Chapter 1

Review
of
literature
1.1 Antibiotics, modes of action and the basic concept of antibiotic resistance

An antibiotic is a drug used to treat infections caused by bacteria and other microorganisms. Originally, antibiotic was defined as a substance produced by one microorganism that selectively inhibited the growth of another. Synthetic antibiotics, usually chemically related to natural antibiotics, have since been produced that accomplish comparable tasks. There are several major classes of antibiotics that can be categorized based on their mode of antibacterial action. In general, antibiotics can be categorized as those that inhibit cell wall synthesis, protein synthesis, and nucleic acid synthesis (Table 1.1).

‘Selective pressure’ refers to the environmental conditions that allow organisms with novel mutations or newly acquired characteristics to survive and proliferate. Mutations that increase an organism’s resistance to antimicrobial agents occur naturally in bacteria. Exposure to a stimulus that inhibits or kills the susceptible majority of a bacterial population allows a resistant subset of strains to grow at the expense of susceptible organisms. A minority of strains present in a given setting may be resistant to the antibiotic being used. The selective factor is the antibiotic (usually) to which the sub-population is resistant. Hence, the phenomenon of antibiotic resistance is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal.

1.1.1 Multidrug resistance (MDR) in bacteria

We have come a long way using antibiotics after the landmark discovery of ‘Penicillin’ in 1928. Bacteria have also got them selected with hardier resistance mechanisms. Infections due to the bacteria belonging to Enterobacteriaceae are increasingly being
Table 1.1 Major antibiotic classes and resistance strategies employed by bacteria against them:

<table>
<thead>
<tr>
<th>Class of antibiotics</th>
<th>Mode of action</th>
<th>Resistance strategy used by bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta lactams: penicillins, cephalosporins</td>
<td>Block cell wall formation</td>
<td>Inactivation, mutation</td>
</tr>
<tr>
<td>Aminoglycosides: gentamycin</td>
<td>Block protein synthesis</td>
<td>Inactivation, mutation</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Inhibit DNA replication</td>
<td>Mutation of binding molecules</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Block protein synthesis</td>
<td>Inactivation, drug efflux</td>
</tr>
<tr>
<td>Glycopeptides: vancomycin</td>
<td>Block cell wall formation</td>
<td>Mutation of binding molecules</td>
</tr>
<tr>
<td>Macrolides</td>
<td>Block protein synthesis</td>
<td>Ribosome protection</td>
</tr>
<tr>
<td>Trimethoprim Sulphonamides</td>
<td>Block formation of nucleic acids and f-met</td>
<td>Mutation of binding molecules</td>
</tr>
<tr>
<td>Glycylcycline (Tigecycline)</td>
<td>Blocks protein synthesis</td>
<td>Over expression of efflux pumps such as MexXY and AcrAB</td>
</tr>
</tbody>
</table>
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reported [Chiu et al., 2009; Huang et al., 2009]. A phenomenon of great concern in the medical community is the rise in multi-drug resistant organisms, defined as bacteria with simultaneous resistance to more than one class of antibiotics [Guyot et al., 1999]. Patients infected with such organisms experience significantly higher degrees of treatment failure, prolonged antibiotic usage and morbidity associated with infection [Gupta & Stamm, 2002; Sobel & Kaye, 2004].

1.2 Mechanisms of antibiotic resistance

The definition of bacteria as resistant or susceptible is critical for clinicians. It is also very important to note the difference between intrinsic and acquired resistance to an antibiotic. Intrinsic resistance can best be described as resistance of an entire species to an antibiotic, based on inherent characteristics requiring no genetic alteration. This is usually due to the absence of a target for the action of a given antibiotic or the inability of a specific drug to reach its target. For example, mycoplasmas are always resistant to β-lactam antibiotics since they lack peptidoglycan (which the β-lactams act upon). Similarly, the outer membrane of gram negative cells can prevent an antibiotic from reaching its target. For example, Pseudomonas aeruginosa exhibits high intrinsic resistance to many antibiotics due to its drug efflux pumps and restricted outer membrane permeability. Acquired resistance can arise either through mutation or horizontal gene transfer. Presence of the antibiotic in question leads to selection for resistant organisms, thereby shifting the population towards resistance.

Mechanisms of antibiotic resistance and the over coming methods are thus an intense area of research. Several mechanisms of antimicrobial resistance are readily
spread to a variety of bacterial genera. First, the organism may acquire genes encoding enzymes, such as \( \beta \)-lactamases, that destroy the antibacterial agent before it can have an effect. Second, bacteria may acquire efflux pumps that extrude the antibacterial agent from the cell before it can reach its target site and exert its effect. Third, bacteria may acquire several genes for a metabolic pathway which ultimately produces altered bacterial cell walls that no longer contain the binding site of the antimicrobial agent. Fourth, bacteria may acquire mutations that limit access of antimicrobial agents to the intracellular target site via down regulation of porin genes [Tenover, 2006]. Thus, normally susceptible populations of bacteria may become resistant to antimicrobial agents through mutation and selection, or by acquiring from other bacteria the genetic information that encodes resistance. Acquired resistance that develops due to chromosomal mutation and selection is termed as vertical evolution.

1.2.1 Inhibition of cell wall synthesis

There are two major groups of cell wall synthesis inhibitors, the \( \beta \)-lactams and the glycopeptides. As bacterial cell walls are wholly unlike the membranes of eukaryotes, they are an obvious target for selectively toxic antibiotics. The \( \beta \)-lactams include the penicillins, cephalosporins, and the carbapenems. These agents bind to the penicillin binding proteins (PBPs) that cross-link strands of peptidoglycan in the cell wall. In gram negative cells, this leads to the formation of fragile spheroplasts that are easily ruptured. In gram positive cells, autolysis is triggered by the release of lipoteichoic acid [Greenwood, 2000].

1.2.1.1 The mechanism of \( \beta \)-lactam resistance
Beta-lactamases play a major role in the development of β-lactam resistance. These enzymes catalyze hydrolysis of the β-lactam ring and, thereby, inactivate these antibiotics. Many bacteria contain chromosomally encoded β-lactamases necessary for cell wall production and it is only the over-production of these enzymes that causes resistance [Greenwood, 2000]. Beta-lactamases encoded on plasmids or other transmissible elements can also lead to resistance [Normark & Normark, 2002]. There are also some bacteria that possess altered PBPs that result in reduced penicillin binding [Greenwood, 2000]. Since 1970s, several compounds, such as clavulanic acid, have been discovered that have the ability to bind irreversibly to β-lactamases and, thereby, inhibit their action. Combinations of these compounds with β-lactam drugs have been very successful in treatment of disease [Bryan, 1984]. The glycopeptides are a group of antibiotics that include vancomycin, avoparcin, and others that bind to acyl-D-alanyl-D-alanine. Binding of this compound prevents the addition of new subunits to the growing peptidoglycan cell wall, thus limiting their action to gram positive organisms.

1.2.1.2 Glycopeptide resistance

Glycopeptide resistance in enterococci has developed through enzymes that use D-alanyl-D-lactate in place of acyl-D-alanyl-D-alanine, allowing cell wall synthesis to continue. Other mechanisms of resistance involve the over-production of peptidoglycan precursors which overwhelm the drug [Greenwood, 2000].

1.2.2 Inhibition of protein synthesis

There are many types of antibiotics that inhibit bacterial protein synthesis. These drugs take advantage of structural differences between bacterial ribosomes and eukaryotic ribosomes. The aminoglycoside antibiotics are a group whose mechanisms of action are
still being investigated. The members of this group contain sugar and consist of streptomycin, neomycin, kanamycin, gentamycin, tobramycin and amikacin. These drugs enter bacterial cells by an active transport that involves quinones that are absent in anaerobes and streptococci, thus excluding these organisms from the spectrum of action. Aminoglycosides irreversibly bind to 16S rRNA and block the initiation complex. By binding to 16S rRNA the aminoglycosides increase the affinity of the A site for tRNA regardless of the anticodon specificity. Aminoglycoside resistance mechanisms include: (a) the deactivation of aminoglycosides by N-acetylation, adenylylation or O-phosphorylation, (b) the reduction of the intracellular concentration of aminoglycosides by changes in outer membrane permeability, decreased inner membrane transport, active efflux, and drug trapping, (c) the alteration of the 30S ribosomal subunit target by mutation, and (d) methylation of the aminoglycoside binding site [Shakil et al., 2008].

Chloramphenicol is a broad-spectrum antibiotic produced by *Streptomyces venezuelae*. Chloramphenicol inhibits peptide bond formation on 70S ribosomes [Bryan, 1984]. This drug is especially useful as it can penetrate eukaryotic cells and cerebrospinal fluid, making it a drug of choice for treatment of meningitis and intracellular bacterial infections such as those caused by chlamydia. It is not in widespread use, however, because of potentially fatal side-effects, namely, aplastic anemia [Greenwood, 2000]. Resistance to chloramphenicol is conferred by the enzyme chloramphenicol acetyl-transferase. A number of these enzymes have been discovered, each altering the chloramphenicol molecule to prevent binding to the bacterial ribosome. Chloramphenicol resistance in gram negative cells can also arise from alteration in outer membrane permeability that prevents the drug from entering the cell [Bryan, 1984].
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The tetracyclines are another group of broad-spectrum antibiotics that inhibit bacterial protein synthesis. They are absorbed into the cell by active transport and, once there, bind to the 30S subunit to prevent binding of aminoacyl tRNA [Roberts, 1996]. Resistance to the tetracyclines occurs via three mechanisms. First, production of a membrane efflux pump removes the drug as rapidly as it enters and there are several genes encoding these pumps. Second, several ribosome protection proteins act to prevent tetracycline from binding to the ribosome, thereby conferring resistance. Third, a protein found only in Bacteroides spp. enzymatically inactivates tetracycline [Roberts et al., 1996]. Interestingly, efflux pump inhibitors have recently been discovered that may allow combinations of these inhibitors and tetracyclines to be used against previously resistant strains [Chopra, 2002].

The macrolides are a group of antibiotics commonly used to treat gram positive and intracellular bacterial pathogens. Erythromycin was the first of these, and several other important macrolides have been discovered since, including clarithromycin and azithromycin. Azithromycin has a longer plasma half-life which allows treatment with a single dose for some pathogens or a once daily dose for others. Clarithromycin has enhanced absorption and causes less gastrointestinal discomfort [Gaynor & Mankin, 2003]. It was originally believed that erythromycin inhibited protein synthesis by competing with amino acids for ribosomal binding sites, but further research showed that several mechanisms were involved. The macrolides are now believed to promote dissociation of tRNA from the ribosome, inhibit peptide bond formation, inhibit ribosome assembly, and prevent amino acid chain elongation [Gaynor & Mankin, 2003]. There are two major mechanisms of macrolide resistance. First, an efflux pump has been found that removes the drug from the cell. Second, modification of the ribosome can confer resistance. Mutations at several sites
of the ribosome can allosterically prevent macrolide binding and a common alteration is
dimethylation of one nucleotide on the 23S rRNA. This dimethylation not only prevents
macrolide binding, but also confers resistance to lincosamide and streptogramin
antibiotics [Gaynor & Mankin, 2003]. The streptogramins are another class of antibiotics
that inhibits bacterial protein synthesis, mostly in gram positive organisms (due to
decreased permeability of the gram negative outer membrane). These antibiotics are
actually combinations of structurally different drugs, types A and B that act
synergistically. These compounds bind to separate sites on the 50S subunit. Type A
drugs block attachment of substrates at two sites on the 50S subunit, whereas type B
drugs cause release of incomplete protein chains. The synergistic effect arises from a
conformational change induced by the binding of a type A drug which significantly
increases affinity of type B drugs [Johnston et al., 2002]. Streptogramins currently in use
include virginiamycin, pristinamycin, and quinupristin/dalfopristin. Resistance to
streptogramin antibiotics can be found in several forms. Efflux pumps for both type A
and B streptogramins have been identified. Type A streptogramins can be inactivated by
one of the virginiamycin acetyl-transferases, and several enzymes have been identified
that can inactivate type B streptogramins. Alteration of bacterial ribosomal proteins or
RNA can also confer resistance. A common mutation is the dimethylation of one
nucleotide on the 23S rRNA, mentioned previously, that gives rise to resistance to type B
drugs, as well as macrolides and lincosamides [Johnston et al., 2002].

1.2.3 Inhibitors of nucleic acid synthesis

The sulfonamides and the diaminopyrimidines should be discussed together, as both the
drugs indirectly inhibit nucleic acid synthesis by inhibiting folate synthesis. Folate is a
coenzyme necessary for the synthesis of purines and pyrimidines. Although both types of drugs are useful on their own, they exhibit a synergistic effect when combined. Sulfonamides are currently not used commonly in medicine, but the combination drug trimethoprim-sulfamethoxazole (TMP/SMX) is sometimes used in the treatment of urinary tract infections. Sulfonamides serve as an analog of p-aminobenzoic acid. Therefore, they competitively inhibit an early step in folate synthesis. Diaminopyrimidines, of which trimethoprim is the most common, inhibit dihydrofolate reductase, the enzyme that catalyzes the final step in folate synthesis [Greenwood, 2000]. Sulfonamides are rendered ineffective by over-production of p-aminobenzoic acid or production of an altered dihydropteroate synthetase. The substrate for dihydropteroate synthetase is p-aminobenzoic acid, and the altered form has a much lower affinity for sulfonamides than for p-aminobenzoic acid [Then, 1982]. Trimethoprim resistance can also result due to over-production of dihydrofolate reductase or production of an altered, drug-resistant form can lead to resistance [Bryan, 1984]. In addition, both drugs can be enzymatically inactivated, resulting in resistance [Then, 1982].

The quinolones are a chemically varied class of broad-spectrum antibiotics widely used to treat many diseases, including gonorrhea and anthrax. Drugs in this class include nalidixic acid, norfloxacin, and ciprofloxacin. Quinolones inhibit bacterial growth by acting on DNA gyrase and topoisomerase IV, which are necessary for correct functioning of supercoiled DNA [Greenwood, 2000]. Although quinolones target both enzymes, in gram negative organisms the primary target is DNA gyrase and, in gram positive organisms, the primary target is topoisomerase IV [Ruiz, 2003]. There are three main mechanisms of resistance to quinolones. Resistance to some quinolones occurs
with decreased expression of membrane porins. Cross-resistance to other drugs requiring these porins for activity also results from these changes. A second mechanism of resistance is expression of efflux pumps in both gram negative and gram positive bacteria [Novak et al., 1999] and the third mechanism is alteration of the target enzymes. Several mutations have been described in both quinolone target proteins that result in reduced binding affinities [Ruiz et al., 2003]. It is believed that high-level quinolone resistance is due to a series of successive mutations in the target genes, rather than a single mutation [Novak et al., 1999].

1.2.4 Genetic mechanisms of resistance

Bacteria also develop resistance through the acquisition of new genetic material from other resistant organisms. This is termed horizontal evolution, and may occur between strains of the same species or between different bacterial species or genera. Mechanisms of genetic exchange include conjugation, transduction, and transformation [McManus, 1997].

1.2.4.1 Transformation involves the passage of DNA to a recipient cell through a specific medium.

1.2.4.2 Transduction is a process whereby DNA is transferred from a donor to a recipient by way of a host, a phage. It is still unknown whether this process causes clinically observed resistance to antibiotics. Because this process is highly dependent on specific phages, it may occur only within certain bacterial species. Only a limited amount of DNA that can be packed into the head of a phage can normally be transferred.
1.2.4.3 **Conjugation** is the process when DNA transfers from a donor to a recipient by simple, direct cell-to-cell contact. The process allows for the passage of more than one functional gene at a time, so that multiple resistance could occur within a single step. Furthermore, many different organisms can act as recipients, allowing DNA (resistant genes) to be donated freely from different sources. Resistance can be passed from commensals in the gut to a pathogen existing in the same environment. Conjugation is thus an important and highly efficient process for transferring genes, and the acquisition of resistance by most pathogens is probably a result of this process. Other mobile genetic elements include transposons and integrons.

1.2.4.4 **Transposons** are sequences of DNA that are capable of transposing or moving from one location to another within the genomic DNA or between plasmids and genomic DNA. Transposons may contain one or more genes, including those necessary for translocation, and often contain either resistance genes or genes necessary for degradative enzymes [Scott, 2002]. These genes are flanked by insertion sequences that allow insertion of the DNA into the plasmid or genomic DNA.

1.2.4.5 **Integrons** are mobile elements that consist of conserved sequences of DNA bordering "cassettes" of genes. The conserved sequences contain the genes necessary for integration, promotion, and capture of cassettes. Gene cassettes are promoter-less units that contain the genes for antibiotic resistance or for virulence factors [Roe & Pillai, 2003]. Integrons are capable of carrying several gene cassettes and are associated with multiple antibiotic resistance and pathogenicity islands.

1.2.4.6 **Plasmids** are double stranded, circular or linear DNA molecules capable of autonomous replication. They are mostly a phenomenon of the prokaryotic world, although they
exist in some groups of primitive eukaryotes as yeast, fungi and cellular slime moulds [Rush & Misra, 1985]. They belong to a special biological category of extrachromosomal, accessory DNA elements [Campbell, 1981]. Natural plasmids have systems guaranteeing their autonomous replication, controlling the copy-number and ensuring stable inheritance during cell division. Many plasmids can promote their horizontal transfer among bacteria of different genera and kingdoms, through the conjugation process. However, the presence of plasmids must have some biological cost for the bacterial host. It is therefore plausible that a selfish element that does not benefit its host can be eliminated from the bacterial population. The plasmid-encoded functions have been extensively investigated and there is ample evidence that natural plasmids have evolved as an integral part of the bacterial genome, providing additional functions to their host [Thomas & Nielsen, 2005]. Resistance genes located on plasmids offer immediate advantage to their host under antimicrobial pressure. Bacterial plasmid genome sequence comparisons provided the historical events through which plasmids are assembled. Their evolution seems to proceed by the assemblage of modular components by transposition, homologous recombination, and illegitimate recombinational events [Bennett, 2004]. This is particularly evident when the phenomenon of antimicrobial resistance is analyzed. Antimicrobial resistance arises from a complex multi-factorial process supported by mobile genetic elements that contain and transfer resistance determinants. Resistance genes located on plasmids move from one bacterium to another, conferring phenotypic characteristics. Several resistance plasmids have been described to carry virulence factors, such as bacteriocins, siderophores, cytotoxins, or adhesion factors [Martinez & Baquero, 2002] and virulence plasmids have been described to carry resistance genes [Herrero et al., 2006]. For
plasmids carrying virulence and resistance linked determinants, an infective population will be selected for antimicrobial resistance, and antimicrobial resistance pressure will select the virulence traits. However, once those determinants have been selected in the bacterial host, they can evolve further and eventually be transferred to other bacterial population. The acquisition of antimicrobial resistance genes on virulence plasmids could represent a novel tool in bacterial evolution, implementing adaptive strategies to explore and colonize novel hosts and environments [Martinez & Baquero, 2002].

Plasmid-mediated resistance to β-lactam antibiotics in E. coli is of major concern in hospitals. Studies show that for both nosocomial and community acquired infections the mortality and morbidity in prolonged hospitalization, are twice as high in patients infected with antibiotic-resistant strains than with susceptible strains [Lautenbach et al., 2001]. Bacteria can possess plasmids that can code for more than one β-lactamase in addition to their expression of chromosomal enzyme. Due to carriage of plasmids and promiscuous exchange of such material between bacteria, these resistance genes have spread widely and are also subject to mutation [Lee et al., 2001]. Plasmid mediated β-lactamases were first recognized in Gram-negative bacteria in the early 1960s, shortly after the introduction of ampicillin [Livermore, 1993].

1.3 β-Lactam resistance

Figure 1.1 shows structures of some of the β-lactam antibiotics and the carbapenems as well. There are three major ways by which bacteria avoid the bactericidal effect of β-lactams:

(a) Production of β-lactamases: β-lactamases are bacterial enzymes that hydrolyze the β-lactam ring and render the antibiotic inactive before it reaches the PBP
Fig 1.1(a) Structures of some of the β-lactams. All structures were retrieved from Drugbank (http://www.drugbank.ca/search/chemquery; accessed on 17/10/09).
Fig 1.1(b) Structures of the carbapenems. All structures were retrieved from Drugbank (http://www.drugbank.ca/search/chemquery) except the structure of 'Doripenem' which was taken from Pubchem (http://www.pubchem.ncbi.nlm.nih.gov/search); both websites were accessed on 17/10/09.
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target. The underlying structural kinship that β-lactamases share with PBPs allows these enzymes to bind, acylate, and use a strategically located water molecule to hydrolyze and thereby inactivate the β-lactam [Massova & Mobashery, 1998].

(b) Altered PBPs that exhibit low affinity for β-lactam antibiotics: Examples are PBP 2x of Streptococcus pneumoniae and PBP 2’ (PBP 2a) of Staphylococcus aureus [Chambers, 1997]. These PBPs are relatively resistant to inactivation by penicillins and are able to assume the functions of other PBPs when the latter are inactivated.

(c) Lack or diminished expression of outer membrane proteins (OMPs) in gram-negative bacteria: The loss of OMPs restricts the entry of certain β-lactams into the periplasmic space of gram-negative bacteria and hence access to PBPs on the inner membrane. Imipenem resistance in Pseudomonas aeruginosa and Klebsiella pneumoniae can arise from the loss of OmpD2 and of OmpK36, respectively [Livermore, 2001; Gootz, 2004; Jacoby et al., 2004]. The destruction of β-lactams by β-lactamases is the most important resistance mechanism in gram-negative bacteria.

1.4 Extended-spectrum-β-lactamases (ESBLs)

ESBLs are globular proteins composed of alpha-helices and beta-pleated sheets [Knox, 1995]. These are β-lactamases that hydrolyze extended-spectrum cephalosporins with an oxyimino side chain. ESBLs are usually capable of hydrolyzing penicillins (e.g., ampicillin and piperacillin), cephalosporins of the first-, second-, third- and fourth-generations, and the monobactam aztreonam (but not the cephamycins or
carbapenems) [Ambler et al., 1991]. The distinctive property of ESBLs (e.g. members of TEM and SHV families) of being inhibited by beta lactamase inhibitors such as clavulanic acid, tazobactam, or sulbactam, is duly exploited in the double-disk synergy test meant for ESBL-detection.

The Ambler scheme and the Bush-Medeiros-Jacoby system [Ambler et al., 1991; Bush et al., 1995] are the two systems commonly used for the classification of beta lactamases (Table 1.2). Typically, the ESBLs derive from genes for TEM-1, TEM-2, or SHV-1 by mutations that result in a change in the amino acid configuration around the active site of these enzymes. ESBLs are classified in group 2be in the Bush-Medeiros-Jacoby system and class A in the Ambler system. Authors have long back observed that point mutations in the SHV and TEM β-lactamases which cause single amino acid substitutions (Asp104→Lys, Arg164→Ser, Arg164→His, Asp179→Asn, Gly238→Ser, and Glu240→Lys) are responsible for this resistance [Philippon & Jacoby, 1989; Jacoby & Medeiros, 1991]. Hence these enzymes are a potent weapon of bacteria and a significant research problem for scientists.

1.4.1 Epidemiology

Although these enzymes were first detected in Germany in 1983 among Klebsiella spp [Knothe et al., 1983], it was not long before that ESBLs were detected in the United States and Asia. The estimated prevalence of ESBL-producing strains of E. coli and K. pneumoniae in the United States is relatively low [Pfaller & Segreti, 2006]. In contrast, the prevalence of ESBL-producing K. pneumoniae is high in the Asian Pacific region and in Latin America [Pfaller & Segreti, 2006]. The SENTRY surveillance program reported the frequency of ESBL-producing K. pneumoniae to be approximately 37% in Latin America.
Table 1.2 Classification schemes for beta lactamases

<table>
<thead>
<tr>
<th>Bush-Jacoby-Medeiros system</th>
<th>Major subgroups</th>
<th>Ambler System</th>
<th>Main attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 cephalosporinases</td>
<td>-</td>
<td>C (cephalosporinases)</td>
<td>Usually chromosomal; Resistance to all β-lactams except carbapenems; Not inhibited</td>
</tr>
<tr>
<td>Group 2 penicillinases (clavulanic acid susceptible)</td>
<td>2a, 2b, 2be, 2br, 2c, 2e, 2f, 2d</td>
<td>A (serine β-lactamases)</td>
<td>Staphylococcal penicillinases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>Broad-spectrum – TEM-1, TEM-2, SHV-1</td>
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<td></td>
<td></td>
<td>A</td>
<td>Inhibitor resistant TEM (IRT)</td>
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<td>A</td>
<td>Carbicillin-hydrolyzing</td>
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<td></td>
<td>A</td>
<td>Cephalosporinases inhibited by clavulanate</td>
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<tr>
<td></td>
<td></td>
<td>A</td>
<td>Carbapenemases inhibited by clavulanate</td>
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<tr>
<td></td>
<td></td>
<td>D(oxacillin-hydrolyzing)</td>
<td>Cloxacillin-hydrolyzing (OXA)</td>
</tr>
<tr>
<td>Group 3 metallo-beta lactamase (metalloenzymes)</td>
<td>3a, 3b, 3c</td>
<td>B (metalloenzymes)</td>
<td>Zinc-dependent carbapenemases</td>
</tr>
<tr>
<td>Group 4</td>
<td>Not classified</td>
<td></td>
<td>Miscellaneous enzymes, most not yet sequenced</td>
</tr>
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vs. 7% in the United States [Sader et al., 1998]. A study [Minarini et al., 2007] found the prevalence of ESBL-producers among *Klebsiella* spp to be as high as 25.8% in Brazil. Prevalences of ESBL-producing *Klebsiella* spp has been reported to be 5%, 21.7%, 31% and 38% in Japan, Taiwan, Philippines and Malaysia/Singapore, respectively. In addition, the prevalence of ESBL-producing *E. coli* varies from 6% to 23% within one region [Parasakthi & Ariffin, 2001]. Several studies from India have also reported a high prevalence of ESBL producing pathogens [Babypadmini & Appalaraju, 2004, Shakil et al., 2009]. Currently, the CTX-M type enzymes have started dominating the globe [Canton & Coque, 2006; Livermore et al., 2007]. PER- and OXA type enzymes are more common in *P. aeruginosa* and *Acinetobacter* spp., but there have been sporadic reports of PER type ESBLs in *Enterobacteriaceae* as well [Luzzaro et al., 2006]. Plasmid-borne KPC enzymes have been reported among *K. pneumoniae* and other enterobacteria from the United States and other countries [Gootz et al., 2009]. These are of due concern because of their ability to inactivate carbapenems.

### 1.4.2 CTX-M: the leading therapeutic challenge

These are now considered the most prevalent ESBLs worldwide [Livermore et al., 2007]. These enzymes show potent hydrolytic activity against cefotaxime and hence they are called as 'CTX-Ms'. Organisms producing CTX-M-type β-lactamases typically have cefotaxime minimum inhibitory concentrations (MICs) in the resistant range (16 to 256 μg/ml), while ceftazidime MICs are usually in the apparently susceptible range (2 to 8 μg/ml). However, some CTX-M-type ESBLs may actually hydrolyze ceftazidime and confer resistance to this cephalosporin (MICs as high as 256 μg/ml) [Poirel et al., 2002]. Aztreonam MICs are variable. CTX-M-type β-lactamases hydrolyze cefepime with high
efficiency [Tzouvelekis et al., 2000], and cefepime MICs are higher than observed in bacteria producing other ESBL types [Yu et al., 2002]. CTX-M-type β-lactamases have 40% or less identity with TEM and SHV-type ESBLs. CTX-M beta-lactamases are commonly found in *K. pneumoniae*, *E. coli*, typhoidal and non typhoidal *Salmonella*, *Shigella*, *Citrobacter freundii*, *Enterobacter*, spp., and *Serratia marcescens* [Walther-Rasmussen & Hoiby, 2004]. Different genetic elements may be involved in the mobilization of genes coding for CTX-M enzymes, designated as ‘bla<sub>CTX-M</sub>‘. Insertion Sequences (e.g., ISEcp1 or ISEcp1-like insertion sequences) have been repeatedly observed upstream of open reading frames (ORFs) encoding the CTX-M-1, CTX-M-2, CTX-M-3, CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-15, CTX-M-17, CTX-M-19, CTX-M-20, and CTX-M-21 β-lactamases [Khalaf et al., 2009; Park et al., 2009; Rodríguez et al., 2009].

### 1.4.3 Structure-function relationships

The study of the atomic structures of class A ESBLs has revealed that the active site is selectively “remodelled and expanded” to accommodate the bulky R₁ side chain of extended spectrum cephalosporins [Knox, 1995].

#### 1.4.3.1 The key players behind the substrate specificity of CTX-Ms

Amino acid residues Asn104, Asn132, Ser237, and Asp240 have been identified as the key players behind the substrate specificity of the CTX-M beta-lactamases by several studies that used comparative sequence analyses, modelling, and mutagenesis techniques [Bauernfeind et al., 1996]. Ser237, has been observed to be involved in the extension of the substrate specificities of TEM and SHV ESBLs to cefotaxime [Knox, 1995]. The Ser237Ala substitution in the CTX-M-4 enzyme induces a decrease both in relative hydrolytic activity against cefotaxime and in susceptibility to inhibition by
clavulanate [Gazouli et al., 1998]. The acyl intermediate structure of Toho-1 in complex
with cefotaxime shows a rotation of the Ser237 side chain, which prevents steric clashes
with the methoxyimino group of cefotaxime and which allows the formation of a
hydrogen bond with the carboxylate group of cefotaxime [Shimamura et al., 2002]. It
has been suggested that this interaction assists in bringing the carbonyl group of the β-
lactam ring of cephalosporins to the optimal position in the oxyanion hole for acylation
[Shimamura et al., 2002]. The relatively low penicillinase activities of CTX-M enzymes
may be caused by Van der Waals contact between residue Ser237 and the methyl group
of the thiazolidine ring. Asn104, Asn132, Ser237, and Asp240 residues establish
hydrogen bonds with the amide and aminothiazole groups of the acyl-amide-cefotaxime
chain. This unusual acyl intermediate of CTX-M enzymes in complex with cefotaxime
may therefore be involved in the activities of the oxyimino-cephalosporinases by fixing
cefotaxime tightly in the binding site [Shimamura et al., 2002].

1.4.3.2 The omega loop

The structure of Toho-1 revealed that the omega loop (amino acid positions 161-179)
has fewer hydrogen bond interactions with the β3 strand in the vicinities of Asn170 and
Asp240 than the restricted-spectrum ß-lactamase of Bacillus licheniformis, the enzyme
most closely related to Toho-1 at the structural level. No hydrogen bond has been
observed between the Phe160 residue and Thr181 and Asp157 residues, which both
connect the N and C termini of the omega loop in restricted-spectrum ß-lactamas
es [Ibuka et al., 1999]. These structural features may increase the flexibility of the omega
loop. The structures of acyl intermediates of the Toho-1 enzyme show a shift of the
omega loop to helix H5 [Shimamura et al., 2002] as a result of a complex structural
Chapter 1

rearrangement in the hydrophobic core in the vicinity of the omega loop (the residues involved in the rearrangement are Cys69, Ser72, Met135, Phe160, and Thr165). This shift narrows the binding site, but the steric contacts of the Pro167 and Asn170 residues with the aminothiazole ring of cefotaxime are avoided.

1.4.3.3 The steps of CTX-M evolutionary ladder: residues at positions 167 and 240

Mutants with point mutations in common CTX-M enzymes exhibiting improved catalytic efficiencies against ceftazidime have been observed [Knox, 1995]. The change in activities of CTX-Ms leading to the evolution of more variants may be due to point mutations present either inside or outside of the active site omega loop (amino acid positions 161 to 179) [Sturenburg et al., 2004]. For example the P167T mutation differentiates CTX-M-23 from CTX-M-1, CTX-M-3 and CTX-M-15. However the CTX-Ms, having identical residues present in the omega loop may still have some difference in their enzymatic activities due to mutations present outside the omega loop. The CTX-M-15, CTX-M-16, and CTX-M-27 enzymes harbor the Asp240Gly substitution. The presence of Lys and Arg residues at position 240 are known to increase the enzymatic activities of the TEM and SHV ESBLs against ceftazidime [Knox, 1995]. The Lys and Arg residues are positively charged and can form an electrostatic bond with the carboxylic acid group on oxyimino substituents of ceftazidime [Huletsky et al., 1993; Cantu et al., 1996]. Neutral residue Gly240 is not able to form electrostatic interactions with β-lactams but could favor the accommodation of the oxyimino-ceftazidime side chain [Bonnet et al., 2001; Bonnet et al., 2003].

1.5 Neonatal infections
Neonates are at much higher risk for developing infections [Fanos et al., 2007] because of their immature immune system, particularly the preterm ones. One reason for the increased risk is that antibodies, which help protect mothers from infections, do not cross through the placenta to the fetus until approximately 30 weeks of gestation. The antibodies present at birth take time to reach optimum levels, which also affects the protection provided to the neonate. Causes of infections in newborns can be divided into three main groups: intrauterine, intrapartum, and postnatal infections. All three groups include factors that increase the infant’s risk of coming in contact with an organism that can cause an infection (Table 1.3).

Intrauterine or factors that increase the risk before birth include the following: poor prenatal care, poor nutrition, recurrent abortions, and substance abuse. Intrauterine infections occur when pathogenic organisms cross the placenta into the fetal circulatory system. The organisms, such as cytomegalovirus (CMV), can reside in the amniotic fluid. Other organisms ascend from the vaginal track, infecting the membranes and causing them to rupture. This rupture of membranes can lead to infections of the respiratory and gastrointestinal tract of a newborn.

Intrapartum or factors that increase the infants chance of becoming infected during the birthing process include: prolonged rupture of membranes (>12 to 18 hours), urinary tract infections, preterm labor, prolonged or difficult labor, maternal fever and maternal infections. Most infections during the birthing process are related to the infant coming into unavoidable contact with an infected birth canal. The birth canal can host bacteria that an infant’s immune system cannot defend against.
Table 1.3 Causes of infections in newborns

<table>
<thead>
<tr>
<th>Infections</th>
<th>Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrauterine</td>
<td>Poor nutrition, Poor prenatal care, Substance abuse</td>
</tr>
<tr>
<td>Intrapartum</td>
<td>Premature rupture of membranes, Maternal fever, Prolonged labor, Maternal UTI</td>
</tr>
<tr>
<td>Postnatal</td>
<td>Male sex, Birth asphyxia, Low birth weight</td>
</tr>
</tbody>
</table>
Postnatal infections may be contracted after delivery, as in the case with infections contracted during resuscitation, or as a result of a nosocomial infection due to improper hand washing. Infections in the postnatal period are more common in those infants who require foreign objects to be introduced into their systems. Items like endotracheal tubes or indwelling catheters increase the risk of an infant becoming septic.

The single most important risk factor for infection in the neonate is prematurity [Seo et al., 1992]. Neonatal factors that increase the infant's chance of becoming sick include: low birth weight, prematurity, birth asphyxia, meconium staining, and resuscitation. There is a significant correlation between gestational age and risk for neonatal infection [Elster et al., 2009]. Infants born at less than 32 weeks gestation are generally supposed to have higher risk of developing infection. Reasons for this include immature immune system, thinner skin, and the frequent need for insertion of foreign objects. Positive cerebrospinal fluid (CSF), urine, and bacterial blood cultures are lab results that confirm infection in a neonate. Other abnormal results which should be checked in an infant suspected of sepsis are hypoglycemia, hyperglycemia, metabolic acidosis, thrombocytopenia, or hyperbilirubinemia. The blood culture and complete blood count (CBC) are the most helpful tests to identify an infection and the causative organism.

CBC findings that indicate an infection is present include: an elevated or decreased white blood count (WBC), a low platelet count, and a high I:T ratio [Aulia et al., 2003]. The I:T ratio is a calculation which is done to show the percentage of
immature to total white blood cells. When the I:T ratio is greater than 0.2, this indicates that there is a “left shift.” This left shift means that there are more immature neutrophils than mature neutrophils circulating around in the bloodstream. A neutrophil is a type of white blood cell that defends the body against organisms that cause infection. When infection is present the neutrophils migrate out of the capillaries and into the infected site, where they ingest and destroy the pathogens causing the infection. When the demand for the neutrophils exceeds the supply in circulation, immature neutrophils are released into the blood to help fight off the infection. This is labeled a “left shift”. As the infection diminishes and neutrophils are replenished, a “shift to the right” occurs, indicating that everything is back to normal.

1.5.1 *E. coli* in neonatal infections

*E. coli* is among the most common causes of gram negative neonatal infections worldwide. This bacterium is found in the mother’s genital tract with a high incidence of colonization in the neonate. The pathogen can cause severe infections that may lead to respiratory distress, cardiovascular collapse, meningitis, multi-organ failure, and even death. *E. coli* is usually treated with gentamicin or amikacin.

1.6 Diabetic foot infections

The Indian diabetic population is expected to increase to 57 million by 2025 [Abdul et al., 1999]. Individuals with diabetes have at least a 10-fold greater risk of being hospitalized for soft tissue and bone infections of the foot than the individuals without diabetes [Boyko & Lipsky, 1995]. Figure 1.2 shows foot ulcer of a diabetic patient.
Fig 1.2 The diabetic foot ulcer [Source: Frykberg, 2002].
1.6.1 Definition of 'Diabetic foot'

Diabetic foot refers to a variety of pathologic conditions which affect the feet of people suffering from diabetes. According to World Health Organization diabetic foot is defined as 'the foot of a diabetic patient that has the potential risk of pathologic consequences, including infection, ulceration, and destruction of deep tissues associated with neurologic abnormalities, various degrees of peripheral arterial disease, and metabolic complications of diabetes in the lower limb' [Frykberg et al., 2006].

1.6.2 Definition of 'Diabetic foot infection'

A diabetic foot infection is most simply defined as any inframalleolar infection in a person with diabetes mellitus [Lipsky et al., 2004]. Spectrum of infection includes paronychia, cellulitis, myositis, abscesses, necrotizing fasciitis, septic arthritis, tendonitis, and osteomyelitis. The most common and classical lesion, however, is the infected diabetic “mal perforans” foot ulcer. This wound results from a complex amalgam of risk factors [Lipsky et al., 2004]. Once the protective layer of skin is breached, underlying tissues are exposed to bacterial colonization. This wound may progress to become actively infected, and, by contiguous extension, the infection can involve deeper tissues. This sequence of events can be rapid (occurring over days or even hours), especially in an ischemic limb. Various immunologic disturbances, especially those that involve polymorphonuclear leukocytes, may affect some diabetic patients, and these likely increase the risk and severity of foot infections [Schubert & Heesemann, 1995]. It is estimated that 15% of diabetic patients would develop a foot ulcer during the course of their disease [Boulton AJM et al., 2004]. Foot ulceration is the most common single
precursor to amputation and has been identified as a component in 85% of lower-extremity amputations [Margolis et al., 2005].

1.6.3 Wagner Classification System

Diabetic patients may develop many types of foot wounds which are due to combination of neuropathy, ischemia and infection. For evaluation and determination of the severity of diabetic foot, various classification systems are in use. It seems that poor clinical outcomes are generally associated with infection, peripheral vascular disease, and increasing wound depth; it also appears that the progressive cumulative effect of these comorbidities contributes to a greater likelihood of a diabetic foot ulcer leading to a lower-limb amputation. Most common and widely used classification system is the Wagner System [Wagner, 1981]. This system is basically anatomical with gradations of superficial ulcer, deep ulcer, abscess osteitis, gangrene of the fore foot, and gangrene of the entire foot. Only grade 3 addresses the problem of infection. In this system, foot lesions are divided into different grades starting from grade 0 to grade 5. Grade 0 includes high risk foot but no active lesion and grade 5 includes gangrene of entire foot. But this system does not mention about ischemia or neuropathy which is the drawback of this system. Otherwise it is a sound predictor of risk of amputation. It is shown in table 1.4.

1.6.4 Risk factors for diabetic foot ulceration and infection

The major risk factors for foot ulceration are: nerve damage (neuropathy) and impaired blood supply in combination with deformities of the feet and the resulting increased pressures on areas of the sole. Other contributing factors include: a history of previous
Table 1.4 Wagner Classification System

<table>
<thead>
<tr>
<th>Grade</th>
<th>Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No open Lesion</td>
</tr>
<tr>
<td>1</td>
<td>Superficial ulcer</td>
</tr>
<tr>
<td>2</td>
<td>Deep ulcer to tendon or joint capsule</td>
</tr>
<tr>
<td>3</td>
<td>Deep ulcer with abscess, osteomyelitis, or joint sepsis</td>
</tr>
<tr>
<td>4</td>
<td>Local gangrene- fore foot or heel</td>
</tr>
<tr>
<td>5</td>
<td>Gangrene of entire foot</td>
</tr>
</tbody>
</table>
foot ulcers and amputation, inadequate footwear, a lack of (or low-quality) podiatry care, poor diabetes management, long duration of diabetes, chronic renal disease, poor visual acuity, psychological factors and behavioral factors, smoking, old age, low social status. Studies have reported 'male sex' as a significant risk factor for non-healing foot ulcers [Prompers et al., 2008].

1.6.5 Role of Infection

Infection is an important complicating factor in ulceration. Diabetic patients are more prone to infection, as they have an altered response to infectious process owing to defects in their immune system [Delamaire et al., 1997]. Wound healing is delayed in diabetic patients as a result of abnormal cellular and inflammatory pathways involving fibroblasts, neutrophils, and advanced glycation end products [Yan et al., 2003]. The rate of infection parallels the level of blood glucose control and it may progress rapidly with devastating consequences. Furthermore, the neuropathic and ischemic deficiencies in diabetic patients predispose them to infection and then compound the problem by potentiating infection once a pathogen has been introduced. Many patients develop abscess formation, osteomyelitis and gangrene. Sepsis can track along the planes of the plantar fascia and flexor tendon sheaths. In the infected foot, edema may be responsible for the thrombosis of digital vessels and gangrene of the toes. Classical signs of infection may not always be present in the infected diabetic foot because of the consequences of neuropathy, alterations in the foot microcirculation, and leukocyte abnormalities. Fever, chills, and leukocytosis may be absent in up to two thirds of patients with diabetes with extensive foot infections, and hyperglycemia is often the sole presenting sign.
1.6.6 Microbes associated with foot ulcers

The microflora of leg and foot ulcers is usually polymicrobial [Citron et al., 2007] and several studies that used molecular techniques have emphasized the complex ecology of these wounds [Davies, 2003].

Knowledge of the etiologic agent(s) that caused the wound infection is helpful in selecting definitive antibiotic therapy. Staphylococci have been the predominant organisms isolated from both prospective, purpose-collected samples and retrospective analysis of clinical investigations. Staphylococci have been reported in varying frequencies from diabetic foot ulcers. Yates et al have recently observed a prevalence of 43% for Staphylococci among the organisms isolated from infected foot ulcers [Yates et al., 2009]. Xu et al., observed a prevalence of 41% and 47% for S. aureus and S. epidermidis among the cases of diabetic foot ulcers, respectively [Xu et al., 2007].

Pseudomonas aeruginosa is another frequently identified organism and has been found in 12% of ulcers in a recent study [Martínez-Gómez et al., 2009]. A number of other aerobic species have also been reported, including E. coli [Varaiya et al., 2008], Enterobacter species [Percival et al., 2008], Klebsiella species [Goldstein et al., 2008], Enterococcus species [Martínez-Gómez et al., 2009], and Proteus species [Raja, 2007] etc.

1.6.7 Treatment and management of diabetic foot infections

One has to make careful choices among the various topical, oral, and parenteral antibiotic agents. Virtually all severe and some moderate infections require parenteral therapy, at least initially. Highly bioavailable oral antibiotics can be used in most mild and in many moderate infections, including some cases of osteomyelitis. Topical therapy
may be used for some mild superficial infections. Sharp debridement and management of underlying infection and ischemia are also critical in the care of foot ulcers. Prompt and aggressive treatment of diabetic foot ulcers can often prevent exacerbation of the problem and eliminate the potential for amputation. Optimal management of diabetic foot infections can potentially reduce the incidence of infection-related morbidities, the need for and duration of hospitalization, and the incidence of major limb amputation. Despite a panoply of studies, the optimal management of diabetic foot infections remains poorly understood. Antibiotics, surgery, rehabilitation and/or off-loading, and glycemic control remain the cornerstones of treatment; alternative therapies remain largely unproven [Miller & Henry, 2009].

Some argue that many apparently uninfected diabetic foot ulcers are actually subclinically infected—that is, they contain a high “bioburden” of bacteria that results in “critical colonization” levels and impairs wound healing [Edmonds & Foster, 2004]. Available published evidence does not support the use of antibiotics for the management of clinically uninfected ulcerations, either to enhance wound healing or as prophylaxis against infection because antibiotic use encourages antimicrobial resistance, incurs financial cost, and may cause drug-related adverse effects. In some circumstances, it is difficult to decide whether a chronic wound is infected, such as when the foot is ischemic, has abnormal coloration or a fetid odor, has friable granulation tissue, is associated with unexpected pain or tenderness, or when an otherwise properly treated ulcer fails to show healing progress [Schultz et al., 2003].

1.6.8 Antibiotic regimens
Selection of the antibiotic regimen initially involves decisions about the route of therapy, the spectrum of microorganisms to be covered, and the specific drugs to administer and later involves choosing the definitive regimen and the duration of treatment. Initial therapy is usually empirical and is based on the severity of the infection and on any available microbiological data, such as recent culture results or current Gram-stained smear findings. For severe infections and for more-extensive, chronic moderate infections, it is safest to commence therapy with broad-spectrum agents. These should have activity against gram-positive cocci (including MRSA in locations where this pathogen is common), as well as gram-negative and obligate anaerobic organisms. Clinical trials suggest that fluoroquinolones, cephalosporins, beta-lactam inhibitor penicillins, and carbapenems are effective. These suggested agents are derived from available published clinical trials and are not meant to be inclusive of all potentially reasonable regimens. Similar agents could be used, depending on various clinical, microbiological, epidemiological, and financial considerations. A study found imipenem to be equally potent against Gram-positive and negative diabetic foot isolates while vancomycin was overall the most effective drug against Gram-positive isolates [Raja, 2007]. Importantly the author concluded that no antimicrobial agent can cover all of the organisms isolated from diabetic foot ulcers [Raja, 2007].

1.7 Urinary tract infections (UTIs)

Urinary tract infections (UTIs) are among the most frequently reported infections among intensive care unit (ICU)-patients [Hassanzadeh et al., 2009]. UTIs are clinically defined as the bacterial colonization of any tissue along the urinary tract, from the urethral opening to the kidneys. The term urinary tract infection encompasses a broad range of
clinical entities that are associated with a common finding of a significant amount of bacteria in urine and a positive urine culture [Dusé & Klugman, 1993]. The infection may be community acquired or nosocomial, often as a consequence of urethral catheterization. Urinary tract infections are very common in women [Pérez-López et al., 2009].

1.7.1 Etiology

Enterobacteriaceae are endogenous to the gastrointestinal tract and are often implicated in UTIs. *E. coli* causes about 80% of all the community acquired UTIs [Stamm, 2006] followed by *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Proteus mirabilis* [Gales et al., 2002]. *S. saprophyticus* [Widerstrom et al., 2007] and group D *Streptococci* (*Enterococci*) are the Gram positive enteric bacteria that commonly cause UTIs [Rebuck et al., 2000]. Infrequent causative agents of UTIs may include bacteria such as *S. aureus*, *Gardnerella vaginalis*, *Corynebacterium* and *Lactobacilli*, yeasts such as *Candida* (in diabetic patients or patients with indwelling urinary catheters) and viruses such as Adenovirus type 2 (associated with acute haemorrhagic cystitis in children) [Dusé & Klugman, 1993]. Non-specific urethritis is frequently caused by sepsis of *Chlamydia*, *Ureaplasma* and *Mycoplasma*, mainly introduced by sexual contact. Gonococci may also invade the urinary tract as well as the reproductive system, by entering the bladder via the urethra with an interim phase or periurethral and distal urethral colonization [Hooton, 2000].

*Acinetobacter baumannii*, a gram-negative coccobacillus, is currently recognized by the Infectious Diseases Society of America as one of the most important pathogens threatening our health care delivery system [Talbot et al., 2006]. It is an
important opportunistic pathogen implicated in urinary tract infections [Bergogne-Berezin & Towner, 1996]. The emergence of carbapenem-resistant $A. baumannii$ was reported in the United States in 1991 [Go et al., 1994]. Since then, many hospital-wide outbreaks have been reported worldwide [Kohlenberg et al., 2009]. Indeed, the Antimicrobial Availability Task Force of the Infectious Diseases Society of America recently identified $A. baumannii$, among “particularly problematic pathogens” for which **there is a desperate need for new drug development** [Talbot et al., 2006].

### 1.7.2 Antibiotic regimens

When choosing an appropriate agent to combat these infections, there are several factors for clinicians to consider. First, the drug of choice should have good in vitro and in vivo activity against many of the organisms known to cause UTIs. Additionally, the agent should be able to achieve high and prolonged concentrations in the urine and surrounding urinary tract tissues without loss of activity [Bergan, 1997; Jancel & Dudas, 2002]. In terms of the patient’s well being, drugs should have the fewest and mildest side effects possible, with minimal effects on normal colonic and vaginal flora [Sobel & Kaye, 2004].

Treatment of uncomplicated UTIs have in the past included aminopenicillins (amoxicillin, ampicillin), first generation cephalosporins (cephalothin, cefazolin), second generation cephalosporins (cefotixin, cefuroxime), third generation cephalosporins (cephotaxime, ceftriaxone, ceftazidime), sulfamethaxazole-trimethoprim combinations (cotrimoxazole), amoxicillin/clavulanic acid (augmentin), and the fluoroquinolones such as ciprofloxacin [Dusé & Klugman, 1993; Hindler et al., 1994; Abdel-Rahman & Kearns, 1998; Bannister et al., 2000]. With their excellent bioavailability when orally administrated, good tissue penetration, low incidence of side effects and broad
spectrum of activity, fluoroquinolones are prescribed to combat a variety of clinical infections and are especially favored in combating complicated UTIs [Mulholland, 1996; Mascaretti, 2003].

1.8 Bioinformatics, drug-discovery and MDR-research

Bioinformatics is the application of tools of computation and analysis to the capture and interpretation of biological data [Bayat, 2002]. Novel approaches to rational drug discovery have been exploding in the last years as high-throughput data (structure, binding affinity and functional effects) become available for both targets and ligands of pharmaceutical interest [Rognan, 2007]. An example of the application of bioinformatics in therapeutics was the development of designer targeted drugs such as imatinib mesylate (Gleevec), which interfered with the abnormal protein made in chronic myeloid leukaemia. The ability to identify and target specific genetic markers by using bioinformatic tools facilitated the discovery of this drug [Bayat, 2002].

Scientists are increasingly using bioinformatics in MDR-research these days [González-Pons et al., 2009]. The rapid diversification of ESBLs capable of hydrolyzing our most potent β-lactams is of serious concern. The efficient inhibition of these enzymes is therefore need of the hour in clinical research. There is a paucity of data concerning ESBLs prevalent in India. To the best of our knowledge there is no study that reports modelling of CTX-M-15, the most frequently encountered ESBL in India. Also, the present study describes docking of carbapenemases like KPC2, SME1, IMI1 and SFC1 with target drugs (carbapenems) and various inhibitors. This information might help in designing versatile inhibitors against the rapidly emerging β-lactamase-variants.
1.9 Objectives of the study

In view of the present background we initiated our study with the following objectives:

1(a) To find the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in the neonatal intensive care unit (NICU) of Aligarh, hospital, India and to identify the risk factors associated with the acquisition of these organisms.

(b) To characterize the mode of transmission of *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub> among the ESBL-producing *E. coli* strains of the NICU and to identify risk factors for “sex-associated ESBL-producing *E. coli* acquisition status” of the neonates.

2(a) To assess potential risk factors for diabetic foot ulcers infected with multidrug resistant organisms (MDROs) and to investigate antibiotic susceptibility patterns and ESBL-production in bacteria isolated from these ulcers.

(b) To characterize the mode of transmission of *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub> among ESBL-producing *E. coli* strains isolated from infected diabetic foot ulcers, and to identify the risk factors for “sex-associated MDRGNB infection status” of the ulcers.

3 To screen *A. baumannii* strains isolated from patients having community-acquired urinary tract infections for the presence of *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, *armA*, *rmtA*, *rmtB* and integrons and to characterize the mode of transmission of *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub> in the ESBL-producers among them.

4 To identify the amino acid residues crucial to the “enzyme-drug” and “enzyme-inhibitor” interactions with reference to CTX-M variants as well as carbapenemase enzymes by using homology modelling and docking studies.