methods described by Pantophlet et al. (1999) is promising and helps in assigning clinically important species into definitive DNA group.

CHAPTER 2

2. HABITAT AND ISOLATION

2.1 Species of Clinical Importance:

Numerous studies are now able to appreciate the original observation, (Bouvet and Grimont, 1987) that A. baumannii is the main genomic species associated with outbreaks of nosocomial infection. Apart from their carriage by patients and staff, various strains of Acinetobacter can be isolated from a wide range of both moist and dry inanimate sources in the hospital environment including pillows (Weernink et al., 1995), tabletops, ventilator equipment, linen, humidifiers, mattresses, urinals and wash basins (Bergogne-Berezin and Towner, 1996). Joly-Guillou et al. (1990b) reported that Acinetobacter strains comprised 1-9% of all bacterial species isolated from clinical specimens between 1984 and 1988. The major sites of infection do not differ from those of other nosocomial gram negative pathogens, with the lower respiratory tract and the urinary tract accounting for 15 - 28% of total Acinetobacter infections (Retailleau et al., 1979; Joly-Guillou et al., 1990b). Nosocomial pneumonia caused by Acinetobacter spp. in the intensive care units is posing a major problem, as effective management of these infections seems to be difficult due to their multidrug resistance (Cunha et al., 1980; Vieu et al., 1980; Muller-Serieys et al., 1989). An
important study by Siefert et al., (1993a) to know the distribution of Acinetobacter spp. from clinical sources using data from twelve different hospitals reports 584 Acinetobacter isolates encountered from 420 patients over a period of 12 months. From this, 426 strains were identified as A. baumannii (72.9%), with 208 A. baumannii isolates being recovered from respiratory tract specimens, 113 from blood cultures and central venous lines, 70 from wound swabs and 35 from other miscellaneous specimens. Further it also identified 158 isolates belonging to species other than A. baumannii, of which the most common were Acinetobacter species 3 (55 isolates), A. johnsonii (29 isolates) and A. lwofii (21 isolates). However, A. baumannii is rarely recovered from the skin of patients or healthy European subjects as other genospecies predominate, but it is a significant nosocomial pathogen (Berlau et al., 1999 a&b). The natural reservoir of this organism in this area is therefore uncertain. Berlau et al. (1999) studied the isolation rates of Acinetobacter spp. from vegetables, as an indicator of the natural environment using a selective technique and classified the genospecies by Amplified ribosomal DNA restriction analysis. Of the 177 samples of vegetables examined, 30 yielded Acinetobacter, with genospecies 2 (A. baumannii) and 11 being the most common, each with a frequency of 27%. Vegetables may therefore be a natural habitat of A. baumannii in this geographical area and this might provide a route by which these bacteria are introduced into hospitals.

Very less information is available about the clinical significance of Acinetobacter spp. other than A. baumannii, and further detailed investigations are required on this aspect. Data accumulated from Seifert et al (1994) suggests some
specific epidemiologic aspects regarding the *Acinetobacter* spp. other than *A. baumannii*, like patient to patient transmission, acquisition of strains from a common source, or outbreaks of infections; all these seems to be rather unusual. The ubiquitous occurrence of acinetobacters in the environment, and as commensals on human skin, means that such isolates in clinical specimens are often considered to be contaminants. This kind of consideration may overlook the fact that there are numerous reports on infections caused by *Acinetobacter* spp other than *A. baumannii* (Seifert et al., 1993; Sherertz et al., 1985; Ku et al., 2000). However, diagnosis of infection with 'unusual' *Acinetobacter* genomic species therefore often depends on clinical indication and repeated isolation of the same strain from a single patient. *Acinetobacter* spp 3 and 13 (as proposed by Tjernberg and Ursing, 1989) have been implicated in documented outbreaks of infection involving nosocomial spread (Dijkshoorn et al., 1993), while *A. johnsonii* has been associated with catheter-related bacteraemia (Seifert et al., 1993b). *Acinetobacter* genomic species 3 is predominant among clinical isolates in Sweden (Tjernberg and Ursing, 1989). Both *A. haemolyticus* and *A. lwaffii* are also known to cause systemic infections, though their incidences are not frequent (Seifert et al., 1994; Castellanos Martinez E., 1995; Ku et al., 2000).

The close relationship between groups 1 (*A. calcoaceticus*), 2 (*A. baumannii*), and 3 of Bouvet and Grimont (1986) and DNA group 13 of Tjernberg and Ursing (1989) is worth to re-emphasize. This 'Acb- complex' (Gerner-Smidt et al., 1991; Kampfer et al., 1993) contains isolates that are mostly glucose-acidifying, and therefore corresponds quite well with the *A. calcoaceticus* var. *anitratus* designation.
that was often used earlier. The majority of glucose negative, non-haemolytic strains recovered from clinical specimens are A. lwaffii, A. johnsonii and Acinetobacter spp 12, and it appears that these species are natural inhabitants of human skin (Berlau et al., 1999). Off late outbreaks due to genomic species 5 (A. junii) were also being reported (Kappstein et al., 2000). Many of the haemolytic isolates identified as A. haemolyticus or Acinetobacter spp.6 and other groups (5,14) seem to be implicated rarely in human infections. However, the overall knowledge regarding the clinical importance of genomic species other than A. baumannii is poor.

2.1.1 Species from the Environment:

Most taxonomic studies with in the genus Acinetobacter have been performed with clinical isolates recovered from different geographical areas. However, acinetobacters are ubiquitous organisms that can be isolated easily from soil, water and sewage with appropriate enrichment techniques (Baumann, 1968), and it has been estimated that acinetobacters may constitute 0.001% of the total heterotrophic aerobic population of soil and water (Baumann, 1968). Some members of Acinetobacter genus are also found in a variety of foodstuffs, including various poultry, meats, and milk products (Koburger, 1964; Barnes and Thornley, 1966; Eribo and Jay, 1985). There have been surprisingly few studies on Acinetobacter isolates recovered from environment sources and their grouping according to new taxonomy, even though it is known that acinetobacters are responsible for spoilage of economically important foods (Shewan et al., 1960; Thornley et al., 1960; Gardner, 1971; Jay, 1982). This
spoilage occurs even when food was stored under refrigeration or treated with irradiation (Firstenberg- Eden et al., 1980).

Gennari and Lombardi (1993) compared 170 Acinetobacter isolates from various food sources with 83 clinical isolates and the results showed a clear difference in the distribution of genomic species between these two environments. A. johnsonii and A. lwoffii were predominating in foods with 45.9 and 14.7 percentage of isolates respectively. Gram negative, nonfermentative, non-motile bacteria very much resembling A. johnsonii were encountered and studied in detail by Shaw et al., 1988 & Kampfer et al., 1992; Knight et al., 1993 from food and waste water treatment plants respectively. Another recent study also described major difference in occurrence of genomic species among clinical and aquatic habitats, wherein species other then A. baumannii were predominant in aquatic habitats (Guardabassi et al., 2000). In addition, an important observation in this study was the inability of clinical strains to transfer tetracycline resistance in vitro to aquatic strains, which contraindicated any important flow of tetracycline resistance genes between clinical and aquatic acinetobacter populations. The above observations in different studies are in contrast with occurrence genomic species like A. baumannii and Acinetobacter species 3 predominantly from clinical sources in majority of clinical studies.

In conclusion, it appears that there is a significant population difference between the acinetobacters found in clinical and other environments. Each population appears to be characterized by predominant groups of genomic species; A. baumannii (DNA group 2), the species which is of greatest clinical significance, genomic spp. 3 &
5 in clinical environments and spp. 7 (A. johnsonii), 8/9 (A. lwoffii) and genomic species 12 in other environments. From clinical point of view, repeated isolation of any genomic species other than A. baumannii from a patient should be a cause for suspicion of infection, especially if clinical symptoms are also present. As well, from a clinical epidemiological viewpoint, it is apparent that the isolation of strains belonging to spp. 2 and 3 from environmental cross infection studies could be considered to be greater epidemiological significance than the detection of genomic spp, which are in association with non-human sources (Towner, 1996).

2.1.2 Correlation between clinical and environmental habitats:

The ecological niche of different Acinetobacter spp. seems to vary depending upon the environmental conditions. However, the natural reservoir for clinically important A. baumannii has not been detected except, one study wherein vegetables were implicated as the main reservoir for this bacteria (Berlau et al., 1999b). Hospital environment seems to be very conducive for the colonisation of A. baumannii as it is the genospecies, which can resist desiccation, radiation and selective antibiotic pressure. Persistence of these species in this environment has been also proved in numerous studies (Webster et al., 1998; McDonald et al., 1999a). Once colonised, A. baumannii and Acinetobacter 13TU, seems to be very difficult to eradicate them from the hospital environment (Trilla et al., 1995; Humphreys et al., 1995). Another recent study also described major difference in occurrence of genomic species among clinical and aquatic habitats (Guardabassi et al., 2000). In this investigation, 50 tetracycline-resistant (MIC > or = 16 mg/L) Acinetobacter strains from clinical (n
and aquatic (n = 15) samples were analysed by PCR for tetracycline resistance (Tet) determinants of classes A-E. Almost all clinical strains that were A. baumannii had Tet A (n = 16) or B (n = 17) determinants. The aquatic strains were belonged to the genomic species other than A. baumannii, and most of them (12 of 15) did not contain determinants for Tet A-E. Further, transfer of tetracycline resistance was tested for 20 strains with three aquatic Acinetobacter strains and Escherichia coli K-12 as recipients. Transfer of resistance was demonstrated between aquatic strains from distinct ecological niches, but not from clinical to aquatic strains, nor from any Acinetobacter strain to E. coli K-12. This observation confirms the inherent difference in genomic species belonging to different ecological niches. A. calcoaceticus has been regarded environmental genospecies where it has been exclusively isolated from the soil environment. The majority of glucose negative, non-haemolytic strains recovered from clinical specimens are A. lwofii, A. johnsonii and Acinetobacter species 12, and it appears that these species are natural inhabitants of human skin (Berlau et al., 1999a). This group are exclusively associated with infection in patients who are immunologically less competent (Ku et al., 2000).

2.2 LABORATORY IDENTIFICATION OF ACINETOBACTER SPP

2.2.1 Isolation from Clinical specimens:

Acinetobacter can be grown readily on simple common laboratory media such as nutrient agar, however defined media consisting of a mineral base containing ammonium or nitrate salts and one or more carbon sources have been used earlier for
specific purposes (Baumann, 1968b; Juni, 1978; Bouvet and Grimont 1986 & 1987; Gerner-Smidt et al., 1991). It is better, for direct isolation from clinical specimen to use a more selective medium that suppresses the growth of other microorganisms. Commercially available Herellea agar from Difco is a selective and differential medium containing bile salts, sugars and bromocresol purple (Mandel, 1964). Holton, (1983) modified this medium by adding various antibiotics. Common media having one or more additional antibiotics has also been used in the investigation of several outbreaks of Acinetobacter infection (Allen et al., 1987; Buisson et al., 1990). Recently, a novel antibiotic containing selective and differential medium, Leeds Acinetobacter medium (LAM) has been described (Jawad et al., 1994) which is useful for the recovery of most Acinetobacter spp. from both clinical and environmental sources.

2.2.2 Isolation from the Environment:

Liquid enrichment cultivation may be useful for the isolation of Acinetobacter spp. from the environment, where in their number is small (Baumann, 1968). For liquid enrichment cultivation, specimens contaminated with a variety of microorganisms can be used, where these are inoculated to a liquid mineral medium containing a single carbon and energy source & ammonium or nitrate salt as a nitrogen source, with a final pH of 5.5 to 6.5. Vigorous shaking of this liquid medium during the incubation is necessary so that acinetobacters present can out grow the Pseudomonads. After incubation for 24 to 48 hrs, a loopful of the culture broth is inoculated on to a selective medium and identified further. The above method has been