the present study, plasmid analysis was found to be rapid, stable, reproducible and highly specific. This has also been noted by a number of other workers (Townsend et al., 1987). None of the epidemiologically correlated isolates were misidentified. Archer and Mayhall (1983) found plasmid pattern analysis to be more helpful than biotyping, antibiograms, serotyping or phage typing in their study of a MRSA outbreak. Our findings were in agreement with the reports of Archer and Mayhall (1983).

Typing of nosocomial strains by genomic fingerprinting with REA has been shown to be reproducible and accurate on the basis of studies with large number of
strains (Ojeniyi et al, 1991). The present study demonstrated a diversity of REA patterns among postoperative wound isolates from different patients, environment and carriers. Although some apparently unrelated strains showed great similarity by REA, this appeared to be a relatively infrequent event and may be offset by the speed, availability and other useful discriminatory powers of this technique. Alternatively, the type of REA described here could be enhanced by using a different restriction endonuclease to confirm the identities or uncover differences within a REA group. In the present study, 5 of the 80 S.aureus strains tested contained no plasmids. Therefore, REA was considered as an effective epidemiological marker. This study showed a considerable diversity in chromosomal digestion pattern of E.coli, P.aeruginosa and S.aureus isolates. The chromosomal digestion pattern permitted us to clearly differentiate these above isolates and even those showing same biochemical profile or the same plasmid profile. Our study also demonstrated a good reproducibility in the chromosomal digestion patterns from isolates of E.coli, P.aeruginosa and S.aureus.

Thus REA may be an additional useful marker for epidemiological studies of infections caused by nosocomial pathogens. The diversity and stability of REA profiles provide an effective means for discriminating between strains. Rapid methods for DNA extraction and data handling may make it suitable and cost-effective method for hospital clinical laboratories. REA confirms or specifies the identities of isolates when they demonstrate variations in some of their biochemical characters. This method appears to be reproducible and discriminating. However, the problem of similar profiles can be minimized with the use of different restriction enzyme so as to reveal minor differences undetected by the first endonuclease. In addition, with the use of the combination of two molecular typing methods such as profile analysis, which examines the most recent genetic events and REA which examines the more stable genetic element in the bacterial chromosome, it may be possible to overcome these difficulties.
In the present investigation there was a good correlation between biochemical profile, phage typing, serotyping, plasmid profile analysis and REA of chromosomal DNA methods. The availability of plasmid analysis or REA on a routine basis will be helpful in characterizing nosocomial isolates that are causing several outbreaks.

**Molecular epidemiology:**

Molecular methods in epidemiological analysis of infectious disease will help in detection of common source of transmission, persistence of the infectious agent in a particular environment, distinct clone involved in the outbreak and formulating infection control measures to curtail outbreaks in the particular set up. The dissemination and persistence of *E.coli*, *P.aeruginosa* and *S.aureus* in the hospital environment probably accounts for their specific role in higher wound infection rates. These species have been found contaminating inanimate materials in hospital and they act as reservoir of infection. In some persistent outbreaks, contaminated materials have been shown to act as a source in the outbreak. The source can be animate (patients, medical staff) or inanimate objects. Epidemiological investigation suggest that genotypic methods such as biotyping, plasmid profiling, REA of chromosomal DNA, RAPD and PFGE are now being used widely for the detection of such common source of outbreak (Webster et al, 1998).

Molecular techniques have proved to be additional useful tools in recent epidemiologic studies. Plasmid profile analysis has enabled tracing of hospital transmission of strains, although problems related to plasmid instability or to poor discrimination among the few conserved plasmids commonly found are significant limitations of this marker system. Additional discrimination may be achieved by REA of plasmid DNA or genomic DNA.

REA of genomic DNA can detect nucleotide sequence variations randomly distributed along the bacterial chromosomes and this approach has been found superior to conventional typing in studies of many bacterial species. A problem
arises with high frequency cleaving enzymes, because hundreds of restriction fragments are obtained, often leading to poorly resolved patterns in conventional electrophoresis. For overcoming the difficulty of comparing these complex patterns, densitometric analysis has been proposed or DNA fragments have been subjected to southern hybridisation with rRNA gene probes, random chromosomal DNA probes or aminoglycoside resistance gene probes, the last showing superior discriminatory power. In summary, analysis of restriction fragment length polymorphisms of totally digested genomic DNA allows one to reliably identify particular species and strains.

The present study makes an attempt to investigate the predominant sources and mode of transmission of endemic nosocomial wound infections in surgical wards of JIPMER hospital. The reservoir of strains of E.coli, P.aeruginosa and S.aureus that cause postoperative wound infection has not been established in many studies. Epidemiologic investigation suggest that general routes of transmission of wound infection include direct contact, equipment (Millership, Patel and Chattopadhyay, 1986), aerosols (Dandalides, Rutala and Sarubbi, 1984), air (Bengtsson, Hambraeus and Laurell, 1979) and health care personnel and patient carriers (Cookson et al, 1989). Colonization of E.coli, P.aeruginosa and S.aureus in human subjects irrespective of patients and healthy medical personnel may have an important bearing in transmission of these infectious agents among patients. High colonisation rates of the anterior nares, respiratory tract, throat, GI tract, axilla, perineum having varying degrees of importance are reported in several studies (Agrawal, 1980; Larson et al, 1986; Andenaes et al, 1996). Many recent studies have used molecular methods such as PCR, PFGE, REA that were able to clearly establish the relationship between the strains obtained from carriers and the patients (Andenaes et al, 1996; Struelens et al, 1992).

This study was an extensive survey of the total aerobic bacteria of the environment of the OTs, wards and dressing rooms and also the total aerobic bacterial flora of 3 skin sites in both postoperative wound infected patients and 3
groups of health care personnel. Of the 210 samples collected from OT environment, 33 (15.71%) samples yielded aerobic bacteria, on 11 occasions (52.38%) the OT was unsatisfactory. On 83.33% times the air of the ward and dressing room were unsatisfactory respectively. On all the occasions S.aureus and Coagulase negative Staphylococci were isolated. These findings were consistent with earlier studies. Thakur et al (1990) reported that 61.35% times, 82.45% times, 68.75% times the air was unsatisfactory in their hospital, major OTs, postoperative ward and dressing rooms respectively. On all occasions, S.aureus was the aerobic bacteria isolated as sole contaminant in their study. Increasing operating room air contamination was observed with increasing human activity in the operating room (Nahmias and Bickhoff, 1961). Burke (1963), Lidwell et al (1971), Ako-Nai et al (1992) and Bauer et al (1990) also made same observation and concluded that air immediately above the wound contributed the large proportion of bacteria entering the wound during operation. In one instance in the present study a strain of S.aureus (Table 2.23; Isolate no.223) was obtained from the air of the operation theatre that was identical phenotypically and genotypically with that of the S.aureus strains isolated, from wounds of the 4 patients wounds (Table 2.23; Isolate No.194, 200, 205, 210). This confirms that contaminated OT air can be a potential source for recurrent infections (Agarwal, 1980; Amit Das, 1985) and illustrates the need for environmental surveillance and control measures. Other areas in the OT, ward and dressing rooms include scrub tub, OT floor, OT table, A/C grill, anaesthetic apparatus mark, patients' clothing, bedding, window curtains, ward floor, ward bathroom floor, preparation tray and sink in the dressing room were found to be contaminated on more than two occasions of 18 to 21 occasions tested.

The predominant aerobic bacteria isolated from above mentioned areas were S.aureus, Klebsiella spp, P.aeruginosa, E.coli, Acinetobacter spp, Coagulase negative Staphylococci, Aerobic spore bearers, micrococcii and diphtheroids. Solutions like cheatle forceps water, saline, IV fluid, savlon were also found to be contaminated atleast on one occasion usually involving one or two organisms (P.aeruginosa, Klebsiella spp, Proteus spp and Acinetobacter spp). This finding
was consistent with the earlier reports (Thakur et al, 1990) which describes the isolation of *E.coli, P.aeruginosa*, Klebsiella spp and Proteus spp as the main contaminants of disinfectants and solutions from the OTs, ICU and wards respectively.

In our study sampling of different areas in the OT, ward and dressing room persistently yielded *S.aureus, E.coli* and *P.aeruginosa* even after prolonged period, probably suggesting successful colonization which was leading to recurrent episodes of endemic infections in the ward patients.

Numerous investigations have documented the persistence and survival capacity of *S.aureus, E.coli* and *P.aeruginosa* in the hospital environment (Burke and Corrigen, 1961; Foster et al, 1960; Allen et al, 1987; Agarwal, 1980; Garcia et al, 1989).

Various equipment and surfaces (telephone handle, door push plates, patient charts, table tops, floor of the wards, patient clothes, bedding, etc.) were shown to be contaminated during an outbreak in an IUC in different studies. These were found to be contaminated by the hands of staff and patients (Getschell-White et al, 1989). Our study documented the contamination of bathroom floor by strains of *E.coli*. These strains were found to be causing infection in many patients. Burke and Corrigan (1961) and Ravichandran (1985) reported that identical strains were obtained from inanimate areas like ward floor, patients’ bedding and clothing, dressing room floor and infected postoperative wounds on different occasions. Most of the earlier studies used only phenotypic methods for epidemiological typing. In recent years such findings are becoming more accurate and assertive due to new molecular techniques employed (Tankovic et al, 1994; Mc Donald et al, 1998). This was again proved by the above observations made in the present study.

In one instance in the present study a strain of *E.coli* (Table 2.21; Isolate No.139) was obtained from bathroom floor which was identical genotypically and
phenotypically with that of \textit{E.coli} strains isolated from the wounds of 4 patients (Table 2.21; Isolate No.110, 115, 124, 131) which confirms contaminated ward environment can be potential source for recurrent infection in the patients.

This finding throws light on the extent of contamination in the ward bathroom, which can play important role by becoming one of the reservoir for large number of pathogenic bacteria and transmit the same to the patients, staff and surrounding ward environment.

JIPMER hospital is the only referral hospital with free medical facilities in the union territory of Pondicherry and neighbouring districts of Tamilnadu. Also people who get treatment in the general wards are people who live below poverty line. Unhygienic practice like walking barefoot adopted by these patients while using the bathroom might have led to themselves getting colonised with the same pathogenic bacteria.

In the present study, the \textit{E.coli} strains from the patients' infected wounds were traced down to the ward bathroom floor. This can be explained by poor hygiene of patients including walking barefoot in the hospital (in the ward as well as bathroom). These patients have more chances of acquiring and getting colonized by bacterial strains from the contaminated bathroom environment and further contaminating the ward environment including their own clothing, bedding. This indicates a need for constant, continuous monitoring of the ward environment.

Skin flora of the 3 skin sites in both wound infected patients and health care personnel, revealed several important differences between these groups. Patients had high number of organisms in the sites sampled. Differences in the composition of the microflora were striking.

In the present study, Coagulase negative Staphylococci were isolated from nares, axilla and perineum of patients, surgeons, sisters and ancillary staff in high
frequency (25-76%). Coagulase negative Staphylococci are of particular concern, because the incidence of infections caused by these organisms is increasing in the hospitals (Bergan et al, 1983) especially among isolates from cases of septicemia (Riedman et al, 1984).

In the present study, only 33.6% of the patients were carrying \textit{S.aureus} in the nares as compared to 66.7%, 57.1% and 58.3% of the surgeons, sisters and ancillary staff respectively. Similar findings have been reported by Williams et al (1986) who found that paramedical personnel working in the hospital wards and operation theatres had higher carrier rates (59.6%) of \textit{S.aureus} than did the patients (46.1%). This could be due to the constant exposure of hospital staff to an environment contaminated with \textit{S.aureus}. Taylors et al (1989) and Chuard et al (1991) reported respectively that 23.8% and 44% of health care personnel studied were nasal carriers of \textit{S.aureus}. In the present study patients were found to be carrying \textit{S.aureus} in axilla and perineum on 27.7% and 2% occasions respectively. Similar findings have been reported by other workers (William, 1960; Agarwal et al, 1980), the skin carriage rate of \textit{S.aureus} being as high as 40%. In the present study gram negative bacteria isolated from surgeons, sisters, ancillary staff’s nose, axilla ar’d perineum on 16.7%, 12.5% and 30% occasions, 14.3%, 12.5% and 25% occasions and 16.7%, 20% and 25% of occasions respectively.

These findings were consistent with findings of Larson et al (1986) which describes the gram negative bacteria isolated from the nose, axilla and perineum of the patients and health care personnel on 27&, 26% and 26% occasions and 17.7%, 23.3% and 7.7% occasions respectively. Rahal et al (1970) recovered GNB from the nares of 12.8% of physicians, nurses and nurses aides, isolates included Klebsiella, Enterobacteria spp, Serratia spp and \textit{P.mirabilis}. Adams et al (1982) recovered GNB from the hands of 31% of a group of hospital workers, most of whom participated in patient care.
Cruse (1980) in his comprehensive surveillance study found auto-infection as a probable contributory mechanism in the development of PWI. Williams et al (1960) and Weinstein (1959) and Morales et al (1994) found a substantial positive correlation to be present between carriage of S.aureus and rate of PWI. Sorensen et al (1991) found there was an association between colonized surgical wounds and increase rates of infections. Experimental studies in animals have shown that inoculum size influences the development of PWI (Kaiser et al, 1992; Citak, 1992). In the present study, 3 different strains of E.coli isolated from the wounds of different patients (Table 2.21; Isolate No.104, 106, 119) were identical genotypically with that of the corresponding skin carrier E.coli isolates of the respective patients (Table 2.21; Isolate No.143, 145, 141). Similarly, 4 patients wound P.aeruginosa strains (Table 2.22; Isolate No.146, 152, 163, 165) were identical to corresponding patient carrier strains (Table 2.22; Isolate No.183, 188, 187, 186). Similarly, 3 patient wound S.aureus isolates (Table 2.23; Isolate No.191, 192, 193) and corresponding carrier patient S.aureus isolates (Isolate No.247, 248, 249). Therefore, in the present study, auto-infection was found to be commonest mode of infection, the most common source being perineum followed by axilla and nares. Almost identical findings were observed by Mary et al (1996) who reported common source of postoperative wound as being rectum following by perinimum. Similar findings were also observed by Amit Das (1985) and Ravichandran (1985). Thus, the present study has proved the endogenous acquisition as one of mode for nosocomial wound infections.

Gram negative bacteria such as E.coli, P.aeruginosa and gram positive bacteria like S.aureus, all have a common feature of intrinsic resistance to multiple antibiotics (Hancock, 1998). They can be easily recovered from the environment, are often resistant to disinfectants and have the potential to spread from patient to patient via container formites or from the hand of medical personnel (Larson, 1981; Quinn, 1988). Extensive and indiscriminate use of broad spectrum antibiotics in many developed countries has served to eliminate competing bacteria and created a vacant ecological niche that could enhance the ability of resistant clones of E.coli,
*P. aeruginosa* and *S. aureus* to colonize and subsequently causing infection in the hospital patients. The commonest species isolated from our patients were *E. coli*, *S. aureus* and *P. aeruginosa* and only few distinct resistant isolates were more common among these species. This may be due to selective antibiotic pressure and effective disinfection procedures. These conditions may also have resulted in the selection of highly adapted strains that were capable of surviving in this environment.

However, the conditions in all the other wards were not similar in the hospital. A large number of beds and high turnover of patients in medical, surgical and pediatric wards makes the hospital overcrowded, thereby good standards of infection control is difficult to achieve. Thus, it might have resulted in a greater variety of strains acquiring various levels of resistance to a wider range of antibiotics, possibly due to overuse of these antimicrobials in this setup with patients easily acquiring these strains. It is quite possible to assume that many endemic unrelated MDR strains of different Escherichia, Pseudomonas and Staphylococcus genomic species are circulating uninterruptedly among patients, staff and different areas in these wards, dressing room and OTs. Some of the samples taken from health care staff were positive for multiresistant *E. coli*, *S. aureus* and *P. aeruginosa* in this study.

Continuous surveillance of patients and the environment many a times help in identifying persistent sources of infection. One should re-emphasize the need for hand washing before and after patient contact (Trilla et al, 1995; Schrock Snadel et al, 1995; Humphreys et al, 1995). Appropriate disinfection procedures supports infection control to a large extent. However, this is particularly relevant in overcrowded hospitals of developing countries like ours, where one can notice inadequate routine cleaning of clinical areas as a result of inadequate budget allocation for domestic cleaning (Webster et al, 1999).