CHAPTER 16

16. DISCUSSION

16.1 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 decades, the epidemiological analysis of hospital acquired infections (HAI) has undergone remarkable evolutionary changes. While earlier, relying purely on phenotypic characterization, recent years have seen a variety of approaches to epidemiological analysis from developments in molecular biology, culminating in the advent of what is conventionally termed 'molecular epidemiology'.

Most important bacterial pathogens involved in hospital acquired infections (HAI) are methicillin resistant Staphylococcus aureus (MRSA), vancomycin resistant Enterococcus (VRE), gram-negative organisms such as Escherichia coli, Klebsiella, Serratia, Proteus, Pseudomonas, Acinetobacter. HAI is also increasing out of proportion due to viral agents like hepatitis B and C. A multitude of new advanced therapies and technologies have prolonged the life of patients, at the same time, they have created situations in which many patients are rendered immunologically less competent. Consequently, opportunistic bacterial pathogens such as Pseudomonas and Acinetobacter have emerged as the most important cause of nosocomial infections.
Acinetobacters are small, non-motile, Gram-negative rods that are ubiquitous in nature. In the past, they have only been of limited clinical interest. However, acinetobacters are now been recognized as important nosocomial pathogens as they are being implicated in numerous outbreaks in various hospitals worldwide (Glew et al., 1977; Allen and Green 1987; Hartstein et al., 1988; Vila et al., 1989; Traub, 1989; Kelkar et al., 1989; Sakata et al., 1989; Joly-Guillou et al., 1992; Kropec et al., 1993; Graser et al., 1993; Tankovic et al., 1994; Vaneechoutte et al., 1995; Debast et al., 1996; McDonald et al., 1998; Prashanth et al., 2000).

16.1.2 Acinetobacter spp. as nosocomial pathogens:

During the last two decades, among nosocomial pathogens, Acinetobacter spp. has given rise to an increasing number of nosocomial infections. Acinetobacter strains are widely distributed in nature; in hospitals, the human skin is the likely source for most outbreaks of hospital infections. The organism has been frequently found in the inanimate environment, especially in moist areas and it has been isolated from various types of opportunistic infections. Nosocomial infections due to Acinetobacter spp. include respiratory tract infections, urinary tract infections, meningitis, bacteraemia, endocarditis, wound and burn infections, but they are frequently isolated from ventilator-associated nosocomial pneumonia (VAP). The increasing frequency of hospital outbreaks due to Acinetobacter spp. has serious implications and there is a need to develop simple and rapid reliable typing methods. The conventional 'phenotypic' methods such as serology, biotyping, phage typing, electrophoretic protein profiles and 'genotypic' systems, namely
ribotyping, plasmid profiles, PCR-based fingerprinting, pulsed-field gel electrophoresis have so far been utilized for strain identification. These typing systems should allow a better understanding of the epidemiology of *Acinetobacter* in the hospital environment, such as sources, modes of transmission, which in turn will result in more efficient preventive measures. *Acinetobacter* infections are difficult to treat owing to their frequent multiple resistance to the antibiotics currently available for the treatment of nosocomial infections. Various mechanisms of resistance to beta-lactams and amino-glycosides have been identified in this genus. Combination therapy is usually recommended for treatment of *Acinetobacter* nosocomial infections. Active antibacterials include imipenem, ceftazidime, amikacin and the newer fluoroquinolones.

The purpose of the present study was to consider the advantages and disadvantages of the variety of typing methods in conjunction with phenotypic characterization of *Acinetobacter* isolates collected over a period of time from JIPMER tertiary care hospital. In addition, issues related to their feasibility, simplicity, affordability were also evaluated. The present study also addresses the prevalence and range of infection caused by *Acinetobacter* species in this particular geographical area. Resistance levels acquired by these organisms to various antimicrobials were also evaluated.

16.1.3 Over view of the Results:

The genus *Acinetobacter* has been increasingly associated with hospital infection. In the present study, *Acinetobacter* spp. was a common bacteria
encountered in our hospital among the gram-negative bacteria. The *A. calcoaceticus-A.baumannii* complex (*Acb*-complex) was frequently involved in nosocomial infections in Respiratory intensive care unit (RICU), surgical and pediatric wards of JIPMER hospital, with the respiratory tract and blood stream being the predominant sites of infection. However, infections in other sites have also been encountered (peritoneal fluid, ascitic fluid, cornea and catheter). Using the CDC criteria (Garner et al., 1988), 36 patients were found infected and 7 patients presented with colonization without any related clinical findings for infection. Few instances of systemic infections in immunosuppressed subjects due to *Acinetobacter lwoffii* and *Acinetobacter johnsonii* were also encountered.

*Acinetobacter baumannii* biotypes 6, 9, 10, 15 and *Acinetobacter* genospecies 13 were recovered as strains responsible for outbreaks. *A. baumannii* biotypes 16, 19 were recovered from endemic sporadic infections. The occurrence of *A. baumannii* biotypes isolated from clinical specimens differed slightly with other studies from diverse geographic areas (Dijkshoorn et al., 1993; Jolly-Guillou et al., 1990; Gerner-Smidt et al., 1992). Multiple drug resistance were encountered in the strains belonging to *Acb*-complex. Community acquired *Acinetobacter* infections were also encountered in the present study. One case of recurrent bronchiectasis caused by *Acinetobacter baumannii* biotype 9, having moderate susceptibility was diagnosed. A case of corneal perforation due to *Acinetobacter junii* was reported (Prashanth et al., 2000).
16.2 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 *Acinetobacter* in the present day. However, if used correctly with caution, they may yield useful information (Dijkshoorn et al., 1993; Tjernberg, 1990; Gerner-Smidt, 1995). In the present study we have used some of these methods cautiously and have been able to correlate certain findings.

The customary practice has been to consider all acinetobacters as members of a single species, *A. calcoaceticus* with two biovars. Most of the times they are identified only up to the genus or biovar level in many microbiology laboratories. Acinetobacters are metabolically inert in commonly used biochemical tests. Only the glucose oxidizing strains contain a non-specific glucose aldosedehydrogenase that acidifies glucose and related aldoses (Bouvet and Bouvet, 1989; Cleton-Jansen et al., 1988). Thus, it is not useful to type acinetobacters by means of carbohydrate oxidation tests except for glucose oxidation only. The production of urease varies among *Acinetobacter* isolates. Proteolytic isolates are seldom encountered from the clinical
sources. Vast majority of isolates are of non-proteolytic biovars *anitratus* and *lwoffii* (Gerner-Smidt et al., 1985, Gerner-Smidt et al., 1987; Gerner-Smidt and Frederiksen, 1993). Isolates belonging to *A. junii*, *A. lwoffii*, and DNA group 13 are only able to give strong and reproducible reactions (Gerner-Smidt et al., 1994). Thus, traditional phenotypic tests are only of limited use in biotyping *Acinetobacter* isolates. However, further emphasis on phenotyping has been done due to the advent of most valuable assimilation tests as an extension to traditional biotyping (Baumann et al. 1968; Bouvet and Grimont 1986).

The new molecular method of identification (using DNA-DNA hybridisation) has delineated genus *Acinetobacter* into 21 DNA-DNA homologous groups (Schreckenberger, 1999). Phenotypic identification of the genus *Acinetobacter* to the species or DNA group level is very difficult. The confusion that the new taxonomy has caused in the clinical laboratory is compounded by the fact that identification of the various species or DNA groups by phenotypic characteristics, many of which are assimilation of numerous carbon sources, is very difficult (Weaver and Actis, 1994). As *Acinetobacter* spp. is extremely versatile in its ability to use single agents as its sole source of carbon or nitrogen (Baumann et al., 1968), Bouvet & Grimont (1986) used it taxonomically for biotyping *Acinetobacter*. The tests for identification of acinetobacters selected by Bouvet & Grimont (1986) seems to be appropriate, but still their large panel of 28 phenotypic tests is tedious and time consuming. In 1987, they simplified this scheme by reducing the number of tests to 19. The reliability of this identification scheme was evaluated by Gerner-smidt et al (1991). Using a numerical approach they could identify more than 90% of strains in the following DNA groups: DNA groups 2,
A considerable problem was present in identification of strains of other DNA groups, as there was significant phenotypic overlap between strains that were genotypically closely related DNA groups namely DNA groups 1, 2, 3 and 13TU. Therefore, the name *A. calcoaceticus - A. baumannii* complex (Acb-complex) was proposed by Gerner-Smidt et al. (1991). Later studies from Gerner-Smidt et al. (1993, 1994) reduced this panel of phenotypic tests still further to 14 tests, which did not result in much variation. DNA group 15 was indistinguishable from *A. lwoffii*. Only extended phenotypic tests can distinguish these groups (Bouvet & Grimont, 1986 and Bouvet and Jeanjean, 1989). Commercial phenotypic identification kit such as API 20NE has been used in many laboratories (Towner and Chopade, 1987). However, most authors consider this method as insensitive and not reproducible (Kropec et al., 1993; Gerner-Smidt, 1995). Hence, every future phenotypic identification system adopted should be tested against genotypically well characterized strains, so that it adds to the validity of the proposed phenotypic tests.

The present study initially started with phenotypic identification scheme for *Acinetobacter* spp. using limited number (14) of tests. Simple identification scheme discussed by Gerner-Smidt et al. (1993) and Tjernberg (1990), along with antimicrobial susceptibility testing was used initially in this study. The increasing clinical association with *Acinetobacter* is attributed mainly to its capacity to cause nosocomial infections, particularly outbreaks in ICUs. Intensive care unit was strictly monitored for *Acinetobacter* infections. The most important nosocomial acinetobacters belonged to Acb-complex (Gerner-Smidt, 1995, Bergogne-Berezin and Towner, 1996). The first phase of the present study also identified more strains belonging to Acb-complex than
other phenotypes (Prashanth and Badrinath, 2000). A total of 28 isolates were typed using two methods, biotyping and antibiotyping, which helped in delineating *Acinetobacter* spp. into 12 phenotypes and two distinct antibiotypes respectively. Among them 6 phenotypes belonged to Acb-complex. A sudden increase in cases of *Acinetobacter* infection suggesting an outbreak in few months during the study period was due to phenotypes 1 & 2 of Acb- complex. The ICU environment was recognised as an important reservoir for the resistant outbreak strain (Acb-1), which was probably leading to persistent colonisation and recurrent infections. This has been the case in many earlier studies (Gerner-Smidt 1987; Webster et al., 1998). Almost all strains belonging to Acb-complex were of resistant antibiotype.

It was realised later that a more pragmatic approach was needed to monitor the *Acinetobacter* infections and to elucidate their epidemiological features. It was thought that extended phenotypic identification in conjunction with electrophoretic methods could give definitive as well as comparative results, which could help in control of this infections in our hospital. Similar kinds of approaches have given better results in many instances (Dijkshoorn et al., 1993; Tankovic et al., 1994; Seifert et al., 1994). An extended phenotypic characterization (Bouvet & Grimont, 1986; Gerner-Smidt et al., 1991) and cell envelope protein analysis (Dijkshoorn et al., 1987) was therefore performed for all our isolates collected over a period of 21 months.

As repeated isolation of organism from blood is a strong indication of infection with related clinical findings (Garner et al., 1988), our investigation had thirty six patients with infection and seven cases of colonisation. Increased infections due to Acb-complex in ICU occurred only during some months, and rest of the year there were less
isolations suggesting outbreaks during certain periods only. Infrequent occurrence of infections with *Acinetobacter spp.*, is punctuated with outbreaks lasting for a month at different time intervals. Outbreaks encountered during the month of Oct'96, Sep'97, Feb'98 and July 1998 were due to multidrug resistant strains of *Acb*-complex. (*A.baumannii* and DNA group13). The source of infection was from the ICU environment where the epidemic strain, *A. baumannii*, biotype 10 (*Acb*-1) was isolated over a prolonged period. *Acb*-1 phenotype that was later identified, as *A. baumannii* biotype 10 strain from environment was phenotypically indistinguishable from strains isolated from few patients and was distinct from all other phenotypes. The outbreak strain *A. baumannii*, biotype 10 was distinct from other *Acb*-complex strains by their inability of utilising malonate (Prashanth and Badrinath, 2000).

The phenotypic tests for identification of acinetobacters designed by Bouvet and Grimont (1987) and later simplified by Gerner-Smidt et al. (1991), adopted later was able to differentiate majority of our isolates. All except 7 strains, in the present study could be assigned to various DNA groups. There was difficulty in the identification mainly between DNA groups 2 and 13 as well between 8/9 and 15. Other workers (Gerner-Smidt et al., 1991; Bouvet and Jeanjean 1989) have also encountered this problem. Three isolates either belonged to DNA group 2 or DNA group 13. However, these isolates were later identified as belonging to DNA group 13 through PFGE. Four isolates could not be assigned to any DNA group. Hence, only 89% of them were identified correctly among all the isolates and 11% of isolates were not identified. This percentage is high when compared with the earlier studies (78%)(Gerner-Smidt et al., 1991). However, our investigation had less number of
isolates. In addition, two schemes were adopted for identification along with biotyping of Acb-complex.

The most important tests for biotyping clinical isolates of *A. baumannii* seem to be assimilation reactions. Bouvet and Grimont (1986) used this for elucidating the taxonomy of *Acinetobacter*. They also devised a biotyping system for *A. baumannii* (Bouvet and Grimont, 1987) based on the assimilation of levulinate, citraconate, L-phenylalanine, 4-hydroxybenzoate, and L-tartrate. Many investigators have used this system for biotyping of the whole Acb-complex (Bouvet et al., 1990; Gerner-Smidt et al., 1991; Dominguez et al., 1995; Bello et al., 1997).

Most of the studies consider *A. baumannii* isolates as a homogeneous taxonomic group (Bergogne-Berezin and Joly-Guillou 1986; Okpara and Maswoswe, 1994), even though 19 biotypes within *A. baumannii* have been recognized (Bouvet and Grimont, 1987). A comparative study of the properties of various biotypes along with their incidence and prevalence may be more meaningful. In the present study, *A. baumannii* and closely related DNA groups such as 1,2,3, and 13 (Acb-complex) were typed using biotyping scheme of Bouvet and Grimont (1987). A total of 42 Acb-complex strains were subjected to biotyping. Biotype 10 and biotype 6 were most prevalent in this geographical area. Biotype 10 has not been encountered frequently in other studies (Tankovic et al., 1994; Dominguez et al., 1995; Bello et al., 1997; Nemec et al., 1999). Six strains belonged to biotype 9. Biotype 16 and biotype 11 constituted 4 and 3 strains respectively. Biotype 2, 3, 7, 8, 15, 19 all comprised of two strains each (Graph-14.1). Biotypes 6, 9, 10, 15 were all responsible for the outbreaks. These results were quite different from various other studies wherein
biotypes 1, 2, 3, 8, 11 were predominantly responsible for many outbreaks (Tankovic et al., 1994; Dominguez et al., 1995; Bello et al., 1997; Nemec et al., 1999). In Chile, *A. baumannii* Biotype 6 incidence was less frequent when compared with Biotype 9 and 8, which contributed 57% and 24% of nosocomial infections respectively (Dominguez et al., 1995). There were quite different observations in our investigations where biotype 10 and 6 were involved in maximum number of nosocomial infections. However, in two studies biotypes 6 and 9 represented majority of strains (Bouvet and Grimont, 1987; Seifert et al., 1993). Only one study reported highest incidence of Biotype 11 (Nemec et al., 1999). Interestingly, in a study from Pune, India, biotype 7 constituted maximum number of strains followed by biotype 2 (Chopade et al., 1994). This observation confirms that varying distribution of different biotypes of *A. baumannii* occur in different regions. Moreover, there is a possibility that such variation occurring in different locals of same geographical area. While comparing the properties of *A. baumannii* isolates belonging to the same biotype, the frequent biotypes 6 and 9 were found to comprise genotypically heterogeneous isolates (Seifert et al., 1993; Nemec et al., 1999). However, with our isolates there was more heterogeneity in biotype 9 isolates, whereas all biotype 6 isolates were indistinguishable by CEPA and PFGE typing i.e they belonged to B1 and VII respectively.

Thus, biotyping system suggested for *A. baumannii* (Bouvet and Grimont, 1987) was helpful in differentiating the groups within the *Acb*-complex. This biotyping system allows the differentiation of all 19 biotypes. In the present study only 11 biotypes were encountered. However, the discriminatory power of biotyping
is not sufficient enough, especially because only few biotypes like biotypes 6, 9, 10 and 15 have been involved in hospital outbreaks. Although to a larger extent biotyping proved to be a stable marker to detect the course of the outbreak in our study.

In nosocomial outbreaks, the simplified identification schemes of Bouvet and Grimont (1986) and Gerner-Smidt et al. (1991) along with biotyping is very useful in typing strains. Different approaches using various schemes for phenotypic identification (including biotyping of Acb-complex) in the present study yielded a good discriminatory power. When all the isolates were included for the DI calculation, a DI of 0.93 was obtained. When problematic strains, which were selected for typing by other molecular methods, were only analyzed, the DI decreased to 0.91. Inclusion of additional method of typing to phenotyping increased the discriminatory index (Table-14.13). Thus, phenotyping results would be more meaningful and accurate if it is used in conjunction with protein profiles or antibiotyping. We were able to detect three outbreaks occurring in ICU during the first 16 months of the study period with the help of this simple identification scheme (Prashanth and Badrinath, 2000). Later protein profiles substantiated these results. Protein profiling can be used for the relative classification of strains in well defined clinical situations, as it was found useful for hospital epidemiology rather than for a general classification of acinetobacters (Dijskhoorn et al., 1987). In conclusion, for epidemiological purposes it may be very essential to identify the genus Acinetobacter spp. up to the species and clonal level. However, it may not be possible for many clinical laboratories with minimal facilities, to perform the molecular techniques and
large panel of assimilation tests in routine practice. Hence, a presumptive identification based upon growth at 44°C, 41°C and 37°C along with acid production from glucose would help in the identification of Acb-complex. In addition, biotyping of Acb-complex using only 5 assimilation tests seem to be good alternative methods for these laboratories, which could be suffice under the present circumstances.

16.3 Antibiotyping:

Antibiogram typing is probably the most widely used method for typing Acinetobacter spp. It is possible to recognize an outbreak on the basis of constant monitoring of the resistance profiles of Acinetobacter isolates (Biendo et al., 1999). Although antibiotyping may alert us to the emergence of a multiresistant Acinetobacter outbreak, distinguishing between strains with slight differences in their resistance profiles may be difficult. However, clustering of isolates can still be achieved by interpreting MICs, breakpoints or zone diffusion sizes very cautiously. Numerous studies have used antibiotic susceptibility patterns to detect emerging resistance and to group similar isolates (Allen and Green, 1987; Bergogne-Berezin and Joly-Guillou, 1985; Vila et al., 1989; Joly-Guillou et al., 1991; Struelens et al., 1993; Thurm and Ritter, 1993). Often this clustering is on the basis of MICs, breakpoints or zone diffusion sizes. In the present study, distribution of the MICs and inhibition zones of each isolate was studied by means of histograms (Gerner-Smidt, 1994) and interpreted. Six antibiotypes were observed among all the Acinetobacter isolates, when tested for their antimicrobial susceptibility for 5 important second line drugs that are commonly used in this hospital (A-F). Antibiotype A had resistance for
all broad antimicrobials tested, such as cefotaxime, ceftazidime, amikacin, ciprofloxacin and ofloxacin. Antibiotype A was responsible for most of the outbreaks in the present study. This is in concordance with other hospitals where A. baumannii showing resistance to almost all broad-spectrum antibiotics except imipenem has been implicated in hospital outbreaks (Struelens et al., 1993; Tankovic et al., 1994). Antibiotype B group of isolates had resistance to only cefotaxime and were sensitive to others. Antibiotype C group of isolates were resistant to ceftazidime, amikacin, cefotaxime and were susceptible only to quinolones. Antibiotype D group of isolates were susceptible to ceftazidime, amikacin, cefotaxime and showed resistance to both the quinolones tested. Group E isolates were susceptible to all the antibiotics tested. Antibiotype F had resistance to amikacin and ciprofloxacin only. Three resistant antibiotypes (A, C, D) were common among the outbreak isolates (Table-14.9). Six A. baumannii biotype 6 isolates showed the antibiotype A and one the antibiotype D. Those isolates classified as A. baumannii biotype 9 or Acinetobacter genospecies 13 exhibited antibiotypes A and C. A. baumannii biotype 10, the most common biotype found in the present study exhibited different antibiotypes A, C, D. However, all the outbreak isolates in this biotype 10 were of antibiotype A. Two isolates of A. baumannii biotype 15 which represented an outbreak showed antibiotype E. These two isolates slightly differed from rest of the antibiotype E isolates by exhibiting intermediate susceptibility to cefotaxime. All the outbreak strains exhibited resistance antibiotic profiles except antibiotype E. Many other studies also reported multiple resistance in outbreak strains (Thurm et al., 1993; Ratto et al., 1995; Webster et al., 1999).
There were only six clusters of antibiotypes in the present study, indicating less diversity in our antibiotic profiles. This is true when compared to the study of Webster et al., (1999) where 19 clusters were reported in a South African hospital. The same study also referred to Nottingham hospital, which had few clusters and a single multiresistant strain of A. baumannii accounting for over 50% of the isolates recovered over a period of 11 years. The contrast in strain diversity found in the two hospitals located at different geographical areas provides an important illustration for remarkable ability of Acinetobacter to gain as well as to loose the resistant genes according to environment in which it is thriving. However, many studies have also reported only few antibiotypes (Dijkshoorn et al., 1993; Ratto et al., 1995; Biendo et al., 1999). Among those few antibiotypes in their investigations, one multiple drug resistant antibiotype was a common feature. This was again a similar finding with that of our investigation.

Antibiotyping was helpful in typing the Acinetobacter species, although it has many drawbacks like risk of similar isolates being placed in different clusters due to the natural variability of the testing method, coincidence of the therapeutic and typing breakpoints in many instances and lastly, antimicrobial resistance markers are not always stable (Gerner-Smidt, 1994). To overcome the first two drawbacks, the present study used histograms for distribution of the MICs and inhibition zones of every isolate and clustering was performed based on this methods (Dijkshoorn et al., 1993).

Although, antibiotyping independently was less helpful in the epidemiological study of Acinetobacter species, it was helpful in initial detection of outbreak. Most
strains belonging to the Acb-complex showed multiple resistance as compared to the strains belonging to other DNA groups. All strains of A. baumannii biotype-6 showed resistance to almost all antibiotics tested. However, they had their uniqueness in showing susceptibility or intermediate profile for chloramphenicol. This may be due to loss of transposon that encodes for chloramphenicol resistance that might have resulted due to nonuse of this drug in our hospital for over a decade. Similar phenomenon has often been seen in other Gram-negative bacteria (Elisha and Steyn, 1991). This transposon may be foreign in origin acquired from different bacteria. Some of these strains were responsible for the outbreaks showing unique resistance patterns that were clearly distinguishable from all other strains. Minor variations were observed among outbreak isolates and were difficult to interpret without the help of a complementary typing system. In addition the discriminatory power of this method was moderate (DI=0.76) when compared to the other typing methods. However, an addition of any one typing technique to this method increased the discriminatory power. Thus, antibiotyping was also helpful in characterizing Acinetobacter isolates in conjunction with phenotypic identification and protein profiling. It can be suitably used for screening method in epidemiological investigations but it may require confirmation of results by other complementary techniques.

16.4 Cell Envelope Protein Analysis:

Whole cell protein as well as cell envelope protein patterns have been used in a number of epidemiological and taxonomic studies of Acinetobacter species
(Alexander et al., 1984; Dijkshoorn et al., 1987a, 1987b; Crombach et al., 1989; Dijkshoorn et al., 1993; Weernink et al., 1995). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) protein profile typing is a useful technique for the relative comparison of strains in small scale epidemiological surveys. Analysis of cell envelope protein patterns (CEPA) by SDS-PAGE has shown heterogeneity in unrelated strains, but multiple isolates from patients or outbreaks were indistinguishable as reported elsewhere (Dijkshoorn et al., 1987a, 1987b).

There was a great heterogeneity of cell envelope protein profiles in the strains investigated in the present study also, although we were able detect similar profiles on few occasions. Totally, 18 different banding patterns were observed in all the phenotypically characterized Acinetobacter isolates obtained from clinical specimens and environmental samples. In conjunction with biotyping and antibiotyping present study was able to trace four outbreaks occurring at different time intervals during the study period. Protein profiling has been successfully used to trace specific strains during endemic episodes and outbreaks in different hospitals (Crombach et al., 1989; Dijkshoorn et al., 1993; Weernink et al., 1995). In the present investigation, protein profiling traced endemic episodes due to two strains of A. baumannii (Patterns B3, B4) that were causing infection sporadically as and when there is breakdown of optimal infection control procedures in ICU.

Electrophoretic analysis of whole-cell protein fractions has the advantage that the sample preparation is simpler than preparation of cell envelopes. However, whole cell protein fractions produce more number of bands and sometimes difficult to interpret. Similarities between Acinetobacter isolates from outbreaks and
dissimilarities in unrelated control strains have been reported in different studies (Alexander et al., 1984, 1988; Mortensen et al., 1987). Isolates having protein profiles B1, B2, B5 and B6 were implicated in the outbreaks in the present study. Protein profiles of strains involved in the outbreaks (B1, B2, B5 and B6) showed similar and identical patterns and were clearly different from strains belonging to different groups that were epidemiologically unrelated. This was in concordance with investigations performed in various other hospitals (Alexander et al., 1984, 1988; Mortensen et al., 1987; Crombach et al., 1989; Dijkshoorn et al., 1993; Weernink et al., 1995).

Biotypes with in the Acb-complex could be distinguished by observing the minor differences in the overall banding patterns. However, accurate identification and correlation with biotyping was not accomplished on few occasions. Among 35 A. baumannii isolates five unique identical clusters were encountered with minor detectable differences (B1-B5). Remaining isolates of A. baumannii were grouped simply as cluster B that included many biotypes, which were similar but not identical. These minor differences in the profiles may be attributed many local conditions including minor changes in growth conditions. There were major differences in patterns of other species when compared to A. baumannii. By comparing standard reference strain profiles and biotyping results, the isolates of profiles B1, B2, B5 tested belonged to DNA group 2 (A. baumannii, biotypes - 6, 10 and 15 respectively). However, strains exhibiting profile B6 were classified as either A. baumannii biotype 9 or Acinetobacter genospecies 13 (strains that were identified as biotype 9 from biotyping), since there was no much difference in this cluster of patterns with that of
B cluster. Profile A represented *Acinetobacter lwaffii*. Patterns B3, B4, were isolates that belonged to DNA group 2 (*A. baumannii*) biotypes 19 and 16 respectively. Other profiles were seen in single or in a few strains. The discriminatory power of this method was good with a DI of 0.89 in the present study.

However, like other phenotypic based methods, apparent differences between isolates from common origin should be interpreted with caution as few isolates in our study originating from a common source showed different profiles. Protein profiling can be used for the relative classification of strains in well defined clinical situations, as it was found useful for hospital epidemiology rather than for a general classification of acinetobacters (Dijkshoorn et al., 1987). Protein profiles though able to presumptively identify DNA groups, when performed alone is insufficient to judge the epidemiological data and to elucidate the outbreaks, hence it should be used in conjunction with other typing methods (Dijkshoorn et al., 1993). According to Achtman et al (1983), membrane protein patterns and other parameters such as biotype are not casually related. In their view, protein profiles are more suitable for identifying clonal relationship. These findings should not be ignored, since all the protein patterns obtained in the present study were stable. In addition, it was found that there is a relationship with particular geographical sources for some protein patterns (Dijkshoorn et al., 1987). These observations were in concordance with our investigations. Altogether electrophoretic protein profiling is very useful and a combined use with any other method could contribute to the identification of strains of epidemiological and clinical importance.
16.5 Arbitrarily primed PCR (AP-PCR):

The most widely used PCR-based epidemiological method to date is commonly referred to as either randomly amplified polymorphic DNA (RAPD) or arbitrarily primed PCR (AP-PCR). However, improved and newer PCR based methods have also evolved recently like the multiplex PCR, ERIC-PCR, REP-PCR, infrequent-restriction-site PCR (IRS-PCR) and amplified fragment length polymorphism (Liu et al., 1997; Grundmann et al., 1997; Janssen et al., 1997; Jin-Hong Yoo et al., 1999). AP-PCR still enjoys greater acceptability owing to its simplicity and feasibility. Arbitrary primed polymerase chain reaction is based on random amplification DNA fragments (RAPD) with a single primer of arbitrary nucleotide sequence. AP-PCR is a simple and rapid method with a high discriminatory power, which can be used as a complementary technique for the epidemiological study of clinical isolates of Acinetobacter (Vila et al., 1994; Vaneechoutee et al., 1995). In the present study, only significant isolates were subjected to random polymorphic DNA fingerprinting. AP-PCR was done for 28 selected strains, which included clinical and environmental strains. Reference strains of clinically important DNA groups were also subjected to PCR in conjunction with M13 forward primer and M13 reverse primer. M13 primer has been used by many authors earlier and it seems to be good in generating more distinct fingerprints (Vila et al., 1994; Grundmann et al., 1997). Earlier, Graser et al. (1993) successfully used amplified DNA from A. baumannii with the core sequence of the M13 phage as a single primer.
In this study, eight different banding patterns were distinguished, comprising 2 to 6 DNA fragments of 0.1 to 1.2 kb in size. These banding patterns were designated using small case alphabets in bold as a-h. The results from one assay to another were reproducible which is in concordance with other studies (Graser et al., 1993; Grundmann et al., 1997). However, the intensity of the bands varied occasionally as it was true with many other documented studies (Reboli et al., 1994; Grundmann et al., 1997). Epidemiologically related strains showed identical patterns and were clearly distinct from that of unrelated isolates. PCR profiles of strains involved in outbreaks showed the same pattern for strains of the same group (a, d, g & h), but were clearly different for strains belonging to different groups (b, c, e, f). M13 primers very well discriminated the epidemic and endemic strains of A. baumannii in the present study. Similar findings have been reported elsewhere (Reboli et al., 1994). However, on few occasions the difference within the biotypes A. baumannii could not be detected by AP-PCR technique. This is in contrast with that of earlier study wherein RAPD was able to discriminate all the biotypes studied (Garcia et al., 1996). UPGMA clustering dendrogram of PCR profiles created using dice coefficient, of Acinetobacter isolates obtained from endemic, epidemic infections as well as environmental origin are shown in the figure 14.8. This cluster diagram clearly demonstrated the unique clusters among outbreak and endemic isolates. Comparisons of relative front and dice similarity coefficient values of different species belonging to different DNA groups are depicted in the graphs 14.5 and 14.7. Graphs were elucidated from the values generated from the densitometric analysis. Densitometric scan can detect any two DNA fragments of same size comigrated at the
same position (Dolzani et al., 1995). Thus, densitometric scan in this study was able
discriminate closely related genospecies of Acb-complex. Identical strains
representative of the outbreak at different time intervals are shown in graph 14.8.

The discriminatory index (DI) for different primers used in PCR
fingerprinting was thoroughly investigated in one study (Liu et al., 1997). It was 0.71
for M13, 0.75 for ERIC1, 0.77 for REP-PCR. The multiplex PCR had a 0.87 DI in
this study. However, M13 primer had a better DI of 0.87 in another study (Vila et
al., 1996). Similar results were obtained in the present study where in for AP-PCR
the DI was 0.88. Many comparative studies using different PCR-based methods
confirm Amplified ribosomal DNA restriction analysis (ARDRA) has least DI and
REP amplification has maximum discriminatory power for the identification of
Acinetobacter genomic species (Vila et al., 1996; Koeleman et al., 1998). Although
the PCR approach is quicker and less labour intensive than PFGE, the latter is more
discriminative especially for differentiating genetically related clonal subtypes (Liu et
al., 1997).

In conclusion, AP-PCR is a simple and rapid technique with a high
discriminatory power and had good agreement with other molecular methods
employed in the present study. This method can be used as a complementary
technique for the epidemiological investigations of Acinetobacter clinical isolates.

16.6 Pulse Field Gel Electrophoresis:

Pulse field gel electrophoresis (PFGE) is the method of choice for the
epidemiological analysis of most pathogens of clinical concern. It is also regarded as
'gold standard' and a technique of third-generation in molecular epidemiology
(Arbeit, 1999; Goering, 2000). The chromosomal macro-restriction patterns resulting from PFGE analysis are based on the spatial distribution of rare, repeated, restriction sequences around the bacterial chromosome (Goering, 2000).

Pulse field gel electrophoresis technique was used first time by Alladert-Servent et al. (1989), to investigate an A. calcoaceticus outbreak in a urology department and bronchial colonization of artificially ventilated patients by Pseudomonas aeruginosa in an ICU. This method allowed a clear distinction between epidemic and self-contaminating strains in different epidemiological conditions. Twenty-eight phenotypically characterized representative Acinetobacter strains were subjected to PFGE in the present study. Earlier, few studies have already established the diversity of PFGE-generated Apal profiles obtained from Acinetobacter species (Gouby et al., 1992; Graser et al., 1993). The fingerprints generated by macro-restriction with either Smal or Apal in this study comprised of approximately 20 to 25 bands with sizes approximately 5 to 300 kb. According to the interpretive criteria of Tenovar et al (1995), eight and nine distinct PFGE patterns were distinguished among these 28 isolates of Acinetobacter by using Smal and Apal, respectively. PFGE generated 15 to 25 bands per strain. By using Smal, 8 patterns (I to VIII) could be detected. By using Apal, one more additional pattern (IX) could be detected. Among A. baumannii (DNA group 2) strains 6 & 5 major restriction patterns could be distinguished using Apal and Smal respectively. DNA group specific PFGE profiles could be established in the present study, which was in contrast with the study conducted by Seifert et al (1995).
In this study, the results of PFGE analysis confirmed those of biotyping and showed that four distinct group of strains, designated I, IV, VII & VIII, were involved in the outbreaks. Outbreak strains were clearly distinguished from epidemiologically unrelated strains by PFGE micro-restriction. Isolates with identical restriction profiles were assigned to same type. Isolates that differed by one or two bands were considered as epidemiologically related (Tenovar et al., 1995). All unrelated strains demonstrated distinct PFGE profiles. There were marked restriction polymorphisms among all these profiles. Earlier, Gouby et al. (1992) demonstrated considerable DNA polymorphisms among A. baumannii strains isolated in different parts of the world. Seifert et al. (1994) observed similar results in their study. Isolates obtained during months of October 1996 and September 1997 from RICU, which were responsible for the prolonged outbreak in ICU gave virtually indistinguishable pattern (PFGE type VII).

All isolates demonstrating identical PFGE profiles by ApaI were regarded as, belonging to same biotype. Conversely, when SmaI was used two strains belonging to different biotype showed same PFGE pattern (biotype 6 and 7). In the present study ApaI digestion resulted in more diverse banding pattern than SmaI. Even the study by Seifert et al. (1994) showed a similar diversity wherein each epidemiologically unrelated strain exhibited a unique banding pattern it is therefore assumed that isolates with identical or almost identical patterns represent a single clone. One distinct pattern was found for Acinetobacter DNA group 3 strains in this study. Two DNA group 13 strains showed difference in one band shift and were regarded as only one PFGE pattern. Among A. lwoffii all the strains exhibited a single pattern. All
outbreak related strains were accurately identified (I, IV, VII & VIII). Outbreak strains were clearly distinguished from epidemiologically unrelated strains by PFGE macro-restriction (Figure 14.10). Epidemiologically unrelated representative strains showed distinct PFGE types. These strains were easily distinguishable from one another and from those of outbreak isolates. These results were almost similar to that of Christie et al. (1995).

Although the results of macro-restriction with SmaI and ApaI were similar, the PFGE after genomic DNA digestion with ApaI had a higher discriminatory index of 0.96 in this study. This was similar to the results of Liu et al (1997). In contrast, Siefert and Gerner-Smidt (1995) found that their isolates were more typable by PFGE using SmaI for restriction, than that using ApaI. On repeat testing, patterns were reproducible in the present study. The PFGE profiles of each isolate on two different occasions were also consistent. Reproducibility as a property allows this technique to be useful for delineation of the clonal relationship of different isolates by dendrogram analysis (Liu et al., 1997).

PFGE has been used by many groups to study epidemiological aspects of Acinetobacter infections (Allardet-Servent et al., 1989; Gouby et al., 1992; Seifert et al., 1994; Struelens 1993). In one of the recent study (Christie et al., 1995), PFGE typing showed that 59 (83%) of 71 typed isolates had distinct patterns. This shows the degree of diversity that this technique can have. Three small clusters of isolates with the same PFGE patterns were observed in the present study, suggesting cross-transmission. Although there were isolated instances of cross-transmission, most
isolates had distinct PFGE patterns. PFGE typing can be useful in directing infection control efforts.

The discriminatory power of PFGE was high in many studies. Marcos et al. (1995) compared PFGE with other typing methods such as biotyping, plasmid profiling, whole cell protein analysis, PCR and antibiotyping and observed that the discriminatory power of PFGE was best with DI of 0.9623. Present investigation also showed PFGE having a very high discriminatory power with DI of 0.96.

PFGE technique was shown to be most suitable method for differentiating strains from hospital outbreaks. However, PFGE is laborious, time consuming and in addition it is very expensive. PFGE may be reserved for situations in which clinical and other conventional typing methods data (biotyping and antibiotyping) are inconclusive.

16.7 Antimicrobial susceptibility testing:

Resistance patterns among gram-negative nosocomial bacterial pathogens may vary widely from country to country at any given point and within the same country over time (Colombian antimicrobial resistance study group, 1999). Because of these variations, a surveillance of important nosocomial pathogens for antimicrobial susceptibility is needed for every country in order to guide appropriate selection of empiric therapy. Antimicrobial susceptibility monitoring could also be a primary pointer for the emergence of an outbreak. Detection of resistance for important drugs in a particular pattern may suggest an ongoing epidemic in the hospital.
However, antibiogram may not be sufficient to distinguish two strains that are responsible for outbreaks (Tankovic et al., 1994).

Antibiotic resistance is a major problem for patients infected with all *Acinetobacter* species, especially those with *A. baumannii* (Devaud et al., 1982; Tjernberg, 1990; Seifert et al., 1993; Bergogne-Berezin and Towner, 1996). This resistance affects the selection of appropriate antibiotics for treating such patients. However, a limited amount of data have been published on the in vitro susceptibility of clinical isolates of *A. baumannii* all over, and limited data are available regarding this in India (De and Deodhar, 1995; Chopade et al., 1994a & b; Pandey et al., 1998; Prashanth and Badrinath, 2000). Only few studies reported the efficacy of antibiotic combinations against this problematic pathogen (Glew et al., 1977; Chang et al., 1995; Pandey et al., 1998; Appleman et al., 2000). The first line antibiotic therapy for *Acinetobacter* infections adopted now includes amikacin, imipenem, ceftazidime, or a quinolone (Joly-Guillou et al., 1992). In 56% of cases, imipenem was prescribed either as a single agent or in combination with amikacin (18%)(Joly-Guillou et al., 1992). Imipenem monotherapy was also proved to be effective in one study (Muller-Serieys et al, 1989). However, most of the recent studies recommend combination therapy in present context to avoid further development of resistance to imipenem, the antibiotic once regarded as drug of choice *Acinetobacter* infections (Joly-Guillou et al., 1992; Bergogne-Berezin et al., 1996; Bauernfeind et al., 1997; Brown et al., 1998). In the present study, imipenem testing was not included due to following reasons. Firstly, the non-availability of the drug in the pure form and second, this drug has not been commercially introduced in India.
The first part of this study (Prashanth and Badrinath, 2000), had twenty-eight isolates characterized, for their antimicrobial susceptibility when tested showed that the strains of *Acb*-complex could be clearly distinguished from that of non-*Acb* phenotypes by the antimicrobial susceptibility pattern. Though it did not help much in the delineation of species, it gave a clear indication of possible grouping of strains between *Acb*-complex and other DNA groups by showing multiple resistance in *Acb* when compared to others, which is characteristic of *Acb*-complex strains (Bergogne-Berezin and Towner, 1996; Nemec et al., 1999). However, *Acb*-1 and *Acb*-2 within *Acb*-complex (later identified as biotypes 6, 19, 16) were more resistant than other *Acb* strains, as the other phenotypes were susceptible to three or more antibiotics tested and hence there was no steep increase in overall percentage of resistance in *Acb*-complex. Varying resistance pattern similar to this were also observed earlier (Bello et al., 1997), highlighting that not all biotypes of *Acb*-complex are multidrug resistant. Disk diffusion technique showed that members of *Acb*-complex were resistant to almost all antibiotics except for newer aminoglycosides and cephalosporins viz amikacin, netilmicin and ceftazidime. Comparatively high level of resistance was noted against amikacin (48%). This is probably due to extensive use of this antibiotic in the ICU. Ceftazidime was one of the second line antibiotic used in our hospital to treat infections due to multidrug resistant gram-negative bacteria. Ceftazidime was also most efficacious drug in this study as only 24% of strains were resistant, which could be attributed to its judicious use. In concordance with that of earlier studies (Bello et al., 1997) the present study also had strains of *Acb*-complex encountered showing resistance to all drugs tested, which is of much concern as infection caused by them pose great therapeutic problem. It is
surprising that the level of resistance development for cefotaxime was moderate even though it was the commonest antibiotic used in our hospital. Although both outbreak phenotypes 1 and 2 of Acb-complex (later identified as A. baumannii biotype 10 and 6 respectively) showed resistance to almost all the antibiotics tested by both the methods, strains of Acb-1 phenotype were distinct through their susceptibility to amikacin and netilmicin unlike strains of Acb phenotype-2.

Second phase of study included another 38 isolates obtained from both clinical and environmental sources. The antimicrobial susceptibility results of the total 66 isolates are interpreted and discussed in the following sections. Kirby-Bauer disc diffusion method results showed high levels of resistance for ampicillin (85%), cefozolin (92.2), gentamicin (63%), piperacillin (88%) and norfloxacine (69%). Almost all the strains were resistant to cephalaxin (98%). Older agents such as ampicillicin, carbenicillin, first and second generation cephalosporins which were used in 1970s to treat Acinetobacter infections are now not effective against this bacterium (Traub and Spohr, 1989; Tjernberg, 1990; Seifert et al., 1993). Present study also documented higher resistance for these older agents and as a consequence these antibiotics are useless in treating Acinetobacter infections.

Amikacin and netilmicin showed maximum level of activity with susceptibility of 55 & 60% respectively. Netilmicin was superior than amikacin among aminoglycosides, perhaps because this is least used in this hospital. This kind of observation was in concordance with the investigation of Cisneros et al (1996). Intermediate levels of susceptibility were encountered more with cefotaxime (37.5%)
and ceftazidime (30%). There was a decrease in susceptibility to both cefotaxime and ceftazidime in later part of the study when compared with initial study. These results were very similar to that of some of the earlier multicentric studies (Colombian antimicrobial resistance study group, 1999). Resistance of Acinetobacter spp to cephalosporins has been attributed to chromosomely encoded cephalosporinases (Hood and Amyes, 1991). The synthesis of some extended spectrum β-lactamases has also been suggested in A. baumannii (Joly-Guillou and Bergogne-Berezin, 1990). Present study has been unable to check this in our isolates. In our study we found that amikacin and ceftazidime were still active against most of the Acinetobacter strains irrespective of species they belonged to, unlike other studies. Although we noted that the MIC ranges of our strains were higher than those found in other studies (Chang et al., 1995).

Netilmicin and amikacin, which showed maximum activity with an overall, low level resistance of 32.8% and 34.4% respectively. Strains of Acb-complex were found to be more resistant to all the antibiotics tested as compared to A. Iwoffii, A. hamolyticus, A. junii and strains of other DNA groups. Piperacillin, gentamicin and amikacin showed highest activity against the Acinetobacter species other than A. baumannii. Only netilmicin and amikacin were effective against A. baumannii (Table-14.10).

The MIC results for the Acinetobacter isolates showed amikacin and ceftazidime having highest activity with susceptibility percentage of 60% and 58% respectively. However, 17 strains of A. baumannii showed high level resistance to amikacin (>128ug/mL) and only six strains showed MIC>64ug/mL for ceftazidime.
This is inconsistent with the results reported from Turkey and Greece, where 67.5% and 96% resistance respectively were shown for ceftazidime (Turkish antimicrobial resistance study group, 1999; Sofianou et al., 2000). In a recent investigation conducted in New York City, higher susceptibility rates for amikacin (87%) has been attributed to the decline in its use (Manikal et al., 2000). High frequency of resistant isolates were found against ciprofloxacin and cefotaxime (58% and 42% respectively) in the present study. Even though amikacin was effective the MICs for amikacin among Acinetobacter isolates were higher. The MIC<sub>50</sub> & MIC<sub>90</sub> of amikacin were 4 & >256 µg/mL respectively. Among the cephalosporins, ceftazidime was superior to cefotaxime, as their MIC<sub>90</sub> was 4 and 32 µg/mL respectively. Very Poor activity was exhibited by ciprofloxacin (MIC<sub>50</sub>-8, MIC<sub>90</sub> - 256 µg/mL). Maximum number of isolates (n=38) were resistant to ciprofloxacin in the present study as compared to other antimicrobial agents tested.

High percentage of isolates belonging to A. baumannii were resistant to ciprofloxacin, ofloxacin and cefotaxime (79%, 76% & 54% respectively) by agar dilution method. As far as susceptibility of A. baumannii to quinolones was concerned, our strains were comparatively more resistant than the strains reported from studies in Chile (Bello et al., 1997; Chang et al., 1995). Chang et al (1995) reported highest activity of quinolones against A. baumannii having susceptibility percentage of 97.8. In contrast, quinolone resistance was quite high in our study. This was very similar with the results obtained in a German study (Traub et al., 1990; Seifert et al., 1993). These may be explained by the fact that ciprofloxacin was
introduced in late 1970's in our region against Salmonella typhi, and even now it is the most extensively used antibiotic in our hospital. Half of our A. baumannii isolates were resistant to amikacin (50% resistance). Chang et al. (1995) reported comparatively higher susceptibility (74.5%) among A. baumannii strains for amikacin. Amikacin was introduced in our hospital only in 1997. The development of moderate resistance with in a short period may be due to extensive use of this drug in our intensive care unit. In comparison less resistance levels were seen with Ceftazidime (37%). However, both ceftazidime & cefotaxime had more isolates showing intermediate range of susceptibility (20%). This relatively high frequency of A. baumannii isolates with moderate resistance in fact anticipates future problems with increased development of resistance as a consequence of uninterrupted indiscriminate use of these antimicrobials.

The proportion of strains susceptible to ceftazidime irrespective of different species of Acinetobacter was only 58% at ≤ 32μg/mL. This is slightly lesser than earlier reports by Visalli et al (71%) (1997) for strains collected from The Netherlands, Pennsylvania, Ohio, California, Switzerland, and Jones et al (63%) (1997) for strains collected from 50 U.S medical centers. All other isolates (n=31) belonging to the groups other than DNA group 2 (A. baumannii) were susceptible to all the antibiotics tested except ciprofloxacin, where a comparatively high resistance level (45%) was recorded. Interestingly, irrespective of species or different DNA groups of Acinetobacter tested, all our isolates showed higher resistance to ciprofloxacin (58% resistance overall; 79% among A. baumannii). This was in concordance with the study of Jones et al. (1997). However, ofloxacin was slightly
more active than ciprofloxacin against *Acinetobacter* species in our study as there was one fold decrease in MICs for ofloxacin as against ciprofloxacin.

Most of the studies consider *A. baumannii* isolates as homogeneous taxonomic group (Bergogne-Berezin and Joly-Guillou 1986; Okpara and Maswoswe, 1994), even though 19 biotypes within *A. baumannii* have been recognized (Bouvet and Grimont, 1987). A comparative study of the properties of various biotypes along with their incidence and prevalence will be meaningful. Resistance patterns of different biotypes may help in the initial tracing of an epidemic. In the present study it was found that biotype 10 and biotype 6 were most prevalent. Biotype 10 has not been encountered frequently in other studies (Tankovic et al., 1994; Dominguez et al., 1995; Bello et al., 1997; Nemec et al., 1999). High frequency of resistant isolates of *A. baumannii* mainly of biotypes 6 and 19 were found (Graph-14.15) as compared to Biotype 9 in our study. This is in contrast with the study of Seifert et al. (1993) from Germany wherein they reported isolates of Biotype 9 of *A. baumannii* were more resistant as compared to Biotype 6. In fact, in one study more than 70% of Biotype 9 and 8 isolates were resistant to third generation cephalosporin, whereas most isolates of Biotype 6 were susceptible (Bello et al., 1997). Almost all isolates of Biotype 6 were resistant to amikacin and ciprofloxacin in the present study, where as Bello et al. (1997) had highest frequency of susceptible biotype 6 isolates for amikacin and ciprofloxacin in their collection, accounting for 85% and 80% susceptibility respectively. Comparatively high level resistance was observed among Biotype 10 isolates (50%). All other biotypes were susceptible to most of antibiotics except ciprofloxacin and ofloxacin. However, Bello et al. (1997) reported that 'other
biotypes' were more susceptible to ciprofloxacin. Since prevalent biotypes of *A. baumannii* differ according to the country in which the study has been conducted and it is evident from the above description, an additional and more exhaustive comparative information dealing with antimicrobial susceptibility and other properties of isolates of different biotypes of *A. baumannii* is needed.

In summary, isolates of *A. baumannii* from patients in JIPMER Hospital, Pondicherry, India were generally more resistant to quinolones, β-lactam antibiotics, first and second-generation cephalosporins and partially resistant to third generation cephalosporins and aminoglycosides. The strains belonging to other DNA groups of genus *Acinetobacter* were comparatively less resistant than *A. baumannii*, except ciprofloxacin. However, despite such resistance, combination therapy using a third generation cephalosporin and amikacin could be the best choice for treating *Acinetobacter* infections in this hospital.

16.8 Clinical investigations:

In the past two decades, *Acinetobacter* spp. has emerged as an important nosocomial pathogen that is often resistant to many broad-spectrum antimicrobials. It is also associated with life-threatening infections in patients with altered host-defense mechanisms (Bergogne-Berezin and Towner, 1996). In addition *Acinetobacter baumannii*, a clinically important species has a tendency toward cross-transmission, particularly in ICUs, where numerous outbreaks are encountered (Bergogne-Berezin and Towner, 1996; Beck-Sague et al., 1990; Struelens et al., 1993; Bou et al., 2000). Nosocomial respiratory infections or colonization due to *Acinetobacter* species in mechanically ventilated patients in ICU setting was also high during 1993 to 1996 in
JIPMER hospital (44.7%), India (Dutta TK, unpublished data). In one recent study high proportion of *Acinetobacter* spp (35%) was responsible for ventilator associated pneumonia, and it was predominant among all the other bacteria encountered (Sofianou et al., 2000). Only few investigations have identified various risk factors for *A. baumannii* infection or colonization with their case-control studies (Lortholary et al., 1995; Mulin et al., 1995; Villers et al., 1998; Gomez et al., 1999).

Univariate analysis of the cases to assess the prognostic and risk factors associated with *Acinetobacter* infection revealed some important findings in this study. All factors significantly associated with a bad prognosis are shown in Table 14.4. No statistical significance was found in relation to sex, age, hospital stay, prior surgery or with prior use of broad-spectrum antibiotics. This is in contrast with earlier studies (Villers et al., 1998; Gomez et al., 1999), where prior use of broad-spectrum antibiotics was the main risk factor. Inappropriate antibiotic therapy was associated with death in 23% of our patients. This percentage is quite less when compared with figures (48%) reported by Gomez et al (1999). However, identification of inappropriate antibiotic therapy as a poor prognostic factor may help in lessening its contribution for infection in the due course of time in the hospital. Mechanical ventilation was associated with higher mortality ($P = 0.02$) in our investigation. This observation is quite similar with the studies of Seifert et al. (1995) and Cisneros et al (1996) and different from the investigations of Gomez et al (1999). The resistant antibiotype was significantly associated with mortality ($P = 0.015$) in the present study.
By multivariate analysis only resistant antibiotype (Resistant to third generation cephalosporins) {\(\text{Czld- OR, 7.13 [95% CI, 1 to 46]; } P = 0.044\); \(\text{Ctx- OR, 6.09 [CI, 0.87 to 30]; } P = 0.045\}) and mechanical ventilation (OR, 5.84 [CI, 0.83 to 31]; \(P = 0.05\)) were independent risk factors for the mortality in the present study.

Because of the continuous occurrence of nosocomial \textit{Acinetobacter} infections or colonization among patients in high-risk wards and ICUs, factors that are associated with infection were assessed. All the factors significantly associated with infection due to \textit{Acinetobacter} spp are shown in Table 14.5. The resistant strain isolated from the patients was significantly associated with infection (\(P=0.04\)). All patients who expired had severe infection with \textit{Acinetobacter} spp (\(P=0.04\)). Fourteen patients died during their stay in the hospital (33% mortality rate). This data is similar with that of study of Cisneros et al. (1996), where in 34% mortality was reported. Fifty percent mortality rate was noted in our ICU admissions alone due to \textit{Acinetobacter} infections.

The following variables were considered to be biologically plausible risk factors for infection and were evaluated in a logistic regression model: prior use of broad-spectrum antibiotics, outcome and infection with resistant antibiotype. By multivariate analysis only resistant antibiotype (\text{Ctx- OR, 9.4 [95% CI, 1.3 to 109]; } P = 0.047) was an independent risk factor for occurrence of infection due to \textit{Acinetobacter} spp. This may be probably due to extensive and indiscriminate use of broad spectrum antibiotics in our set up which has served to eliminate competing
bacteria and created a vacant ecological niche that might have enhanced the ability of particular resistant *Acinetobacter* clones to cause infection.

Earlier, it was emphasized that progressive decrease in the effectiveness of third generation cephalosporins against *Acinetobacter* species has been coupled with the increased use of these antimicrobial agents (Joly-Guillou et al., 1990). Our results re-emphasizes that cefotaxime and broad-spectrum antimicrobials should be used intravenously with caution or ceftazidime and cefotaxime use should be discontinued in units where resistant gram-negative strains for these two antibiotics are being reported increasingly. In this hospital other combinations along with amikacin were administered after cefotaxime resistant strains were increasingly reported. Since inappropriate antibiotic therapy was the risk factor in the present study cautious antibiotic selection and administration that could avoid multi-resistant *A. baumannii*-outbreaks was advised to physicians.

16.8.1 *Respiratory infection*:

The precise diagnosis of pneumonia in critically ill and mechanically ventilated patients is somewhat difficult (Fagon et al., 1989). Quantitative culturing of bronchial secretions using fiberoptic bronchoscopy (BAL) and protected specimen brush (PSB) described by Fagon and colleagues (1989) might allow a more reliable distinction between colonization of tracheobronchial tree and nosocomial pneumonia. However controversy regarding the use of PSB and BAL, and their advantage over routine endotracheal aspirate as a better clinical specimen still exists. Few reports claim
that there is no definitive advantage of protected specimens over endotracheal aspirates 
(Niederman et al., 1994; Sanchez-Nieto et al., 1998; Ruiz et al., 2000) for the diagnosis 
of nosocomial pneumonia. This study used only quantitative endotracheal aspirates for 
the diagnosis of nosocomial pneumonia. The incidence of respiratory tract infections 
(n=21) was 48.8% in this study. Majority of the strains responsible for respiratory 
infection were A. baumannii, however strains of DNA group 13TU were also 
isolated from these infections. Only endemic resistant antibiotype ($P = 0.047$) and 
mechanical ventilation ($P = 0.05$) were independent predisposing factors for 
occurrence of respiratory infection due to Acinetobacter spp in our intensive care 
unit. This observation was similar to that of other investigators (Beck-sague et al., 
1990; Peacock et al., 1988; Smego, 1985; Seifert et al., 1995; Trouillet et al., 1998). 
However, they also implicated many other risk factors such as prior antibiotic use, 
ICU, I.V. catheters for respiratory infection, which were not significant in our 
investigation. Crude mortality rates of 30 to 75% have been reported for nosocomial 
pneumonia due to Acinetobacter spp. with highest rates encountered in ventilator- 
dependent patients (Bergogne-Berezin et al., 1991; Fagon et al., 1993 and 1996; Torres 
et al., 1990). In the present study 50% mortality rate was noted in ICU admissions 
alone due to Acinetobacter species.

16.8.2 Bacteremia:

The source of bacteremias due to Acinetobacter species varies depending on 
the risk factors. The largest outbreak of A. baumannii bacteremia described so far 
was due to inaccurate sterilization of pressure transducer, which was led to vascular 
infection (Beck-Sague et al., 1990). However, the most common source for
*Acinetobacter* bacteremia was the respiratory tract (65%) in the present study. This figure was less when compared with the study conducted by Cisneros et al (1996) (71%). Another study also reported respiratory infection as the most common source for bacteremia (Tilley and Roberts, 1994). This is understandable as majority of clinical *Acinetobacter* isolates are from respiratory samples (Seifert et al., 1993). *A. baumannii* blood stream infections are most common among patients with severely impaired host defenses and breakdown of natural defense barriers such as skin and mucous membranes (Seifert et al., 1995). Majority of our patients (31) had undergone surgery (72%); 19 patients were intubated and ventilated (44%) as they had respiratory distress. In contrast, other investigators have noted that *A. baumannii* blood stream infections were more frequent in cancer patients and in patients with severe underlying malignancies (Fuchs et al., 1986; Rolston et al., 1985; Tilley and Roberts, 1994).

16.8.3 *Infection due to Species other than A. baumannii:*

*A. baumannii* was the species responsible for most of the *Acinetobacter* infections in the present study. However, other species belonging to different genomic species were also encountered. Second common species that has been implicated in many outbreaks is DNA group 13 (Dijkshoorn et al., 1993; Seifert and Gerner-Smidt, 1995; McDonald et al., 1999). Three DNA group 13 TU strains isolated from our study were epidemiologically related and involved in outbreak occurred during 1996. Few instances of systemic infections in immunosuppressed subjects due to *Acinetobacter lwaffii* and *Acinetobacter johnsonii* were implicated in the present study. Five patients developed secondary meningitis due to *A. lwaffii* in our series. Of them,
one developed deep keratitis due to the same organism. The other patient who was septicaemic had repeated blood culture positive for *A. lwofii*. Similar studies wherein infection due to *A. lwofii* in immunosuppressed patients have been documented earlier (Melki and Sramek, 1992; Seifert et al., 1994; Ku et al., 2000). Only one strain of DNA group 3 causing peritonitis in a patient who was on continuous ambulatory peritoneal dialysis (CAPD) was observed. Similar cases of *Acinetobacter* peritonitis in CAPD patients have been reported earlier (Valdez et al., 1991; Lye et al., 1991). The occurrence of genomic species 3 seems to be less frequent in this geographical region as compared to other regions where it is one of the important cause for nosocomial infection (Tjernberg and Ursing, 1989; Dijkshoorn et al., 1993; Chu et al., 1999). In one study in Sweden it was found that *Acinetobacter* genomic species 3 was the predominant species among the clinical isolates (Tjernberg and Ursing, 1989). One patient developed secondary meningitis due *Acinetobacter johnsonii*, wherein CSF from the four-year-old male patient grew this organism twice. In this case it was suspected that infection might have been induced iatrogenically during lumbar puncture. Earlier, 13 cases of *Acinetobacter johnsonii* blood stream infections due to vascular catheterization have been reported (Seifert et al., 1993). *Acinetobacter haemolyticus* was earlier implicated as a cause for infective endocarditis. In our study, *A. haemolyticus* was isolated from an 86-year old male patient suffering from recurrent bronchiectasis. One strain belonging to DNA group 14 was isolated from a patient suffering from fever of unknown origin. This was regarded either as skin colonization or contamination, since there was no strong evidence for infection in this case. However, DNA group14 strains were not found in clinical materials frequently
Corneal perforation due to *A. junii* diagnosed in this hospital was reported during the study period (Prashanth et al., 2000). Only few reports on *A. junii* causing outbreaks have been documented (Bernards et al., 1997; Kappstein et al., 2000). Our report has its significance owing to its acquisition. Community acquired infections due to *Acinetobacter* are rare. The present investigation suggested that *A. junii* infection might have been acquired from the community.

16.9 *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 the outbreaks in the particular setup. The dissemination and persistence of *Acinetobacter* spp. in the hospital environment probably accounts for the specific role in epidemiology. *Acinetobacter* spp have been found contaminating inanimate materials in hospital, and these act as reservoir of infection, especially during outbreaks. In some persistent outbreaks, contaminated materials have been shown to act as a source for the outbreak. The source can be animate (patients, medical staff) or inanimate objects. Genotypic methods such as plasmid profiling, RAPD, ribotyping, PFGE are now being used widely for the detection of such common source of outbreak (Hartstein et al., 1988; Cefai et al., 1990; Gerner-Smidt, 1992; Seifert et al., 1994; Gouby et al., 1992; Webster et al., 1998).
Colonization of *Acinetobacter* in human subjects irrespective of patients and healthy medical personnel may have important bearing in transmission of this infectious agent among patients. There are only 3 studies reported in the literature on skin and mucous membrane carriage and colonization of *Acinetobacter* with the new molecular classification scheme. In the first two studies, (Seifert et al., 1997; Berlau et al., 1999) *A. baumannii* was not the predominant species on skin and mucous whereas Chu et al. (1999) reported *A. baumannii* and DNA group 13TU as frequent colonizers. Chu et al., 1999 concluded that, low density, variation in genospecies, strains, short duration, and the typicality of a given locality characterized the skin carriages in the majority of healthy subjects. In contrast, the carriage rate may be much higher in hospitalized patients, especially during outbreaks of infection (Chu et al., 1999). In one study tracheostomy swabs were positive for *Acinetobacter* spp. in 45% of hospitalized patients while same study revealed throat swabs positive for *Acinetobacter* spp. in 7 to 18% in hospitalized patients (Patterson et al., 1991). High colonization rates of the skin, throat, respiratory system, or digestive tract, having varying degree of importance, are reported in several outbreaks. In particular, outbreaks involving mechanically ventilated ICU patients are associated with a high colonization of respiratory tract (Allen and Green, 1987; Buxton et al., 1978; Gerner-Smidt et al., 1987; Peacock et al., 1988), which may suggest contamination of the ventilatory equipment as the possible source of an outbreak. In our study, repeated cultures of endotracheal aspirate in seven patients (16%) with out infection indicated heavy colonization by *Acinetobacter*. In addition, patients may often have their skin colonized by *Acinetobacter* spp during outbreaks. Such type of colonization
plays an important role in subsequent contamination of the hands of hospital staff during simple contacts, thereby contributing to the spread and persistence of outbreaks (Getschell-White et al., 1989). In one instance in the present study a strain of *A. baumannii* was obtained from the hands of the attending clinician that was identical genotypically with that of outbreak strain confirms hands can be a potential source for recurrent infections (Prashanth and Badrinath, 2000). However, oropharyngeal and digestive tract colonization of patients by *Acinetobacter* spp is a rare phenomenon (Grehn et al., 1978; Sakata et al., 1989; Wise et al., 1990).

Several conclusions regarding colonization in hospitalized patients can be drawn from published studies. A high rate of colonization can be found in debilitated hospitalized patients, particularly during outbreaks. Moreover, the predominant site of colonization is skin, but other sites such as the respiratory or digestive tract might also be involved and may increase on certain occasions. Finally the observed discrepancies between carriage rates for outpatients and hospitalized patients indicates that infecting or colonizing organisms in hospital acquired infections (HAI) may have been derived more often from cross-transmission or hospital environment, rather than from endogenous sources in patients.

Materials used for respiratory therapy or support have been implicated as source for *Acinetobacter* in many outbreaks (Smith and Massanari, 1977; Hartstein et al., 1988; Cefai et al., 1990). An outbreak of 24 cases of infection with *Acinetobacter* spp, occurring mostly in debilitated patients with intravascular catheters, was traced to contaminated room humidifiers (Smith and Massanari, 1977). Contaminated air samples up to 10m were found from these humidifiers, and
probable skin colonization of patients in the vicinity of the devices resulted in intravascular catheter infection. A similar outbreak occurred in patients undergoing peritoneal dialysis (Abrutyn et al., 1978). The contamination of dialysis fluid bottles, via a contaminated water bath used to warm the dialysis fluid was demonstrated. This outbreak was controlled after revision of the decontamination procedures for heating buckets and for starting infusion of dialysis fluids. Most of the above studies used only phenotypic methods for typing. In recent years such findings are becoming more accurate and assertive due to new molecular techniques employed (Cefai et al., 1990; Tankovic et al., 1994; McDonald et al., 1998). Outbreaks due to inadequate sterilization of autoclavable, reusable needles for administration of intrathecal methotrexate in patients with leukemia was reported from India (Kelkar et al., 1989). Plasmid profiling was used to elucidate this outbreak. Other outbreaks involving, defective heating of a washing machine used for decontamination of reusable ventilator tubings (Cefai et al., 1990), and inadequate decontamination of respiratory monitoring devices and resuscitation bags have been reported (Stone and Das, 1985; Hartstein et al., 1988; Vandenbroucke-Grauls et al., 1988). Hartstein et al. (1988) detected the contaminated source using plasmid profiling. Ethylene oxide sterilization of such contaminated equipment has been shown to be effective. In our study humidifier sampling persistently yielded A. baumannii even after prolonged period, probably suggesting successful colonization, which was leading to recurrent episodes of endemic infections in the ICU. This long-term persistence of a potentially epidemic strain in the ICU, even during a non-outbreak period, indicates a need for constant, continuous monitoring of the ICU environment. Similar observations were
reported in many studies (Gerner-Smidt, 1987; Weernink et al., 1995; Mulin et al., 1995; Webster et al., 1998, 1999). Radiation resistance of *Acinetobacter* clinical isolates may enhance their ability to survive for longer periods as many irradiation sterilizing procedures may fail to eradicate this resistant organisms. One of the recent reports describes radiation resistance of *Acinetobacter* species (Christensen et al., 1991). These observations indicate that special attention should be paid to medical devices that are normally sterilized by irradiation, particularly devices used in ICUs.

Numerous investigations documented the persistence and survival capacity of *Acinetobacter* spp. in hospital environment (Sherertz et al., 1985; Allen and Green, 1987; Crombach et al., 1989; Weernink et al., 1995; Jawad et al., 1996). These observations indicate that certain *Acinetobacter* spp. can persist in the environment for several days, even in dry conditions on particles and dust, thereby probably contributing to the development and persistence of outbreaks. It has been reported that *Acinetobacter* can survive on dry surfaces for duration’s even longer than that found for *Staphylococcus aureus* (Getschell-White et al., 1989; Musa et al., 1990; Jawad et al., 1996). In one particular study, apart from desiccation resistance of Acb-complex strains, it has also been demonstrated that the survival time of strains increased when kept at higher relative humidity (Jawad et al., 1996). Environmental contamination during an outbreak in a pediatric ICU was demonstrated on various equipment and surfaces in the unit (telephone handles, door pushplates, patient charts, tabletops, etc.), all of which were probably contaminated by the hands of staff (Getschell-White et al., 1989). In the present study *A. baumannii* was isolated from floor, phone handle, temperature probe and ventilator surfaces. An epidemic strain of
multiresistant \textit{Acinetobacter} spp. has been shown to survive for up to six days after inoculation on to dry filter paper. This duration is similar to that found with \textit{Staphylococcus aureus}, which persisted for seven days, but significantly greater than the survival times for \textit{Escherichia coli} and \textit{Pseudomonas} spp., both of which persisted for 24 hours or less (Allen and Green, 1987). Prolonged survival of \textit{Acinetobacter} spp. on hospital floors and air-dried wash clothes has also been described (Buxton et al., 1978). Survival is probably also helped by the ability of \textit{Acinetobacter} spp. to grow at a range of different temperatures and pH values (Baumann, 1968; Baumann et al., 1968a; Hugh, 1978; Towner et al., 1991).

Gram-negative bacteria such as \textit{Pseudomonas aeruginosa}, \textit{A. baumannii}, \textit{Stenotrophomonas maltophilia} and \textit{Burkholderia cepacia}, 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 fomites or from the hands of medical personnel (Larson, 1981; Quinn, 1988). Members of the genus \textit{Acinetobacter} have a remarkable ability to develop resistance to even the potent antimicrobial agents. 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 particular \textit{Acinetobacter} clones to colonize and subsequently causing infection in immunosuppressed patients (Webster et al., 1998). The commonest species isolated from our patients was \textit{A. baumannii}, and only few distinct resistant clones were more common among this group. The predominance of resistant biotypes 6, 10, 16 of \textit{A. baumannii} in our hospital ICU
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 other wards are not similar in the hospital. Large number of beds and high turn over of patients in medical, surgical and pediatric wards makes the hospital overcrowded there in good standards of infection control is difficult to achieve. Thus, it might have resulted in a greater variety of strains and biotypes 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 Acinetobacter genomic species are circulating uninterruptedly among patients and staff in these wards. Some of the samples taken from staff were positive multiresistant Acinetobacter in our study.

Continuous surveillance of patients and the environment many a time’s 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; Schrocksnadel 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).
16.10 Correlation of five typing methods:

Identification and typing of microorganisms are extremely important in efforts to monitor the nosocomial infections and their control. It is also important to know the pathogens distribution geographically. It is certain that for a better understanding of the role of the various Acinetobacter genomic species in hospital acquired infections and in human pathology, identification of acinetobacters according to the recent taxonomy is imperative. Many typing methods have been used by different investigators for elucidating the hospital outbreaks. However, only few comparative studies on different typing methods have been reported (Joly-Guillou et al., 1990; Dijkshoorn et al., 1993; Marcos et al., 1995; Liu et al., 1997). A comparative study of phenotyping, antibiotyping, cell envelope protein analysis, PFGE of chromosomal DNA and PCR with arbitrary primers was performed for the isolates of Acinetobacter spp. obtained from our hospital to determine the best markers for epidemiological analysis. Discriminatory power of initial three methods used in our study to type sixty-six isolates Acinetobacter spp. showed antibiotyping to have the least discriminatory power (DI=0.76), whereas phenotyping using 21-carbon sources (additional 4 for biotyping) and cell envelope protein analysis showed good to moderate discriminatory power (DI=0.93 and 0.89 respectively). Dijkshoorn and colleagues (1993) had good agreement between CEPA and ribotyping but biotyping and antibiotyping were least discriminatory in their study. Our study showed that good discrimination could be obtained if one uses combinations of phenotypic schemes. However, in the present study also antibiotyping was least discriminatory. The addition of phenotyping or cell envelope protein analysis to antibiotyping was
able to further increase the discriminatory power in the study (DI = 0.95 and 0.96 respectively). In contrast, Marcos et al. (1995) were not able to get an increase in DI by the inclusion of an additional typing method. However, the addition of phenotyping to cell envelope protein analysis did not increase discriminatory index much (DI = 0.94) in this study. On the basis of the data presented here, strain differentiation appears to be acceptable if one uses phenotyping supplemented by any one molecular method.

Discriminatory power of five methods used to type selected twenty-eight isolates of *Acinetobacter* spp showed PFGE to have the best discriminatory power among all these methods (0.96). Similar results were observed by Marcos et al. (1995) and Liu et al. (1997) with a DI of 0.96 and 0.98 respectively. Antibiotyping and cell envelope protein analysis were the least discriminatory methods (DI=0.76 and 0.82 respectively), whereas AP-PCR and phenotyping using 21-carbon sources showed good discriminatory power (DI=0.91 and 0.88 respectively). Graser et al. (1993) had good discriminatory results from AP-PCR that was on par with PFGE results. Moreover, compared with other DNA-based identification methods, PCR fingerprinting offers the advantages of simplicity and rapidity. In contrast, in our study PFGE and biotyping proved to have better discriminatory power. In the present study, the addition of phenotyping or antibiotyping to pulse field gel electrophoresis of chromosomal DNA further increased the discriminatory power (DI = 0.97). This was not the case in the investigations of Marcos et al., 1995. However, this DI was not improved further by the inclusion of PCR with arbitrary primers as an additional method to the PFGE in our study. Both AP-PCR and *PFGE showed excellent
reproducibility in our study. This was in concordance with other studies (Graser et al., 1993; Marcos et al., 1995; Liu et al., 1997). Overall, the best discriminatory power indicated by a discriminatory index of 0.97 was achieved by combining phenotyping or antibiotyping with pulse field gel electrophoresis. Hence, PFGE with any one additional method is enough for an epidemiological analysis where in one can easily elucidate outbreak isolates. Moreover, its reproducibility as a property allows PFGE to be useful for delineation of the clonal relationship of different isolates by dendrogram analysis (Liu et al., 1997).

One study compared ribotyping with PFGE, wherein ribotyping proved to be useful in taxonomic identification of the strains up to DNA group level (Seifert and Gerner-Smidt, 1995) but PFGE lacked this property. However, both methods were useful for strain differentiation in epidemiological studies of Acinetobacter isolates. PFGE and ribotyping both are cumbersome and time consuming. Hence, PCR in conjunction with protein analysis or phenotyping with carbon assimilation tests seems to be more economical and simpler and any clinical laboratory can adapt this methods. However, rigorous standardization for these techniques is of paramount importance to achieve good and reproducible results in a particular set up (Grundmann et al., 1997; Gerner-smidt, 1994; Dijkshoorn et al., 1993).

Since PFGE is expensive and time consuming it may be reserved for situations in which clinical and other typing methods data (biotyping, antibiotyping and PCR) are inconclusive. However, these methods are not in reach for most of the clinical laboratories in India. Moreover a better approach for this problem is to, setting up a national reference laboratory for this kind of molecular typing which can
help the smaller clinical laboratories countrywide in elucidating epidemiology of various infectious diseases, especially nosocomial infections. Until such kind of facilities are realized, one can still rely on traditional phenotypic methods like biotyping, antibiotyping and cell envelope protein analysis which alone may be good enough to discriminate outbreak isolates from that of epidemiologically unrelated strains. These conventional methods adopted in the present study helped us to a greater extent in epidemiological analysis of Acinetobacter infections.