REVIEW OF LITERATURE
CHAPTER 1

1. INTRODUCTION

Research on hospital acquired infections (HAI) has made unprecedented progress in the last three decades. Enormous literature has accumulated on this aspect in the recent years. The field of hospital infection control has emerged as an important facet of the broader discipline of hospital epidemiology through the incorporation of epidemiological principles and statistical analysis. Much importance is now given to nosocomial infections (HAI) as it results in an extension of hospital stay on an average of 5 days, which ultimately leads to increase in national health care expenditure. Despite many advances in modern medicine nosocomial infections still pose a potential risk of morbidity and mortality in patients. The major thrust is thus given, for an effective infection control that may reduce many of above mentioned eventualities.

Most important bacterial pathogens involved in hospital acquired infections 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 major viral agents like hepatitis B and C. A multitude of new 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 organisms such as *Pseudomonas* and *Acinetobacter* have emerged as the most important cause of nosocomial infections. Moreover, the widespread use of specific antimicrobial agents against bacteria in hospitals and indiscriminate use in community has resulted in the emergence of multiple resistance in these organisms. Nosocomial pneumonia that is second common HAI along with primary bacteremia is the leading cause of death. Infections with *Pseudomonas* and *Acinetobacter*, is usually high and higher mortality rates are also associated with these organisms when compared to other causes of nosocomial pneumonia (Fagon et al., 1993; Kollef et al., 1995).

The genus *Acinetobacter* originally described by Brisou and Prevot (1954) comprised of heterogeneous collection of non-fermentative, non-motile gram negative organisms which could be differentiated from other similar organisms by their lack of pigmentation (Ingram and Shewan, 1960). The genus *Acinetobacter* have been classified previously under a variety of different names, and it is only recently that rational taxonomic approaches for these organisms have emerged. Delineation of species within the genus is still the subject of ongoing research, and there is a need for universally acceptable and rapid method for assigning new isolates to individual species. Genus *Acinetobacter* is now defined to include gram-negative coccobacilli, which is sometimes difficult to destain. It has the G+C content of 39 to 47 mol%. They are strictly aerobic, non-motile, catalase positive and oxidase negative organisms.
The acinetobacters are free-living saprophytes found in soil, water and foods. They are also ubiquitous organisms in the hospital environment, where they play a significant role in the colonization and infection of patients admitted in hospitals. The members of genus have been increasingly recognized as important nosocomial pathogens as they cause pneumonia, bacteremia, urinary tract infection, wound infection and secondary meningitis. They have a predominant role as infectious agents of nosocomial pneumonia, particularly in intensive care units (ICU). Increased use of advanced invasive diagnostic and interventional therapeutic procedures in hospital ICUs during recent times may be, in part, responsible for the emergence of Acinetobacter species as a important nosocomial organism in the ICUs. Such infections pose a great therapeutic problem for the clinician, because of resistance of these bacteria to major groups of antibiotics.

Epidemiological studies have become dire necessity as these organisms are increasingly being incriminated in numerous outbreaks that occur across the world in various hospitals. Although some rare cases of community acquired infections caused by Acinetobacter spp. have been reported (Achar et al., 1993; Towner et al., 1996; Prashanth et al., 2000), the primary pathogenic role of these bacteria is, as nosocomial pathogen in hospitals. All three kinds of genetic transfers are seen in this genus. As the transfers occur both within species and between species experimentally as evidenced by reports, rapid transfer of resistant markers between different species in environment is one of the factor most clinical microbiologists feared of as it poses problem in treatment.
Acinetobacter has been implicated in a variety of food spoilage in addition to human infections. They can be easily grown in the laboratory in a simple mineral medium containing a single carbon as energy source. The vast majority of isolates resemble saprophytic Pseudomonads in being able to use any one of a large number of organic compounds as a carbon and energy source. Although the utilization of carbohydrates is relatively uncommon, a major biochemical feature of the genus is the range of compounds that can be metabolized. This range includes aliphatic alcohols, aminoacids, dicarboxylic and fattyacids, n-alkanes, alicyclic compounds, many aromatic compounds. This property has helped the taxonomist to clear the confusion that existed in their taxonomy, by developing phenotypic assimilation tests to differentiate the species. This is now the main stay for identification of these organisms in all clinical laboratories. Members of the genus are thus, particularly suitable for studying unusual peripheral biochemical pathways. These metabolic pathways may also have a significant role to play in biodegradation of a variety of hazardous pollutants and industrial residues, thus helping to keep the environment clean (Towner et al., 1996).

Very little is known about the various species circulating in hospital environments in India, resulting in a vacuum of knowledge about Acinetobacter. Identification of Acinetobacter to its species level, through different typing methods, will enhance our knowledge about their role in infection in the Indian setup. This study was designed to elicit information, regarding the prevalence of the Acinetobacter spp. as a nosocomial pathogen and to elucidate their epidemiology.
through molecular methods. In addition, to document the range of infections, caused by *Acinetobacter* spp. and their resistance patterns to various antibiotics.

1.1 TAXONOMY

1.1.1 History of the genus:

Members of the genus *Acinetobacter* have left a trail of taxonomic confusion. New rational scientific foundation, which has been established recently, has cleared the confusion to some extent regarding their taxonomy. *Acinetobacter* is ubiquitous in nature and hence all types of microbiologists have been encountering them one time or other. Their morphology resembles that of bacteria of many other genera, leading to a taxonomical confusion. A wealth of 'generic' names, which were synonyms for *Acinetobacter* spp, has been used in the past. At least fifteen different 'generic' names gained importance; among them the most well known are *Bacterium anitratum* (Schaub and Hauber, 1948), *Herellea vaginicola*, *Mima polymorpha* (De Bord, 1939), *Achromobacter*, *Alcaligenes*, *Micrococcus calcoaceticus*, 'BSW' (Juni, 1978), *Morexella glucidolytica* and *Moraxella lwoffii* (Piechaud et al., 1956; Brisou, 1957). Least known names were *Diplococcus mucosus* (Von Lingelshiens, 1908), *Neisseria winogradskyi* (Lemoigne et al., 1952), *Cytophaga anitrrata* and *Cytophaga lwoffii* (Lautrop, 1961).

The genus *Acinetobacter*, as in original concept (Brisou, 1957; Piechaud et al., 1956) included both oxidase positive and oxidase negative strains. In 1971, the subcommittee on the taxonomy of *Morexella* and allied bacteria proposed that the
genus *Acinetobacter* should include only the oxidase negative ones. Classified in the family *Neisseriaceae*, the *Acinetobacter* comprised only one species, *Acinetobacter calcoaceficicus* according to Bergey's Manual of Systemic Bacteriology (Juni, 1984). However, the approved lists of bacterial names (Skermann et al., 1980) noted two species, *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. Although never validated, the name “*Acinetobacter anitratrus*” has been used for glucose - oxidizing acinetobacters, *Acinetobacter lwoffii* being used for the asaccharolytic strains by many workers until now who are in favour of the only two species concept.

Ever since it is known that *Acinetobacter* is heterogeneous (Johnson et al., 1970) both phenotypic and genotypic considerations are made to form the basis for a phylogenetic classification of these bacteria. Hence it is not acceptable to have only one species in the genus *Acinetobacter*. As early as 1968, Baumann et al. tried to separate acinetobacters by devising carbohydrate assimilation tests that could make a gross subdivision of the genus. However, they were unable to devise a suitable scheme to identify various species with in the genus. Almost twenty years later, Bouvet and Grimont (1986) created a new classification of the genus on genotypic grounds. They also succeeded in devising a phenotypic identification scheme with twenty-eight tests, where in they were able to characterize each of these DNA groups. More recent taxonomic developments have resulted in the proposal that members of the genus should be classified in the new family *Moraxellaceae*, which includes *Moraxella*, *Acinetobacter*, *Psychrobacter* and related organisms (Rossau et al., 1991). This family constitutes a discrete phylometric branch in super family II of
the Proteobacteria on the basis of 16SrRNA studies and rRNA-DNA hybridization assays (Van Landschoot et al., 1986; Rossau et al., 1989).

The genus *Acinetobacter* is now defined as gram-negative coccobacilli, with a DNA G+C content of 39 - 47 mol %, that are strictly aerobic, non-motile, catalase positive and oxidase negative organisms. Sufficient growth can be achieved on complex media between 20°C to 30°C without growth factor requirements, while nitrates are reduced rarely. Crucially, extracted DNA is able to transform mutant strain BD413 trpE 27 to the wild type phenotype (Juni, 1972). Most strains of *Acinetobacter* can grow in a simple mineral medium containing a single carbon and energy source such as acetate, and pyruvate.

1.1.2 **Delineation of species and Current Taxonomic status:**

A genotypic definition has been proposed by an adhoc committee on reconciliation of approaches to bacterial systematics (Wayne et al., 1987). According to this committee a species ("Genospecies") is a set of bacteria that are 70% or more related to each other by DNA/DNA hybridization with a ΔTm (difference in thermal denaturation midpoint of hybrids) of 5 °C or less. Nucleic acid hybridization and sequencing studies are now able to provide the rational methods for designating species, as compared to traditional method of grouping strains, where in a high degree of similarity in terms of their phenotypic properties are grouped under particular species. However, genomic species that can be distinguished by phenotypic characteristics can be only given a formal species name.

Early DNA hybridization experiments with nitrocellulose filter method although showed the genus *Acinetobacter* is heterogeneous (Johnson et al., 1970),
only two species, *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii* were included in the approved lists of bacterial names (Skermann et al., 1980), and only one species was described in Bergey’s Manual of Systematic Bacteriology (Juni, 1984). More exhaustive recent studies using the DNA relatedness criteria described above have resulted in the recognition, to date, of 21 DNA-DNA homology groups (genomic species) with in the *Acinetobacter* genus (Bouvet and Grimont, 1986; Nishimura et al., 1988; Bouvet and Jeanjean, 1989; Tjernberg and Ursing, 1989; Ibrahim et al., 1997; Schreckenberger and Von Graevenitz, 1999). However, there are some minor discrepancies in the numbering schemes adopted for genomic species by different research groups from different laboratories and a definitive scheme has yet to be finalized. Seven of the genomic groups have been given formal species names, where in four new species names were introduced viz *Acinetobacter baumannii*, *Acinetobacter haemolyticus*, *Acinetobacter junii*, *Acinetobacter johnsonii* and description of the two old species *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii* were emended. The species *Acinetobacter radioresistens* (Nishimura et al., 1988) was being shown to be equivalent to genomic species 12 of Bouvet and Grimont (1986) by Tjernberg and Ursing (1989). DNA groups 1, 2, 3 and 13TU (Tjernberg and Ursing, 1989) have been shown to have a extremely close relationship and are referred to by some research groups as the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex (*Acb*-complex)(Gerner-Smidt et al., 1991). There is a need for a rapid and reliable method of assigning new isolates to individual genomic species. Ongoing efforts for devising rapid method envisage a possible direction for future research avenues that one can explore. One of the serotyping