CHAPTER 9

9. CLINICAL ANTIBIOTIC RESISTANCE:

High rates of antibiotic resistance found in Acinetobacter spp. have been documented in numerous reports (Bergogne-Berezin and Joly-Guillou, 1985; Buisson et al., 1990; French et al., 1980; Joly-Guillou et al., 1992; Larson 1984; Struelens et al., 1993; Tankovic et al., 1994; Prashanth et al., 2000). The main concern has been the frequent multiple antibiotic resistance shown by nosocomial acinetobacters which results in greater therapeutic problem while treating the patients with Acinetobacter infections in ICUs. Until the early 1970s, nosocomial Acinetobacter could be treated successfully with gentamicin, nalidixic acid, ampicillin or one of the tetracycline derivatives viz minocycline. These were used as single agents or in combinations, but increasing rates of resistance began to occur between 1971 and 1974. Successive surveys since then have been showing increasing resistance in clinical isolates of Acinetobacter spp. (Garcia et al., 1983; Godineau-Gauthey et al., 1988; Joly-Guillou and Bergogne-Berezin, 1985; Obana et al., 1985). Currently high proportion of strains have become resistant to older antibiotics and many acinetobacters are resistant to clinically achievable levels of most commonly used antibacterial agents such as aminopenicillins, ureidopenicillins, narrow and broad spectrum cephalosporins (Joly-Guillou and Bergogne-Berezin, 1985; Morohoshi et al., 1977), most aminoglycosides-aminocyclitols (Devaud et al. 1982; Dowding et al., 1979; Goldstein et al., 1983; Joly-Guillou and Bergogne-Berezin, 1985), chloramphenicol,
and tetracyclines: For some relatively new antibiotics, such as broad spectrum cephalosporins (cefotaxime, ceftazidime), imipenem, tobramycin, amikacin, and fluroquinolones, partial susceptibility remains, but the MIC's of these drugs for *Acinetobacter* isolates have increased substantially in the last ten years. However, imipenem still remains as the most active antibiotic. Imipenem showed 100% activity according to several recent reports (Amor et al., 1993; Muller-Serieys et al 1989; Seifert et al., 1993; Vila et al., 1994), however the only other drug with similar activity is polymixins. Unfortunately, the most recent analyses of hospital outbreaks have documented the spread of imipenem-resistant strains (Go et al., 1994; Tankovic et al., 1994; Manikal et al., 2000). The imipenem resistance markers might have been transferred from resistant *Klebsiella pneumoniae* as suspected, in one of the study (Urban et al., 1993). This is of concern as this development threatens the successful treatment of *Acinetobacter* infections. Most resistance to imipenem has been observed in strains identified as *A. baumannii*, while the MIC of carbapenems for *Acinetobacter* spp. other than *A. baumannii* has remained below 0.3mg /L, but the widespread emergence and/or spread of resistance to imipenem will definitely pose a serious threat in the future.

Variations in antibiotic susceptibility patterns among strains belonged to different countries have been observed, probably as a result of environmental factors and different patterns of antimicrobial usage. Differences have been observed in different countries such as USA, Germany and France (Seifert et al., 1993).
Species other than *A. baumannii* isolated from the hospital environment and clinical specimens are generally more susceptible to antibiotics (Gerner-Smidt 1987; Joly-Guillou, 1992; Traub and Spohr, 1989), however these species are less frequently involved in nosocomial infections. Species like *A. lwoffii*, *A. johnsonii*, *A. junii* are shown to have varying susceptibility between countries, but most of them are in susceptible range. Strains of *A. lwoffii* are more susceptible to β-lactams than the strains of *A. baumannii* (Bergogne-Berezin and Joly-Guillou, 1985). *A. haemolyticus* isolates are normally resistant to aminoglycosides and rifampin as documented in certain studies (Vila, 1998).

### 9.1 Mechanisms of Antibiotic Resistance:

*Acinetobacter* is a genus that appears to have a propensity to develop antibiotic resistance rapidly and for broad range, perhaps as a consequence of its long-term evolutionary exposure to antibiotic producing organisms in the soil environment. The above phenomenon is in contrast to more 'traditional' clinical bacteria, which seem to need more time to acquire highly effective resistance mechanisms in response to introduction of more advanced new broad spectrum antibiotic therapy. It is the *Acinetobacter* species ability to respond rapidly to challenge with antibiotics, combined with indiscriminate use of antibiotics in the hospital environment, which is responsible for their success as nosocomial pathogens. Conjugation has been only shown to play important role in the transfer of antibiotic resistance genes between members of this genus, even though all three major modes of chromosomal gene transfer have been documented in *Acinetobacter* spp. (Chopade

Both plasmids and transposons play a vital role in the biology of *Acinetobacter* spp. Numerous studies have documented that more than 80% of *Acinetobacter* isolates carry multiple indigenous plasmids (Gerner-Smidt, 1989: Seifert et al., 1994). However, many were having problems in isolating the plasmid DNA from this species, probably due to difficulty in lysing the cell wall of these organisms. Although many clinical isolates of *A. baumannii* were multidrug resistant, only a few studies have been able to demonstrate the plasmid-mediated transfer of resistance genes (Paton et al., 1993; Scaife et al., 1995). This may simply reflect the absence of a suitable test system for detecting such transfer as postulated by Towner (1991). Complex and varied transfer frequencies of standard plasmids belonging to different incompatibility groups have been observed between *Acinetobacter* strain EBF65/65 and *E. coli* K-12. However, most of these transfers need an additional mobilizing plasmid for retransfer to occur (Chopade et al., 1985, 1994). Hence, most reported cases of indigenous transmissible antibiotic resistance in *Acinetobacter* spp. have truly been associated with plasmids belonging to broad host range incompatibility groups. Transposons may play a significant role, in conjunction with integrons as there have been several reports of chromosomally located transposons carrying multiple antibiotic resistance genes in clinical isolates of *Acinetobacter* spp (Towner, 1991; Vila, 1998). Apart from these genetic mechanisms, it seems that a decrease in membrane permeability and active efflux system in this bacterium confers an important intrinsic resistance on these bacteria. The resistance transfer may take
place by conjugation or natural transformation, and both processes occur not only in animate but also in inanimate reservoirs. In addition, the selective pressure exercised by the antibiotics in these reservoirs may select resistant mutants. Thus, Acinetobacter, and more specifically, clinically important Acinetobacter spp has all the necessary conditions to acquire multi-resistance and it may therefore be considered the paradigm of multidrug resistant bacteria (Vila, 1998).

Clinically important Acinetobacter spp are well known for their multidrug resistance, especially for newer antimicrobials. The known biochemical and genetic mechanisms of resistance in Acinetobacter spp. to all the major groups of antibiotics has been elucidated in detail in the coming sections.

9.1.1 β-Lactams:

Most resistance for β-lactams in Acinetobacter spp. has been associated with the production of β-lactamases (Devaud et al., 1982; Goldstein et al., 1983; Joly-Guillou et al., 1988; Donald et al., 2000). TEM-1 and TEM-2 enzymes are the common β-lactamases in Acinetobacter, which are also widely seen in many gram-negative bacteria. In one study an analysis of 76 ticarcillin-resistant Acinetobacter strains for β-lactamases presence, revealed penicillinase activity in only 41% of the resistant strains (Joly-Guillou et al., 1988), of which majority produced TEM-1 like enzyme with a pI of 5.4, although a few also produced CARB-5, an enzyme with a pI of 6.3.
Some strains also had enzyme with high pI like 8.0. These were enzymes, which were not characterized, that were presumed to be chromosomally encoded cephalosporinases because of their high pI. In another study 98% of the clinical A. baumannii isolates were having cephalosporinase activity (Vila et al., 1993), and it therefore seems that cephalosporinases are the major β-lactamases in this species. Four such cephalosporinases (ACE-1 to ACE-4) were studied in detail (Hood and Amyes, 1991). All 4 enzymes showed their maximum activity against cephaloridine except ACE-4 that showed good activity against cephradine. However, they had little activity against penicillins, aztreonam, cefotaxime and ceftadizime. ACE-1 was most active against cefuroxime. Hence the contribution of these chromosomal β-lactamases appears to be important in the expression of β-lactam resistance along with reduced permeability and altered penicillin-binding proteins that is already conferring inherent resistance to these bacteria (Obara and Nakae, 1991; Sato and Nakae, 1991; Vila, 1998). The plasmid-encoded penicillinases do not seem to play a significant role in acquisition of long term β-lactam resistance in Acinetobacter species. However, few studies strongly suggest the possible role of plasmid encoded extended spectrum β-lactamases in these bacteria for the development of resistance (Paton et al., 1993; Scaife et al., 1995).

The identification of novel β-lactamase ARI-1 in an imipenem resistant strain of A. baumannii recently is one of the worrisome development as it will jeroperodise the efforts of effective treatment of infections caused by MDR Acinetobacter (Paton et al., 1993). This enzyme can hydrolyse both imipenem and azlocillin, however, it cannot hydrolyse cefuroxime, ceftazidime and cefotaxime. Direct conjugative transfer
of ARI-1 was demonstrated between original host *A. baumannii* and to an *A. junii* recipient in one of the study (Scaife et al., 1995). This observation suggests strongly that ARI-1 is a plasmid-encoded carbapenemase, a development that may have extremely serious long-term consequences. The *bla*<sub>ARI-1</sub> gene coding ARI-1 has also been sequenced recently and it was classified as a novel class D β-lactamase that has also been called by an alternative name OXA-23 (Donald et al., 2000). Imipenem resistance due to β-lactamases in *A. baumannii* has subsequently been reported worldwide, and two additional β-lactamases, ARI-2 (Brown et al., 1998) and an oxacillin-hydrolyzing enzyme (Afzal-Shah et al., 1999) have been also elucidated which were responsible for imipenem resistance. Recently one more novel class D β-lactamase that is chromosomally coded named OXA-24 has been characterized (Bou et al., 2000b). They have also sequenced the gene *bla*<sub>OXA-24</sub> that codes for this β-lactamase that is involved in the carbapenem resistance of *A. baumannii* RYC52763/97. Earlier, Bou et al. (2000a) reported the biochemical properties of chromosomally coded AmpC β-lactamase from the same bacterium. Metallo-β-lactamases found in Japanese isolates of *Ps. aeruginosa* and *A. baumannii* (Takahashi et al., 2000) were also detected in *A. baumannii* AC-54/97 a clinical isolate from Italy, wherein an allelic variant of *bla*<sub>IMP-1</sub>, i.e. *bla*<sub>IMP-2</sub> carried by intergon or gene cassettes of different phylogeny was sequenced and characterized recently (Riccio et al., 2000). The repertoire of intergon-associated β-lactamases has been shown to include metallo-β-lactamases (Riccio et al., 2000), which is a most worrisome development in the field of bacterial drug resistance. In fact, these enzymes are able to hydrolyze virtually all β-lactam compounds including carbapenems and expanded
spectrum cephalosporins and are not susceptible to the mechanism based serine-β-lactamase inhibitors (Bush, 1998; Laraki et al., 1999; Riccio et al., 2000). Thus, many studies strongly suggest the role of plasmid as well as chromosome encoded extended spectrum beta-lactamases in the *Acinetobacter* for the development of resistance.

9.1.2 Quinolones:

The development of 4-quinolone resistance is often quite difficult to demonstrate in the clinical laboratory as in the clinical situation many a time it gives contradictory results. This might be true with *E. coli*, but not with *Acinetobacter* and other nonfermentative gram-negative bacteria. *Acinetobacter* spp can develop quinolone resistance rapidly, however the precise mechanism for this resistance has not been elucidated. Usually, gyrA mutations that cause changes in DNA gyrase subunits in bacteria were implicated for 4-quinolone resistance development in many bacteria. PCR has been used to amplify DNA surrounding the active site region of the gyrA gene from 13 clinical isolates of *A. baumannii* having MIC's range 0.25 to 64mg/L for ciprofloxacin (Vila et al., 1998). When amplified PCR fragments were sequenced, it was found that the susceptible bacteria had 87 nucleotide differences, correlating with 13 aminoacid differences, when compared with the same 290-bp DNA fragment from *E. coli*. The residues Gly-81, Ser-83, Ala-84, and Gln-106, whose substitution in *E. coli* leads to quinolone resistance, were all conserved in the susceptible *A. baumannii* strains. All 9 isolates of *A. baumannii* with ciprofloxacin MIC's of >2mg/L showed a substitution of Ser-83 to leucine and Ala-84 to proline.
Only one exhibited a change from Gly-81 to valine. They were well correlated with that of *E. coli* in substitution of Ser-83, which was responsible for ciprofloxacin resistance in *A. baumannii* isolates.

Outer membrane permeability to antibacterial agents is less in *Acinetobacter* when compared with other gram-negative organisms. This inherent decreased uptake may be due to changes in outer membrane. In *Ps. aeruginosa* resistance to quinolones is due to alterations in outer membrane proteins (Vila et al., 1998) and this may also true with *Acinetobacter* spp. In support of this hypothesis, an increase in the proportion of *Acinetobacter* isolates with combined resistance to all β-lactams, all aminoglycosides, and quinolones have been demonstrated (Muller-Serieys et al, 1989).

9.1.3 Aminoglycosides:

Aminoglycosides are now widely used for the treatment of serious *Acinetobacter* infections. From last two decades there have been increasing number of studies reporting highly resistant *Acinetobacter* strains for aminoglycosides. And their number is also increasing in an exponential manner. All three types of aminoglycoside modifying enzymes have been identified in *Acinetobacter* strains. However, there have been geographic variations in their incidence (Shaw et al., 1991 & 1993). Even there are geographic variations in the incidence of resistance genes. The gene AAC(3)-Ia was frequently found in *Acinetobacter* strains that were prevalent in Belgium. However it was observed less frequently in United States and no strains had AAC(3) in Argentina (Shaw et al., 1991 & 1993). More than one
aminoglycoside resistant gene is more common in some strains and up to six different resistance genes are identified in some isolates.

The novel gene aac(6')-Ig was identified only in *A. haemolyticus*, where it confers resistance to amikacin. This phenomenon can be used as simple identification using PCR detection for identification of *A. haemolyticus* (Lambert et al., 1993).

Both plasmid transposon locations for aminoglycoside resistance genes have been identified in few studies (Devaud et al., 1982; Elisha and Steyn, 1991; Goldstein et al., 1983; Gomezilus et al., 1980; Lambert et al., 1990; Murray and Moellering, 1980).

9.1.4 Other antibiotics:

Most clinical isolates of *Acinetobacter* are resistant to chloramphenicol. The chloramphenicol acetyltransferase I (CAT1) gene has been associated with both chromosomal and plasmid DNA in a *Acinetobacter* isolate of clinical origin, suggesting that the CAT1 gene might be transposon encoded and it had improved its survival potential by locating in both replicons (Elisha and Steyn, 1991). High level resistance for trimethoprim (MIC > 1,000mg/L) have been reported in few studies. This resistance might be due large conjugative plasmids or transposons. Resistance to sulfonamides is due to acquisition of plasmids encoding the target protein, dihydropteroate synthetase. Vila (1998) and their colleagues have been able to demonstrate the presence of sull gene that is responsible for sulfonamide resistance in MDR *A. baumannii* strains. There are only few studies on the resistance mechanisms developed for other antibiotics in clinical isolates of *Acinetobacter* (Chirnside et al., 1985; Goldstein et al., 1983; Muller-Serieys et al., 1989).