CHAPTER 7

7. EPIDEMIOLOGY:

Elucidation of epidemiology of *Acinetobacter* largely depends on their persistence in the hospital environment and human carriage. The above two parameters define the source of organism in hospital and possibility of these organisms involving in outbreaks.

7.1 Colonization in Humans:

*Acinetobacter* spp may colonize as normal flora of the skin, particularly in moist regions such as the axillae, groin, and toe webs, and it has been reported that at least 25% of normal individuals carry *Acinetobacter* spp. on their skin (Somerville and Noble, 1970; Taplin et al., 1963). It can also colonize in the oral cavity and respiratory tract of healthy individuals occasionally (Glew, 1977; Rosenthal and Tager, 1975). However, the carriage rate of *Acinetobacter* spp. in nonhospitalised patients, apart from on the skin, is very low or negligible.

There are only 3 studies documented in the literature on skin and mucous membrane carriage of *Acinetobacter* with the new molecular classification scheme. The first study (Seifert et al., 1997) was undertaken to investigate the colonization with *Acinetobacter* spp. of the skin and mucous membranes of 40 hospitalized patients and 40 healthy controls. Nine different body sites were selected. Thirty patients (75%) and 17 controls (42.5%) were found to be colonized with
*Acinetobacter* spp., and the colonization rates of patients increased during their hospital stay. The most frequently isolated species were *A. iwoffii* (47%), *A. johnsonii* (21%), *A. radioresistens* (12%), and DNA group 3 (11%). In contrast, *A. baumannii* and DNA group 13TU were found rarely on skin (0.5 and 1%, respectively).

The second study (Berlau et al., 1999) tried to determine the distribution of the 19 currently known genospecies of *Acinetobacter* on skin of healthy humans. The sites selected were forehead, forearm and toe webs. Over 40% of 192 healthy volunteers carried *Acinetobacter* spp. at one or more body sites. The frequencies of colonization on these sites were as follows: 51% on forearm, 47% on forehead and 34% on toe web. Genospecies 8/9 (*A. iwoffii*) was the most common (61%), followed by genospecies 15BJ and 12 (*A. radioresistens*) having distribution percentage of 12.5% and 8% respectively. Surprisingly strains of *Acb*-complex, which were responsible for nosocomial infections, was found in only one healthy individual. It is clear from the above studies that the natural reservoir of *A. baumannii* remains to be identified.

More recent interesting study in Hongkong (Chu et al., 1999) investigated, the carriage of *Acinetobacter* spp. at 5 superficial sites in 79 patients from 2 hospitals, in 133 healthy controls from the community and in 198 student nurses in different classes. In contrast to earlier European studies they found out the ready presence of clinically significant genospecies (2,3 & 13TU) on superficial sites of both healthy subjects and patients. Short-term duration and the typicality of a given locality characterized the skin carriages in the majority of healthy subjects. In
addition, the strains were present in low density and variation in genospecies and strains were often observed.

The carriage rate seems to be much higher in hospitalized patients, that to when there are outbreaks of infection. 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, have been witnessed 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 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). Colonization of digestive tract of patients by *Acinetobacter* spp is rather unusual phenomenon (Grehn et al., 1978). However, several studies have documented oropharangeal colonization of patients along with respiratory colonization and digestive tract colonization has been shown to be a major reservoir of resistant strains (Sakata et al., 1989; Wise et al., 1990).

Several conclusions regarding colonization in hospitalized patients can be drawn from the published studies. A high rate of colonization can be found in
debilitated hospitalized patients, particularly during outbreak situations. 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 derive more often from cross-transmission or hospital environmental sources rather than from endogenous sources in patients.

A large proportion of colonized patients in a given hospitalised setting means that the differentiation between colonization and infection may not be straightforward. Nosocomial *Acinetobacter* infections may involve any site, but they predominate in the respiratory tract, urinary tract, and wounds. Many isolates from the skin and the respiratory tract should still be considered to be colonizing rather than infecting organisms. A steady increase in the proportion of *Acinetobacter* isolates from superficial wounds has been recorded over a past ten years (Joly-Guillou and Bergogne-Berezin, 1990). Hence the skin, respiratory tract and superficial wounds should therefore be considered to be potential reservoirs for infection caused by this organism during outbreak periods.

### 7.2 Persistence in the hospital environment:

Numerous studies have documented the presence of *Acinetobacter* spp. in the hospital environment, but rates of positive cultures may vary widely, depending on the epidemiological setting. *Acinetobacter* spp. has been found in 27% of hospital sink traps and 20% of hospital floor swab cultures (Rosenthal et al., 1974). Air
contamination in the absence of the colonized patient is comparatively rare, but several studies have documented extensive contamination by *Acinetobacter* spp. of the environment, including respirators and air samples, in the vicinity of infected or colonized patients (Cunha et al., 1980).

During an outbreak of infection originating from an ICU, 12 (11.5%) of 104 air samples from wards harboring colonized patients were positive for *Acinetobacter* spp. as well as seven (8%) of 89 sink trap swabs and 13 (17%) of 75 samples from bedside cupboards in the same areas (Crombach et al., 1989) were also positive for *Acinetobacter* spp. Extensive contamination of the environment of *Acinetobacter* spp. by spreading through air borne mode has been described (Allen and Green, 1987). Bed linen from colonized patients were culture positive consistently, but linen from noncolonized patients were also positive on several occasions, as well as overblankets from empty beds in the vicinity of one ventilated colonized patient. The bed curtains around colonized patients were also contaminated. Persistent environmental contamination was documented for up to 13 days after the discharge of a patient (Allen and Green, 1987).

A further notable example of the role of the vicinity of bed environment in the dissemination of *Acinetobacter* spp. was seen in a large outbreak involving 63 of 103 patients admitted to a burns unit over a 21-month period (Sherertz et al., 1985). The outbreak was traced to contamination of mattresses through breaches in plastic covers that allowed water penetration and persistence of the organism in the wet foam of the mattresses. More recently, feather pillows were found to be contaminated with considerable numbers of acinetobacters in an outbreak of infection which occurred in
Netherlands (Weernink et al., 1995). It is therefore apparent that contaminated bedding materials may play an important role in the nosocomial dissemination of these organisms.

The above data 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 durations 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, they also 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). Similarly, an epidemic strain of multiresistant Acinetobacter spp. has been shown to survive for up to six days after inoculation on to dry filter paper. This duration was similar to that found with Staphylococcus aureus, which persisted for seven days, but significantly greater than the survival times for Escherichia coli and Pseudomonas spp., both of which persisted for 24 hours or less (Allen and Green, 1987). Prolonged survival of 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 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).

In summary, *Acinetobacter* spp. has unique characteristics among nosocomial gram-negative bacteria that favour their persistence in the hospital environment. These organisms spread easily in the environment and colonize in patients and can survive in that environment for many days, a factor that may explain their propensity for causing extended outbreaks. However, it should be noted that acinetobacters are ubiquitous organisms that can also be isolated readily from nonclinical sources such as soil, drinking and surface waters, sewage, and a variety of different food stuffs (Towner et al., 1991). There appears to be a significant population difference between the genomic species found in clinical sources and those found in other environments (Gennari and Lombard, 1993), and it is therefore vital that acinetobacters be identified to the genomic species level and then typed before epidemiological conclusions can be drawn.

7.3 Molecular Epidemiology:

Most of the outbreaks were elucidated using genotypic methods and phenotypic methods, however the latter has its own limitations. The dissemination and persistence of *Acinetobacter* spp. in the hospital environment probably accounts for the specific role in epidemiology. *Acinetobacter* spp has been reported for contaminating inanimate materials in hospital and they act as a reservoir of infection, especially during outbreaks. In some persistent outbreaks, contaminated materials
have been shown to act as a source of the outbreak. Genotypic methods such as RAPD, ribotyping, PFGE were clearly able to detect the source of outbreak.

Several early reports detected the source of outbreaks either through characterizing the isolates by phenotyping or by simple molecular techniques such as protein profiles and plasmid fingerprinting. Materials used for respiratory therapy or support in ICU have been implicated in many such outbreaks (Smith and Massanari, 1977; Hartstein et al., 1988; Cefai et al., 1990). Thus, 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 were found up to 10 m from 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. Other outbreaks have involved inadequate sterilization of autoclavable reusable needles used for administration of intrathecal methotrexate in patients with leukemia (Kelkar et al., 1989), 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 (Hartstein et al., 1988; Stone and Das, 1985; Vandenbroucke-Grauls et al., 1988). Hartstein et al. detected the contaminated source using plasmid profiling. Ethylene oxide sterilization of such contaminated
equipment has been shown to be effective. Radiation resistance of *Acinetobacter* clinical isolates has been described (Christensen et al., 1991), and these results indicate that special attention should be paid to medical devices that are normally sterilized by irradiation, particularly devices used in ICUs.

However, although respiratory equipment may be responsible for persistent outbreaks as a result of inadequate decontamination between use in consecutive patients, such equipment may act in some instances only as an intermediate reservoir of organisms and not as the primary source of infection. Thus, in one outbreak, a respiratory therapist with hand lesions from dermatitis was found to be chronic carrier of *Acinetobacter* spp. and was contaminating the equipment during assembly and testing (Buxton et al., 1978). Medical equipment may therefore become contaminated both by the patients themselves and staff during handling, and the latter possibility should always be considered when outbreaks of infection occur, especially in respiratory ICUs. Hence, a better approach is to screen the patients and patients attender along with environmental sampling for *Acinetobacter* spp. In addition, accurate typing of these isolates might be very useful in detecting the source of outbreak. Few investigations that were able to delineate and detect the source of outbreaks are described below wherein different molecular typing methods have been employed.

Traub (1989) used serotyping for delineation of outbreaks of nosocomial cross-infection. A series of outbreaks were detected in the study period occurring in ICU, surgery, PICU and extramural pediatric hospital. Unfortunately no other group
is working on serotyping, though it might become an additional marker for the
differentiation of phenotypically indistinguishable strains.

Low frequency cleavage using restriction enzyme and PFGE was able detect
an outbreak in urology ward due to *A. calcoaceticus*, in France (Allardet-Servent et
al., 1989). This also allowed a clear distinction between epidemic and self-
contaminating strains in different epidemiological situations existed in that locality.
Gouby et al., (1992) reinforced that PFGE can be an ideal epidemiological tool as it
was able to detect considerable DNA polymorphism within the species *A. baumannii*
and even among strains from different geographical regions that belong to the same
biotype. Four methods such as biotyping, CEPA, ribotyping and antibioticogram were
attempted to delineate outbreaks in different North European hospitals (Dijkshoorn et
al., 1993). The study concluded that biotyping and antibiotyping does not seem good
enough to use alone and it is acceptable to use CEPA and ribotyping for typing as the
latter had the ability to differentiate phenotypically indistinguishable isolates. Vila et
al. (1994) compared ribotyping with arbitrary primed PCR (AP-PCR) for the
investigation of hospital outbreaks caused by the *A. baumannii*. Both these methods
proved very efficient in delineating outbreaks however, AP-PCR was a simple and
rapid method with high discriminatory power when compared with ribotyping, which
is cumbersome and time consuming. One more study compared biotying and
antibiogram with that of plasmid analysis and PFGE (Seifert et al., 1994). Overall,
there was a remarkable degree of uniformity in typing results of plasmid analysis and
PFGE and it allowed differentiation of all sets of outbreak related strains. Hence
plasmid profile analysis can provide a cost-effective approach as a first step in
epidemiological typing. An hospital outbreak due to imipenem resistant *A. baumannii* was detected by Tankovic et al., (1994) using PFGE. ICU environmental contamination was recognized as an important reservoir of this imipenem resistant *A. baumannii* epidemic strain. The outbreak ceased only after the ICUs were closed for complete cleaning and disinfection.

In another study all traditional methods were not useful to detect an apparent outbreak (Reboli et al., 1994). Even enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) was not useful, however repetitive element PCR-mediated DNA fingerprinting using repetitive extragenic palindromic primers (REP) was able discriminate epidemic and sporadic strains of *A. baumannii*. Earlier, PCR fingerprinting was performed using a single primer M13 wherein it was applied to characterize *A. baumannii* isolates from an outbreak in an anesthesia ICU (Graser et al., 1993). Later PCR analysis was made quicker by using automated laser fluorescence (RAPD-ALFA) where in fluorescent primers were used (Grudmann et al., 1995) and on-line generation of high resolution DNA fingerprints of PAGE-RAPD products were achieved. The procedure took less than 8 hours whereas PFGE needs 90 hours.

Sporadic infections punctuated by more prolonged outbreaks involving large number of patients admitted in intensive therapy unit with severe underlying disease which occurred in Nottingham hospitals was reported. Multiple resistant *Acinetobacter* spp were responsible for outbreaks as detected by PFGE (Crowe et al., 1995). An outbreak of respiratory *A. baumannii* infection occurred in 5 ICUs of a tertiary care center, Canada was delineated using PFGE (Kapil et al., 1996).
A. baumannii was subsequently isolated from disinfected temperature probes and ventilators. Proper disinfection using gas sterilization of temperature probes terminated this outbreak.

In another study room humidifiers were responsible for the outbreak which occurred in neonatal intensive care unit (McDonald et al., 1998). PFGE was used to detect this outbreak.

Recently infrequent restriction site PCR (IRS-PCR) was applied to investigate an outbreak due to A. baumannii (Yoo et al., 1999). Hands of 2 health care personnel and Y piece of a mechanical ventilator was the source for persistent outbreak. In another recent work, an outbreak occurring in neonatal unit was linked to contaminated suction catheters (Pillay et al., 1999). The source of this outbreak was determined by ribotyping. Aerators were implicated as a reservoir for A. junii, which was responsible for bacteraemia in paediatric oncology patients. Automatic laser fluorescence analysis of RAPD profiles (RAPD-ALFA) was able delineate this outbreak (Kappstein et al., 2000). The clonal spread and persistence of a single strain of Acinetobacter 13TU in a large Scottish hospital was detected by PFGE. API 20 NE commercial phenotypic system misidentified 53% of these strains as A. junii, which was later showed that, these were Acinetobacter 13TU strains by tDNA finger printing (McDonald et al., 1999)

Many studies on epidemiological typing compared Discriminatory index (DI) of biotyping, antibiotyping, PCR, ribotyping, CEPA and PFGE and it was proved beyond doubt that PFGE offers best discrimination and has DI of 98% and above (Seifert and Gerner-Smidt, 1995; Crowe et al., 1995; McDonald et al., 1998).