SUMMARY AND CONCLUSIONS
17. SUMMARY

a) Patients and bacterial isolates:

The genus *Acinetobacter* has been increasingly associated with nosocomial infections. *Acinetobacter* species is the common bacteria encountered in our hospital among the gram-negative organisms. The present study was conducted in 860 bedded JIPMER hospital, for a period of 21 months from October 1996 to July 1998. A total of 43 patients admitted in RICU, paediatric and other medical wards were studied. Male predominance (58%) was present which was in concordance with other studies.

*Acinetobacter* was isolated from various types of infections such as respiratory tract infections (n=21, 48.8%), blood stream infections (n=7, 16.27%), secondary meningitis (n=6, 14%), UTI (n=4, 9.3%), peritonitis (n=2, 4.65%), corneal infection (n=1), necrotising faciitis (n=1) and osteomyelitis (n=1). Two cases of community acquired respiratory and corneal infection were also included in the present series. Among patients with respiratory tract infections, 19 patients were also bacteremic. Three developed blood stream infection secondary to wound infection. A total of 29 patients (69%) developed bacteremia during the study period. The most common source for *Acinetobacter* bacteremia was the respiratory tract (65%). Seven cases were either regarded as colonization or contamination with no related clinical findings of infection. Fourteen patients died during their stay in the hospital (33% mortality rate). Fifty percent mortality rate was noted in ICU admissions due to *Acinetobacter* infections.
A total of 98 isolates were obtained during this study period. Majority of cases were from patients admitted to ICU (42%). Fifty-five isolates were obtained from 18 patients of RICU. Remaining 43 isolates were from 25 cases, which were admitted in other wards. From 6 patients two different strains were isolated. In cases, where multiple isolates were obtained from the same patient, after preliminary biochemical testing and antibiotyping representative strain/strains taken up for further study. Hence, only 49 isolates were selected for typing and epidemiological analysis. Seventeen Acinetobacter isolates that were obtained from environmental sources were also included for the analysis.

b) Phenotyping:

Phenotypic identification of Acinetobacter isolates using different schemes was able to differentiate most of the isolates. However, these identification schemes failed to distinguish strains of Acb-complex especially strains belonging to DNA group 2 and DNA group 13. According to the phenotypic identification system followed by Gerner-Smidt (1991), our population had 39 strains belonging to Acb-complex. Out of which 32 strains were correctly identified as A. baumannii. Another 3 strains either belonged to DNA group 2 or DNA group 13. The species that followed next was A. lwoffii. Other less common species encountered were A. johnsonii (1), A. heamolyticus (2), A. junii (1). Remaining four strains were belonged to DNA group 3 and group 13 (two each). One strain, which was heamolytic belonged to DNA group 14 (Table- 14.7).
Extended phenotypic tests for identification of Bouvet and Grimont (1987), Bouvet and Jeanjean (1989) was used to identify the isolates, which were inconsistent in their assimilation test results. Ten strains that were inconsistent in their assimilation tests were subjected to extended phenotypic tests repeatedly and only 6 strains out of them were identified. Among these 3 strains belonged to DNA group 2. Other three strains belonged to DNA group 15, *A. lwoffii* and *A. calcoaceticus*. Based on extended phenotypic tests, our population had 43 strains belonging to Acb-complex, of which 35 strains were correctly identified as *A. baumannii*. One strain was identified as *A. calcoaceticus*. Two strains belonged to DNA group 3. Three strains either belonged to DNA group 2 or DNA group 13TU. Remaining two belonged to DNA group 13TU, which were not recognized by Bouvet and Jeanjean (1989) (Table-14.8).

*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 was the most common type found in the present study (10 strains), followed by biotype 6 (7 strains). Six strains belonged to biotype 9. Biotype 16 and biotype 11 constituted 4 and 3 strains respectively. Biotypes 2, 3, 7, 8, 15, 19 all comprised of two strain each (Graph-14.1).

c) Other typing methods:

Antibiotyping yielded six antibiotypes (A-F) among the all *Acinetobacter* isolates tested using agar dilution method. Antibiotype A had resistance for all the 5 broad-spectrum antimicrobials tested and this cluster was predominantly responsible for
the outbreaks in our study. Using cell envelope protein analysis, 18 different banding patterns were observed from phenotypically characterized 66 *Acinetobacter* isolates. Strains showing protein profiles B, B1, B2, B5 and B6 were implicated in the outbreaks. By comparing standard reference strain profiles and biotyping results, the profiles B1, B2, B5 were all obtained from DNA group 2 strains (*A. baumannii*, biotypes - 6, 10 and 15 respectively). Profile B6 was either from *A. baumannii* biotype 9 or from genomic species 13 strains.

Only significant isolates were subjected to random polymorphic DNA fingerprinting (AP-PCR). From AP-PCR, eight different banding patterns were observed, 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. PCR profiles of strains involved in the outbreaks were a, d, g & h. PCR profiles of outbreak strains were clearly different from that of epidemiologically unrelated strains. PCR failed to differentiate biotypes of *A. baumannii* in one instance, and once it showed two profiles for a single biotype.

Twenty-eight phenotypically characterized strains were subjected to PFGE. The fingerprints generated by macro-restriction with either *SmaI* and *ApaI* comprised 15 to 25 bands with sizes approximately 5 to 300 kb. Eight and nine major distinct PFGE patterns were distinguished among these 28 isolates of *Acinetobacter* by using *SmaI* and *ApaI* macro-restriction. In all, 14 PFGE profiles were observed which also included subtypes. Among *A. baumannii* (DNA group2) strains 5 & 6 major restriction patterns
could be distinguished using Smal and Apal respectively. Among A. lwaffii, all strains exhibited a single pattern. Every outbreak-related strains were correctly identified and could be clearly distinguished from epidemiologically unrelated strains by PFGE macro-restriction (Figure 14.10). Epidemiologically unrelated representative strains showed distinct PFGE types. A few subvariants of the major restriction patterns were also seen in the present study.

d) Comparison of typing methods:

Typing methods and their efficiency in discriminating isolates collected over a period of time, especially those involved in outbreaks in our hospital are summarized below. During nosocomial outbreaks, the simplified identification scheme of Bouvet et al (1987) could be used for typing strains, in conjunction with protein profiles. We were able to detect three outbreaks occurring in ICU during the first 16 months of the study period with the help of simple identification scheme (Prashanth and Badrinath, 2000). This was later confirmed by cell envelope protein profiling. Protein profiles though, were able to presumptively identify DNA groups when used alone, it was insufficient to judge the epidemiological data and to elucidate the outbreaks. Hence, it should be used in conjunction with conventional typing methods or at least with one genotypic method such as PCR. Both phenotyping and protein analysis had a good discriminatory power in this study (0.93 and 0.89 respectively).
AP-PCR was a simple and rapid technique with a high discriminatory power (DI = 0.88) and had good agreement with other molecular methods employed in the present study. Thus, PCR can be used as a complementary technique for the epidemiological investigations of Acinetobacter clinical isolates. PFGE was shown to be a more accurate method in differentiating strains from outbreaks in this study. Discriminatory power of five methods used in this study to type selected twenty-eight isolates of Acinetobacter spp showed PFGE having best discriminatory power among all methods employed (DI = 0.96).

e) **Species, biotypes, risk factors and epidemiology:**

Numerous genomic species of Acinetobacter have been identified. However, it has been recognized that *A. baumannii* is the species most responsible for outbreaks of nosocomial infections (Bergogne-Berezin and Towner, 1996). Phenotypic identification of genomic species 1, 2, 3 & 13 is difficult. Hence it has been suggested to group them under *A. calcoaceticus* - *A. baumannii* complex (Acb-complex) (Gerner-Smidt et al., 1991). Based on these criteria, Acb-complex was involved frequently in nosocomial infections in RICU, surgical and pediatric wards of JIPMER hospital, with the respiratory tract and blood stream being the predominant sites of infection. However, other sites have also been observed (pus, urine, peritoneal fluid, ascitic fluid, cornea and catheter). Using the CDC criteria (Garner et al., 1988), we found that 36 patients infected and 7 patients had colonization without any related clinical findings of
infection. Most of the infections due to *Acb*-complex were in severely compromised patients admitted in RICU.

Species other than *A. baumannii* were also encountered in the present study causing different kinds of infection. Few instances of systemic infections in immunosuppressed subjects due to *A. lwoffii* and *A. johnsonii* were encountered. However, *A. lwoffii* strains were predominantly found from environmental sources as compared to clinical specimens. *A. lwoffii* was responsible for many blood stream infections and meningitis. Strains of DNA group 13TU and DNA group 3 were responsible for wound infections and peritonitis respectively. Community acquired *Acinetobacter* infections were also recorded in the present study. One case of recurrent bronchiectasis caused by *A. baumannii* biotype 9 was encountered. A case of corneal perforation due to *A. junii* was diagnosed and reported during the study period (Prashanth et al., 2000).

*A. baumannii* biotypes 6, 9, 10, 15 and *Acinetobacter* genospecies 13TU were recovered as strains responsible for outbreaks. The homogeneity of the outbreak isolates on macrorestriction analysis provides a compelling evidence that outbreaks in different months during three years study period was due to a clonal epidemic *A. baumannii* biotypes 6 and 10 strains. *A. baumannii* biotypes 16, 19 were recovered from endemic sporadic infections. This may be due to their successful colonization in ICU, as observed by their occurrence in clinical specimen at different time intervals. However, this study was unable detect the source of these endemic isolates. Other
biotypes encountered in this study were sporadic ones and their distribution varied from time to time.

*A. baumannii* biotypes isolated in this study differed slightly with other studies from diverse geographic areas (Dijkshoorn et al., 1993; Chopade et al., 1994; Jolly-Guillou et al., 1990; Gerner-Smidt et al., 1992; Nemec et al., 1999). Biotype 10 and 6 were most common in our study. Multiple drug resistance was encountered in the strains that belonged to *Acb*-complex. Some strains of *A. baumannii* biotype 6 and 10 were resistant to all the antibiotics tested in the present study. Species other than *A. baumannii* encountered in our setup were usually susceptible to the majority of the antibiotics tested. However, they showed higher percentage of resistance to ciprofloxacin. Third generation cephalosporins such as cefotaxime, ceftazidime and aminoglycosides like amikacin and netilmicin, were only antibiotics that showed higher percentage of in vitro susceptibilities in the present investigation. However, 50% of *A. baumannii* strains showed resistance to amikacin.

*A. baumannii* was the major species encountered in the present study and was associated often with infections in intensive care units especially in debilitated patients. Fifty percent of mortality rate was noted in ICU admissions, due *Acinetobacter* infections. Twenty three percent of our patients failed to recover who had inappropriate antibiotic therapy. Mechanical ventilation was significant risk factor associated with higher mortality (*P* = 0.02). The resistant antibiotype was significantly associated with mortality (*P* = 0.015). Multivariate logistic regression analysis, showed only resistant antibiotype *(Czd- OR, 7.13 [95% CI, 1 to 46]; *P* = 0.044);*(Cesx- OR, 6.09 [CI, 0.87
to 30]; \( P = 0.045 \}) \) and mechanical ventilation \((\text{OR}, 5.84 [\text{CI}, 0.83 \text{ to } 31]; \ P = 0.05)\) as independent risk factors contributed to the mortality.

The resistant strain isolated from the patients was significantly associated with occurrence of infection \((P=0.04)\). All patients who expired had severe infection with \textit{Acinetobacter} \textit{spp} \((P=0.04)\). The variables such as prior use of broad-spectrum antibiotics, outcome and infection with resistant antibiotype were considered to be biologically plausible risk factors for infection and were evaluated in a logistic regression model. By multivariate analysis only resistant antibiotype \((\text{Ctx- OR}, 9.4 [95\% \text{ CI}, 1.3 \text{ to } 109]; \ P = 0.047)\) was found to be independent risk factor for occurrence of infection due to \textit{Acinetobacter} \textit{spp}.

In the present study, the six-bedded respiratory intensive care unit \((\text{RICU})\) had been contaminated by multidrug resistant \textit{A. baumannii}. Ventilator equipment, humidifier, temperature probe, phone handle and floors of RICU were found contaminated with \textit{Acinetobacter} \textit{spp}. Outbreaks may result from intrinsic contamination of medical equipment and devices and/or contamination from the environment either by contact with patients or by airborne route. Long-term persistence of acinetobacters in the environment provides a conducive atmosphere and opportunities for contamination of patients and medical personnel and this explains prolonged outbreaks \((\text{Webster et al., 1998})\).

Outbreaks encountered during the months of Oct. 1996, Sept 1997, Feb. 1998 and July 1998 in our hospital were due to few clones of multi-drug resistant \textit{A. baumannii} and
genomic species 13TU (Prashanth and Badrinath, 2000). Predominantly strains of genomic species 13TU were responsible for outbreak occurred during October 1996. Our observation confirms that Acinetobacter species can survive for prolonged periods in ICU environment and has the potential to cause subsequent infections. Prompt infection control practices could able to curtail these prolonged outbreaks.