CHAPTER I

REVIEW OF LITERATURE

In the complex microbiological milieu of the hospital there occurs a duel between man and microbe, which often gets tilted in the latter’s favor. Our antibiotic choices seldom remain more than a few drugs ahead of the resistant strains. Resistance can occur even before the clinical use of specific antibiotics e.g. sulfonamides and aminoglycosides resistance can be found in gram negative bacteria isolated long before the use of these compounds (Bennett et. al. 1998).

Antimicrobial agents were introduced in mid 1930’s. In 1941 virtually all strains of Staphylococcus aureus worldwide were susceptible to penicillin G, but by 1944, S. aureus was capable of destroying penicillin by penicillinase, which is now called beta lactamase. Today greater than 95% of S. aureus worldwide are resistant to penicillin, ampicillin and anti-pseudomonal penicillin (Ticaracillin, Piperacillin). In July 1945, Guiseppe Brotzu, an eminent medical scientist at the University of Cagliari, isolated from a local sewage outfall a mould later identified as Cephalosporium acremonium (now called Acremonium chrysogenum). He found that this fungus prevented the growth of several species of pathogenic bacteria (Brotzu 1948, Brumfitt 1999). This fungus was found to produce Cephalosporin C by Newton and Abraham (Newton et. al. 1955). In 1970’s and 1980’s there were outbreaks of methicillin resistant S.aureus (MRSA) (Haley RW et. al. 1982). In 1980’s large families of beta lactamases that mediate resistance to cephazolin antibiotics emerged in Enterbacteriaceae, vancomycin resistance in Gram-positive cocci was described and aminoglycoside resistant betalactamase containing Enterococci surfaced. The 1990’s have witnessed Staphylococcal and Pseudomonal resistance to the fluoroquinolone antibiotic and imipenem resistance in Enterobacteriaceae (Schaberg et. al. 1991).

Beta-lactam antibiotics can be rendered inactive by production of beta-lactamase, alteration in the pre-existing penicillin binding protein (PBP), acquisition of a novel PBP insensitive to beta-lactam, changes in the inter membrane proteins of Gram-
negative organisms and active efflux of the antibiotic, which prevents these compounds from reaching their targets, and can confer resistance (Bradford 2001).

The most common mechanisms of resistance by bacteria are by antibiotic inactivating enzyme such as beta-lactamases, aminoglycoside-modifying enzymes or chloramphenicol acetyltransferase etc. Bacterial chromosomes or plasmids may mediate this resistance. Families of plasmid-borne extended spectrum beta-lactamases were first identified in 1983 in Europe and USA (Philippon 1989) and around the world (Bradford 2001).

The important factors for emergence, persistence and transmission of antibiotic resistance are microbial characteristics, environmental and human reservoirs in which resistant organisms can persist, patterns of antimicrobial used and societal and technological changes also affect the transmission of these resistance organisms (Bennett et al, 1998; McGowan 1983; Stamm et al. 1981). Bacterial resistance has become a fact of hospital life and is so common that it often goes unnoticed until it is either extreme or epidemic.

1.2. Antibiotic resistance in bacteria:

The selection of resistant bacteria began on a global scale in the early 1940s, with the introduction of the first penicillin into clinical use. The first antibiotic, penicillin, was discovered in 1928 by Sir Alexander Fleming and it came into clinical use by 1940s. After World War II, penicillin resistance among Staphylococci and gonococci strains was first noted (Kirby 1944). Staphylococcus aureus strains carrying the beta-lactamase genes had spread worldwide by the late 1950s. The first penicillin resistant pneumococcus was detected in a remote village in Papua, New Guinea in 1967 (Hansman et al, 1974).

Since 1960s reports of antibiotic-resistant bacteria in hospitals have appeared with increasing frequency. During 1965 to 1975, however, 11 of 15 nosocomial epidemics of Enterobacteriaceae studied involved multiple resistance strains (Stamm et al. 1981, Weinstein et al. 1998). Methicillin-resistant (MRSA) S. aureus emerged in 1970s (Tomasz 1994). A study by Centre of Disease Control (CDC) showed ciprofloxacin resistance in MRSA which went from less than 5% to greater than 89% within one year (Neu 1992).
The period of the late 1940s and early 1950s saw the discovery and introduction of streptomycin, chloramphenicol and tetracycline and the age of antibiotic chemotherapy came into full being (Table 1). These antibiotics were effective against the full array of bacterial pathogens including Gram-negative bacteria. But by 1953, a Shigella outbreak in Japan produced a pathogen exhibiting resistance to chloramphenicol, tetracycline, streptomycin and sulfonilamide (Tomasz 1994).

Acquired vancomycin resistance in Enterococci began to appear in the mid-1980s. Data reported to the National Nosocomial Infection Survey of the Centre for Disease Control and Prevention revealed that vancomycin resistance has increased more than 20-fold in Enterococci, from less than 0.5% in 1989 to more than 10% in 1995 (Gold et al. 1996). In the mid-1980s in France and Germany, the failure of therapy for Klebsiella infections suddenly occurred because Klebsiella pneumonias were resistant to cefotaxime, ceftriazone, or ceftazidime, agents considered totally stable to beta-lactamase (Neu 1992). Chakrabarti et al. in 1988 studied the effect of beta-lactamase inhibitor on anaerobic isolates of Calcutta (Chakravarty et al. 1988).

By 1990s E.coli isolated from urine was found to be multi-drug resistant (Neu 1992). Since the mid-1980s the incidence of nosocomial infections caused by enteric Gram-negative bacilli such as Klebsiella pneumonias and Escherichia coli has decreased, whereas the incidence of infections due to more resistant Gram-negative pathogens, Gram-positive bacteria and fungi have increased. Although the reason for this shift is not entirely clear, it may be explained by the widespread use of a variety of antibiotics, such as cephalosporins, which inhibit growth of many Gram-negative bacteria, but have little or no activity against enterococci or coagulase negative Staphylococci (Schaberg et al. 1991). The 1990s also witnessed Staphylococcus and Pseudomonas resistance to the fluoroquinolone antibiotics and imipenem resistance in Enterobacteriaceae (Weinstein 1998). The first vancomycin-resistant S.aureus (VRSA) clinical isolate, defined as a strain for which the vancomycin MIC is > 32g/ml, was reported in Michigan in June 2002 (Liu et al. 2003). Basically each class of antibiotic became resistant by a different mechanism (Table 2).
Table 1: Timeline of Antibiotics

The years show when given antibiotic was released in the pharmaceutical market.

- 1939 sulfacetamide
- 1940 sulfamethizol
- 1942 benzylpenicillin
- 1944 streptomycin
- 1948 chlorotetracycline
- 1949 chloramphenicol
- 1950 penicillin G procaine
- 1952 erythromycin
- 1955 vancomycin
- 1960 methicillin
- 1960 metronidazole
- 1961 ampicillin
- 1961 sulfamethoxazole
- 1961 trimethoprim
- 1962 cloxacillin
- 1964 gentamicin
- 1972 amoxicillin
- 1975 ticarcillin
- 1976 amikacin
- 1977 ceftoxitin
- 1977 cefturoxime
- 1980 ceftotaxime
- 1980 piperacillin
- 1981 amoxicillin/clavulanic acid (co-amoxiclav)
- 1981 ceftazidime
- 1982 ceftriaxone
- 1983 cefuroxime
- 1983 norfloxacin
- 1985 imipenem/cilastatin
- 1985 ofloxacin
- 1986 aztreonam
- 1986 cefoperazone/sulbactam
- 1986 ticarcillin/clavulanic acid
- 1987 ampicillin/sulbactam
- 1987 roxithromycin
- 1987 ciprofloxacin
- 1988 azithromycin
- 1989 ceftibutenoxime
- 1992 ceftizoxime
- 1992 piperacillin/tazobactam
- 1993 panipenem/betamipron
- 1994 ceftazidime
- 1995 quinupristin/dalfopristin
- 2000 linezolid
- 2001 telithromycin
### TABLE 2: Mechanisms of Antibiotic Resistance

<table>
<thead>
<tr>
<th>Antibiotic class</th>
<th>Mechanisms of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporins</td>
<td>Extended-spectrum betalactamases, Chromosomal cephalosporinases.</td>
</tr>
<tr>
<td>Beta lactamase inhibitors</td>
<td>Hyper producers of betalactamases, New betalactamases resistance to inhibitors, chromosomal cephalosporinases.</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>Zinc metalloenzymes and other beta lactamases.</td>
</tr>
<tr>
<td>Vancomycin, Teicoplanin</td>
<td>Modified cell wall precursors with decreased affinity for vancomycin</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Alterations in DNA topoisomerases, efflux mechanisms, permeability changes</td>
</tr>
<tr>
<td>Trimethoprin-sulfamethoxazole</td>
<td>Resistant enzyme in folate synthesis pathway.</td>
</tr>
<tr>
<td>Erythromycin, new macrolides</td>
<td>Methylation of bacterial ribosome producing resistance to macrolides, clindamycin and streptogramin, beta antibiotics.</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Aminoglycosides modifying enzymes.</td>
</tr>
</tbody>
</table>
To combat the new versions of beta-lactamase beta-lactamase inhibitors were used. Clavulanic acid, a naturally occurring beta-lactam, had been the first such inhibitor, which is produced by *Streptomyces clavuligerus* (Reading *et. al.* 1977). Subsequently, a few more viz., sulbactam, a penicillanic acid sulphone and tazobactam (Aswapokee *et. al.* 1978) were found.

Antibiotic resistance has increased over the last 60 years. A large number of antibiotics, against which, distinct mechanism of resistance has developed. The strategy of the pharmaceutical industry is to widen the antibacterial spectrum of each new antibiotic and thus introduced enormous quantities of these potent drugs into the therapeutic environment, resulting in emergence of increasing number of antibiotic resistant bacteria. Maximum resistance was found towards beta-lactam group of antibiotics (Thomson *et. al.* 2000) As one of the important reasons of beta-lactam resistance is the production of the antibiotic inactivating enzyme, beta-lactamase by bacteria, we focused our attention on bacterial beta-lactamases.

### 1.3. Beta-lactamases in bacteria:

Emergence of resistance to beta-lactam antibiotics, began even before the first beta-lactam, penicillin was developed. The first beta-lactamase was identified in *Escherichia coli* prior to the release of penicillin in medical practice (Abraham *et. al.* 1940). The age of penicillin saw the rapid emergence of resistance to *S. aureus* due to plasmid-encoded penicillinase. Beta-lactamases are the leading cause of resistance to beta-lactams among hospital and community isolates. Many genera of Gram-negative bacteria possess a naturally occurring chromosomal mediated beta-lactamase. This may be due to selective pressure exerted by beta-lactam producing soil organism found in the environment (Ghuysen 1991).

Over the last 20 years, many new beta-lactam antibiotics have been developed, that are specifically designed to be resistant to hydrolytic actions of beta-lactamases. However, with the new class that has been used to treat patients, new beta-lactamases emerged that caused resistance to that class of drugs. This is mainly due to the selective pressure of the use and overuse of the new beta-lactams (Bradford 2001).
1.4. Types of beta-lactamases found in clinical strains:

Different types of novel beta-lactamases have emerged during the last few decades. Following are the betalactamases which have become increasingly important:

1.4.1. Extended-Spectrum Beta-lactamases (ESBLs):

The first plasmid-mediated beta-lactamase in Gram-negatives, TEM – 1, was described in early 1960s (Datta 1965). The TEM – 1 enzyme was originally found in a single strain of *E coli* isolated from blood culture from a patient named Temoniera in Greece, hence the designation TEM (Medeiros 1984). Being plasmid and transposon mediated has facilitated the spread of TEM-1 to other species of bacteria within a few years after its first isolation, the TEM-1 beta-lactamase spread worldwide and is now found in many different species of number of the family *Enterobacteriacea*, *Pseudomonas aeruginosa* etc. Another common plasmid mediated beta-lactamase found in *Klebsiella pneumoniae* and *E. coli* is SHV-1 (for sulphydryl variable). The SHV-1 beta-lactamase is chromosomally encoded in majority of isolates of *K. pneumoniae* but same is usually plasmid mediated in *E. coli*.

After this many new beta-lactam antibiotics were developed specially designed to be resistant to the hydrolytic activity of beta-lactamase. But with each new class of antibiotics used to treat patients a new beta-lactamase emerged, that caused resistance to that class of drug. The first of these expanded spectrum beta-lactamase was SHV–2 which was found in a single strain of *Klebsiella ozaenae* isolated in Germany (Kliebe 1985). Because of their increased spectrum of activity, especially against the oxyimino-cephalosporins, these enzymes were called extended spectrum beta-lactamases (ESBLs). Gram-negative bacilli producing ESBL were first described in Germany in 1983 (Bush et. al. 1995).

Now, over 150 different ESBLs have been described (Bradford 2001). The majority of ESBLs contain a serine at the active site and belong to Ambler's molecular Class A (Ambler 1980). A modern classification by Bush, Jacoby, and Medeiros that used, the biochemical properties of the enzyme plus the molecular structure and nucleotide sequence of the genes to place beta-lactamases into functional groups (Bush et. al. 1995). Using this classification, ESBLs are defined as beta-
lactamases capable of hydrolyzing oximino-cephalosporins that are inhibited by clavulanic acid and are placed into functional group 2be (Bush et. al. 1995). These beta-lactamases extend their substrate profile to include drugs such as cefotaxime, ceftriaxone, ceftazidime, ceftizoxime, and aztreonams, but they remain highly susceptible to beta-lactamase inhibition. There are now over 70 derivatives of TEM-1 and TEM-2 and over 20 derivatives of SHV-1.

TEM-1 is the most commonly encountered beta-lactamase in Gram-negative bacteria. Upto 90% of ampicillin resistance in *E coli* is due to the production of TEM-1 (Livermore 1995). TEM-1 is able to hydrolyze penicillins and early cephalosporins such as cephalothin and cephaloridine. TEM-2, the first derivative of TEM-1, had a single amino acid substitution from the original beta-lactamase (Bradford 2001). This caused a shift in the isoelectric point from a pI of 5.4 to 5.6, but it did not change the substrate profile. TEM-3, originally reported in 1989, was the first TEM type beta-lactamase that displayed the ESBL phenotype (Sougakoff et. al. 1988). After this, upto 90 different type of ESBL have been found which have a combination of amino acid changes. These resulted in subtle alteration in substrate and isoelectric points which can range from a pI of 5.2 to 6.5 in ESBL.

The ESBL production has been reported worldwide in *Enterobacteriaceae* like *E coli* and *K. pneumoniae* right from 1983 to date (Livermore 1995, Bradford 2001). But recently they have been described in other organisms like *Morganella morganii, Serratia marcescens, Shigella dysentriae, Salmonella, Proteus* and *Citrobacter* (Philippon et. al. 1989, Jacoby 1991, Thomson 1996). They have also spread beyond the family of *Enterobacteriaceae*, being reported in some rare French isolates of *Pseudomonas aeruginosa* (TEM-4, TEM-42, SHV-2), *Burkholderia cepacia* and others (Mugnier et. al. 1996, Nordmann et. al. 1998, Heritage et. al. 1999 and Naas et. al. 1999). Strains of ESBL began to be reported in United States around 1989-1990, but major outbreaks of producers have since been reported in Chicago (Bradford et. al. 1994), New York (Meyer et. al. 1993), San Francisco (Naumovski et al 1992), Boston (Rice et. al. 1990). By 1994, the Center for Disease Control and Prevention National Nosocomial Infections Surveillance Scheme was reporting that 8% of *Klebsiella* in United States had ESBL (Burwen et. al. 1994). The type TEM-3 is commonest in France (Petit et. al. 1990) whereas TEM-10, TEM-12 and TEM-26 is predominant in United States (Bradford et. al. 1994, Naumovski et. al. 1992).
The SHV-1 beta-lactamase is most commonly found in *K. pneumoniae* and is responsible for up to 20% of the plasmid mediated ampicillin resistance (Tzourdeks *et al.* 1999). The OXA – type enzymes are another growing family of ESBLs. These beta-lactamases differ from the TEM and SHV enzymes in that they belong to molecular class D and functional group 2d (Bush *et al.* 1995). The OXA – type beta-lactamases confer resistance to ampicillin and cephalothin and are characterized by their high hydrolytic activity against oxacillin and cloxacillin and the fact that they are poorly inhibited by clavulanic acid (Bush *et al.* 1995). The OXA type ESBLs have been found mainly in *P. aeruginosa* (Bradford 2001). Resistant organisms harbouring a variety of these enzymes carried on transferable plasmids have been isolated with increasing frequency in Europe over the past several years (Chanal *et al.* 1988, Deschaseaux *et al.* 1988, Gutmann 1988, Jarlier 1988, Petit *et al.* 1988, Sirot 1988, Spencer *et al.* 1987, Vufe *et al.* 1989, Quinn *et al.* 1989).

By 1994, the Center for Disease Control and Prevention as well as National Nosocomial Infections Surveillance System (NNIS) reported that 8% of *Klebsiella* spp. had ESBLs (Burwen *et al.* 1994). A 1995-96 study in Richmond, Virginia reported 1.5% of isolates produced ESBLs. In Europe, in 1995, about 20-25% *Klebsiella* spp in ICUs was found to be ESBL producers, whereas in France it was 30-40% (Sanguinetti *et al.* 2003).

In 1999-2002 ESBL producing *Proteus mirabilis* was reported from Hong Kong (Ho *et al.* 2005). A study from Spain reported ESBLs which were CTX – M – 9 (27.3%), SHV – 12 (23.9%) and CTX – M- 14 (20.5%) for *E-coli* and TEM – 3 (16.7%) and TEM – 4 (25%) for *K. pneumoniae* (Hernandez *et al.* 2005). From Poland a study from 1998 to 2000 showed TEM type ESBL in *Enterobacteriaceae* (Baraniak *et al.* 2005). A SHV–5 ESBL from *K. pneumoniae* reported from Malaysia (Palasubramaniam *et al.* 2005). SHV type beta-lactamase was reported from Hungary among Enterobacteriaceae (Toth *et al.* 2005). In Korea CTX-M and SHV-12 beta-lactamases are the most common ESBLs in clinical isolates of *E. coli* and *K. pneumoniae* (Kim *et al.* 2005). *K. pneumoniae* in Korea showed 28.4% ESBL production (Yum *et al.* 2005). The apparent geographical occurrence of individual Seals varies probably in response local antibiotic prescribing preferences, but it is also
likely that prevalence data reflects the diligence and watchfulness of the microbiologist of different countries.

The first report of ESBL from India was in 1987 (Nandivada et al. 1987) and then a novel beta-lactamase SAR – 2 was identified in a *Escherichia coli* strain in South India which was active against penicillin, cephalosporins, oxacillin and methicillin (Nandivada et al. 1989). In 1993, there were reports of presence of TEM-1 beta-lactamase in *E. coli* isolated from Vellore (Thomson et al. 1993) and in 1995, fifteen strains of *K. pneumoniae* producing ESBL, were reported from Vellore (Abigail et al. 1995). In 1997, 25.8% of *K. pneumoniae* having ESBL reported from Nagpur (Hansotia et al. 1997). In 1998, first report of SHV-5 beta-lactamase producers was made from Delhi (Revathi et al. 1998). In 1999, six non-clonally related Enterobacterial isolates producing the same ESBL CTX-M-15, was found in New Delhi (Karim et al. 2001). In 2001, ESBL producing *Klebsiella* spp. causing nosocomial respiratory infection was reported (Gladstone et al. 2001). 6.6% of *Klebsiella* spp. was reported to be ESBL producers in Chennai (Subha et al. 2001). 68% of ESBL producers were reported in 2002 from Delhi (Mathur et al. 2002). ESBL was detected in 86.6% of *Klebsiella* spp., 73.4% of *Enterobacter* spp. and 63.6% of *E. coli* strains in a study done on neonatal sepsicaemia from Lucknow (Jain et al. 2003). From a *Pseudomonas aeruginosa* clinical strain a bla (VEB-1) gene which codes for ESBL was reported from New Delhi (Aubert et al. 2004). 48.3% ESBL producers *E. coli*, *K. pneumoniae* and *Acinetobacter* was reported from Nagpur, India (Tankhiwali et al. 2004). 70.6% *K. pneumoniae* strains were identified as ESBL producers from a major hospital in New Delhi (Grover et al. 2004). 87% of the isolates were ESBL producers among *K. pneumoniae* from Delhi, India (Manchanda et al. 2005). Whereas Mathur et al. reported 89% ESBL production in Gram-negative bacteria with *Pseudomonas aeruginosa* being the commonest isolate (Mathur et al. 2005). In 173 gram–negative clinical isolates from four tertiary care hospitals in Gurgaon, India, 23% of the isolates were resistant to cefoxitin of which 8% were AmpC beta-lactamase production (Singhal et al. 2005).

Inhibitor resistant beta-lactamases are not ESBLs, but they are discussed with them because they are also derivatives of the classical TEM or SHV – type enzyme. In early 1990s beta-lactamases that are resistant to inhibition by clavulanic acid were discovered, they were given the name IRT for inhibitor resistant TEM beta-
lactamases, but have subsequently been renamed with numerical TEM designations. Inhibitor resistant TEM beta-lactamase have been found mainly in clinical isolates of *E.coli*, but also some strain of *K. pneumoniae, Klebsiella oxytoca, Proteus mirabilis* and *Citrobacter freundi* (Bret *et. al.* 1996, Lemozy 1995). Although the inhibitor-resistant TEM variants are resistant to inhibition by clavulanic acid and sulbactam, thereby showing clinical resistance to the beta-lactam beta-lactamase inhibitor combinations of amoxicillin-clavulate, ticarcillin-clavulanate and ampicillin-sulbactam, they remain susceptible to inhibition by tazobactam and also susceptible to the combination of piperacilin and tazobactam (Bonomo *et. al.* 1997, Chaibi *et. al.* 1999). These beta-lactamases have been detected in Greece and a few other locations within Europe (Chaibi *et. al.* 1999). In a study of amoxicillin – clavulanate resistant *E.coli* in a hospital in France, Lefton – Gaubout et al found that up to 41% of these isolates produced inhibitor-resistant TEM variants (Lefon–Guibout *et. al.* 2000). TEM – 50 is an inhibitor-resistant TEMs recently identified. This enzyme was resistant to inhibition by clavulanate, but it also conferred a slight resistance to expanded spectrum cephalosporins (Sirot *et. al.* 1997). This could indicate the possibility of a new group of beta-lactamases with a complex phenotype showing some characteristics of ESBL and inhibitor resistant enzymes. Inhibitor resistant variant of SHV – 1 have also been detected (Philippon 1989).
**TABLE 3: Time-line of beta-lactamase producers (Livermore 1995)**

<table>
<thead>
<tr>
<th>Year</th>
<th>Place</th>
<th>Type of enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>Germany</td>
<td>TEM-1 TEM-2</td>
</tr>
<tr>
<td>1983-1984</td>
<td>USA</td>
<td>TEM-1 TEM-2</td>
</tr>
<tr>
<td>1983-1986</td>
<td>Spain</td>
<td>TEM-1 OXA-1 SHV-1</td>
</tr>
<tr>
<td>1983-1987</td>
<td>Finland</td>
<td>TEM-1 TEM-2</td>
</tr>
<tr>
<td>1984</td>
<td>Scotland</td>
<td>TEM-1 TEM-2</td>
</tr>
<tr>
<td>1984</td>
<td>India</td>
<td>TEM-1 TEM-2 SHV-1</td>
</tr>
<tr>
<td>1984-1988</td>
<td>HongKong</td>
<td>TEM-1 OXA-1 TEM others</td>
</tr>
<tr>
<td>1986-1987</td>
<td>Spain</td>
<td>TEM-1 OXA-1 SHV-1 TEM others</td>
</tr>
<tr>
<td>1988-1990</td>
<td>Portugal</td>
<td>TEM-1 TEM-2 OXA-1 SHV-1 TEM others</td>
</tr>
<tr>
<td>1990</td>
<td>Sweden</td>
<td>TEM-1 OXA-1 SHV-1 TEM others</td>
</tr>
<tr>
<td>1990-1991</td>
<td>London</td>
<td>TEM-1 TEM-2 OXA-1 SHV-1 TEM others</td>
</tr>
</tbody>
</table>
1.4.2. AmpC Beta-lactamases:

The clinical use of beta-lactams is now the major selective factor influencing beta-lactamase production by pathogens. AmpC cephalosporinase of bacteria occur in organisms long pre-dating the antibiotic era. These enzymes may have some physiological role in peptidoglycan assembly or may have evolved to defend bacteria against beta-lactam produced by environmental bacteria and fungi.

Plasmid-mediated AmpC were detected in late 1980s, providing AmpC beta-lactamase to spread to species such as *Klebsiella pneumoniae*, *Proteus mirabilis* and *E. coli* (Philippon et al. 1999). There are now 19 types of plasmid-mediated AmpC beta-lactamase, with CMY-2 appearing to be the most prevalent and widely distributed (Bauerfeind et al. 1998). Recently strains that could be induced to produce beta-lactamase by adding CA were detected. DHA-1 is the first identified plasmid-encoded inducible cephalosporinase from Saudi Arabia in 1998 (Yan et al. 2002). In Italy, 15% (77 out of 510) clinical isolates have been found to be AmpC beta-lactamase producers in 2003; in the same year in Richmond, Virginia AmpC production was reported (Coudron et al. 2003). The MIC90s for beta-lactams except carbapenems, and cefepime, were 32 µg/ml or more. The MIC 90s for the five quinolones tested ranged from 4 to 16 microg/ml among AmpC beta-lactamase producing *E. coli* isolates from Japan (Yamaski et al. 2005). Among *E. coli* and *Klebsiella* spp four kinds of plasmid-mediated AmpC beta-lactamases, ACT – 1, CMY – 1, CMY-2 AND DHA – 1, were detected from Korea. (Kim 2005). AmpC beta-lactamase enzyme production was detected in 9.3% of *E.coli* and 4.48% of *K. pneumoniae* from a hospital in Shenyang, China (Wang et al. 2005). Respiratory isolates of *K. pneumoniae* in Korea showed 53.5% of plasmid mediated AmpC beta-lactamase (Yum et al. 2005).

In India, 2.6% *K. pneumoniae* were found to be AmpC beta-lactamase producers (Thomson et al. 1993). In 2003, 20.7% AmpC enzyme producers were reported in Delhi (Machanda et al. 2003). In the same year Subha et al. found AmpC production in 24.1% of *Klebsiella* spp. and 37.5% of *E. coli* in Chennai, (Subha et al. 2003). Shahid et al. found 20% of *P. aeruginosa* producing AmpC beta-lactamase in Aligarh (Shahid et al. 2003), in Karnataka Ratna et al found 3.3% of *E. coli*, 2.2% *K.
Structure of Thienamycin
pneumoniae, 5% C. freundii, and 5.5% of E. aerogenes harbouring AmpC enzymes (Ratna et. al. 2003).

1.4.3. Metallo-beta-lactamases:

Metallo-beta-lactamases are emerging worldwide as acquired resistance determinant in clinical strains, which can hydrolyze carbapenems. Imipenem (N-formimidoyl thienamycin), a carbaperem, is a semisynthetic derivative of theinamycin produced by *Streptomyces cattleya* (Barza 1985). Imipenem was approved for clinical use in 1987 in Japan, followed by Panipenem and Meropenem in 1993 and 1995, respectively. But carbapenem resistant strains had already emerged in Japan by 1989 (Kurokawa et al. 1999). These carbapenems have been used in clinical settings as a last resort for their broad-spectrum antibacterial activity and stability against various beta-lactamases produced by Gram-negative bacteria. Two major groups of MBLs are IMP and VIM type. IMP-1 was the first identified acquired MBL (Murphy et. al. 2003). *Pseudomonas aeruginosa* producing MBL (12%) have been reported by Navneeth et. al. in 2002 from Bangalore (Navneet et. al. 2002). In 1984 in our laboratory we found all penicillin-resistant beta-lactamase producing strain were inhibited by N-formimidoyl theinamycin (IPM) at concentration ranging from 0.012- 0.025 µg/ml (Ray et. al. 1984). In 1989, Gram-negative organisms recovered from neutropenic patients (*Klebsiella* spp., *Enterobacter* spp., *E.coli*) in India showed full susceptibility to Imipenem (The Indian Antimicrobial Resistance Study Grcup et al. 2002). Whereas, by 2003, in Brazil, carbapenem use had been limited due to high carbapenem-resistance rates among *Pseudomonas aeruginosa* (Gales 2003).

In a University Hospital in Italy 20% of all *P. aeruginosa* isolates were found to carry bla (VIM) MBL gene (Rossolini 2004). A study on Gram-negative rods from 13 laboratories in Japan showed 0.5% MBL producers (Nishio et. al. 2004). *Pseudomonas aeruginosa* isolates collected from bacteraemic patient from Brazil showed MBL production in 19.7% (Sarder et. al. 2005). 46% MBL positive strains were found by phenotypic method among *P. aeruginosa* in Calgary, Canada (Pitout et. al. 2005). 29% of *Pseudomonas aeruginosa* strains and 100% of *K. pneumoniae* were found to be metallo-beta-lactamase enzyme in Turkey (Toraman 2004).
Sixteen percent of isolated tested were resistant to imipenem in a study conducted in Chennai on *Pseudomonas aeruginosa*. The production of MBL was detected by a 4-fold reduction in MIC with imipenem ethylene diamine tetraacetic acid (EDTA) and zone size enhancement with EDTA impregnated imipenem and ceftazidime discs (Hemalata *et al.* 2005). The dissemination of MBL genes is typified by the spread of bla VIM – 2, believed to originate from a Portuguese patient in 1995, and is now present in over 20 countries (Walsh 2005). Spencer and Walsh studied a new method of inhibition of metallo-beta-lactamase (Spencer *et al.* 2006).

In 1990 report of IMP-1, a metallo-beta-lactamase encoded by a mobile gene (blaIMP) located in gene cassettes inserted in plasmid or chromosome borne integrons. (Koh *et al.* 1999) and a plasmid mediated enzyme was reported in Portugese clinical isolate of *P. aeruginosa* (Cardoso *et al.* 1990).

1.4.4. Characterisation and Classification of Beta-lactamases by Isoelectric Focusing:

Analytical isoelectric focusing is an important tool for characterization of crude beta-lactamases. Proteins being ampholytes, can behave either as acids or bases. Thus at high pH, they are negatively charged and migrate towards the anode in an electric field and at acid pH, they are positively charged and migrate towards the negative electrode (cathode). Between these extremes, there is a characteristic pH for each protein, its isoelectric point (pI), where it does not migrate. The technique of isoelectric focusing takes advantage of these ampholytic properties (Burtis *et al.* 1999).

In the technique described by Mathew *et al.*, 1975 a direct visual comparison of beta lactamases can be made by examination of the patterns that the enzymes give in a pH gradient produced electrophoretically in thin layers of polyacrylamides (Mathew and Harris, 1975). The focused enzymes are located by staining with nitrocefin. Purification of crude preparation is unnecessary and the
technique is sufficiently sensitive to demonstrate beta lactamases in mutants previously reported to lack the enzyme (Mathew and Harris, 1975).

1.4.5. Genetic Elements responsible for beta-lactam resistance:

With the successful introduction of antibiotics as therapeutic agents of infectious diseases, resistant bacteria emerged. Pathogenic bacteria have developed numerous strategies to resist the action of antibiotics. Most of the antimicrobial drugs currently in use are derived from metabolites of soil organisms, mainly fungi and acetinomycetes. The resistance genes probably evolved in the antibiotic-producing bacteria from the detrimental action of its own antibiotic. Subsequently gene transfer might have spread the resistance determinants to other bacteria.

Multiple drug resistant mediated through R-plasmid has often been the cause of hospital-borne infections (Watanbe 1963; Davis 1978). There is always a risk of R-plasmid from the normal bowel bacteria being transferred to the enteric pathogens within the bowel under antibiotic selection pressure (Watanbe 1963).

In 1950s the remarkable finding that multiple antibiotic resistant strains of Shigella were able to transfer the resistance phenotype to other bacterial strains during cell to cell mating (Akiba et al. 1960) dramatically changed our understanding of the molecular basis of antimicrobial resistance. Resistance was linked to the presence of extrachromosomal circles of DNA in bacteria, called plasmids. Subsequently, plasmids have been found in most clinically important bacterial pathogens (Falkow 1975, Bukhari 1977). In 1960s, bacterial viruses were found to move antimicrobial resistance genes between strains of staphylococci in a process called transduction (Novick et al. 1967). Sometimes the entire plasmids could move from one strain of S. aureus to another, enhancing the ability of resistances genes to disseminate. In 1970s, the recognition of transposable elements after containing antimicrobial resistance genes that could move from plasmids to bacteriophage (Berg et al. 1975) or from plasmids to chromosomal locations independent of the usual DNA recombination mechanisms (Rubens et al. 1979) added another dimension to the ability of resistance genes to move among bacteria. This novel mechanism of mobilizing genes among bacterial cells indicated that the likelihood of widespread gene dissemination was high, particularly in
environments where antibiotics were present in high concentrations to offer an advantage to these organisms that processed a resistance mechanism.

81% of Ceftazidime resistance ESBL producing *K. pneumoniae* isolated from Bankok, Thailand, was found to have a VEB -1 gene, located on self-conjugated plasmid (Ca-24 to 200 kb), which was part of class I integrons (Girlich *et. al.*2001). In Mexico, 321 strains of *K. pneumoniae* were studied and found to harbour a 135 kb and showed an ESBL phenotype (Silva *et. al.*2001). Epidemiological study of MDR entoerbacteriaeae from 5 patients in France showed a 100 kb plasmid encoding ESBL TEM-24 (Neuwirth *et. al.*2001). In 2003, studies by Wu Tsu-Lan *et. al.* in Taiwan showed that the SHV-12 and CTX-M-3 producing bacteria were located on large plasmids greater than 96 kb in size (Wu *et. al.*2003). A nosocomial outbreak of *Serratia marcescens* producing inducible AmpC type beta-lactamase enzyme and carrying the trimethoprim-resistant gene and adenyltransferase gene, which confers resistance to streptomycin within a Class I integron (Bagattini *et. al.* 2004). Plasmid encoding AmpC type beta-lactamase was found in 8.5% of *K.pneumoniae*, 6.9% of *K.oxytoca* and 4% *E.coli* (Alvarez *et. al.*2004). There are now 19 plasmid mediated AmpC beta-lactamase, with CMY – 2 appearing to be the most prevalent and widely distributed (Bauernfeind *et. al.*1998).

In India a 48.5 kb plasmid carrying resistance to amikacin was found in 30 isolates (of which 20% produced AmpC) in MDR *P.aeruginosa* (Shahid *et. al.* 2003). Kanta S and others have studied 150 strains of *E.coli* in Patiala (India) and found auto transferable R-plasmids in 80% of hospitalized patients (Kanta *et. al.*1991). A study in Pune in 2001 showed that 40.4% antibiotic resistance in *Klebsiella pneumoniae* was carried by a large plasmid of size 98.7 kb by agarose gel electrophoresis (Misra R, 2001).

The study of the published literature not only indicates the wide range of work already done but also the future trends in antibiotic resistance.