CHAPTER 1
INTRODUCTION

1.1 Introduction

Irresistible infectious illnesses are extremely unsafe to human well-being. While the disappointment of social insurance experts to stop infections could incite scourges and rapid spread of an infection midst majority of individuals. Irresistible illnesses remain an essential reason for dismalness and mortality, particularly in hospitalized quiet. Antimicrobial resistance occurs when microorganisms adapt to antimicrobial medications to which they are exposed. New resistance components are developing and spreading undermining our capacity to treat regular irresistible maladies, bringing about delayed ailment, incapacity and passing. The long, obtrusive and escalated tertiary care healing facility medications bring down the obstructions to microbial intrusion of host tissues and bargain the advancement of a sufficient invulnerable reaction. Prolonged hospital stay and need of more specialised care for infected individuals is a direct result of Antimicrobial resistance and eventually this increases the cost of health care insurance expenditure.

Presence of ESBL producing Escherichia coli, Klebsiella pneumoniae and extensively drug resistant Acinetobactor baumanii even in acute health care settings in very high numbers demands the proper detection of these important organisms in the clinical laboratories.

The perpetually expanding bacterial imperviousness to anti-infectious agents is a standout amongst the most troublesome errands of all the medicinal issues which are being confronted by us today. B-Lactam antibiotics are generally used to treat microbial infections. Expanded utilization of anti-infectious agents, especially the third era of cephalosporins, has been connected with the rise of Beta - Lactamases interceded bacterial resistance, which in this manner prompted to the improvement of ESBL creating microorganisms. ESBLs provides resistance to a wide spectrum of antibiotics such as ceftazidime, cefotaxime, ceftriaxone and aztreonam and is produced by the gram negative bacteria more commonly in Escherichia coli and Klebsiella
pneumoniae. Beta lactamases activates the hydrolysis of the Beta-lactam ring of antibiotic, thereby perishing the antibacterial activity. ESBL producing organism are continuously resistant to different classes of antibiotics, as the plasmids with the gene encoding ESBLs usually bear other resistance alleles. Resistance in *Klebsiella pneumoniae*, a common intestinal bacteria that can cause life-threatening infections has spread to all regions of the world. *K. pneumoniae* is a major cause of nosocomial infections. More than half of the people treated for *K. pneumoniae* infection with carbapenems fails to respond in many countries including India. *E. coli* strains resistant to fluroquinolones, an antibiotic of choice for urinary tract infections are predominant worldwide. But it is more common in hospitalized patients especially in long care facilities, health care manipulations. e. g., use of catheters [1]. Initially ESBL producing organisms were commonly isolated from nosocomial infections but these organisms are now also being isolated from community.

1.1.1 Emergence of drug resistance

Resistance in microbes which are now difficult to treat are mostly of genetic origin and transferable between species and genera of bacteria [2]. The abuse of antimicrobial agent does not accomplish the coveted remedial results and is connected with the rise of resistance. Deficient dosing, poor adherence and substandard antimicrobials may assume a vital part. Every antimicrobial agent can possibly choose antibiotic resistant subpopulations of microorganisms. The broad utilization of antimicrobials, the pervasiveness of imperviousness to each new medication has expanded. The incidence of resistant strains varies with geographical locations. Somehow resistance emerged to all antibiotics. (WHO 2001).

Since the principal anti-microbial, penicillin, was found in 1929, many normally happening anti-infection agents have been distinguished. As their fundamental components of activities are deciphered, the drug–target co-operation have been resolved. This has prompted the advancement of more novel manufactured antibiotics through atomic alteration of previous anti-microbials. These endeavours have extraordinarily improved treatment in clinical practices. However, despite the increasing prevalence of drug resistance, the research and development for new antibiotics has almost halted. This is evident in the fact that there has been no major novel types of antibiotics developed in the last two decades (Fig. 1.1). The antibiotic
candidates currently in the pipeline are variations of previous antibiotics and, due to the similarity between the antibiotic structures and their targets, are likely to provoke new resistance [3]. This ineffectiveness of antibiotics can create horrible situations, even a general infection can be a life taking one. Therefore, the necessity to find advanced antimicrobials with new antimicrobial mechanisms has become a matter of great urgency.

![Timeline of discovery of distinct classes of antibacterial drugs](image)

**Fig. 1.1. Timeline of discovery of distinct classes of antibacterial drugs [3].**

### 1.1.2 Mechanism of resistance to antimicrobials

Evolution occurs naturally when microorganisms replicate themselves erroneously or exchange traits under selective pressure. Drug resistance has evolved in nature since ancient people started to use herb medicine well before the antibiotics era. Phylogenetic researches reveal that some classic resistant genes such as metallo-β-lactamases originated more than two billion years ago [4]. Drug resistance is now widely spread all over the world because of antibiotic abuse and inadequate infection control practices.

Antibiotics inhibit or alter essential cellular functions in bacteria by selective pressure. The major strategies used by bacteria against antibiotics have been
reported and are well understood (Fig. 1.2 and Fig. 1.3). A possible strategy is antibiotic destruction. B-lactam antibiotics, such as penicillin, can be hydrolyzed by bacteria bearing the ESBL gene coding for a β-lactamase. Bacteria also use mutations to overcome antibiotics. Streptomycin resistance requires special mutations within the bacterial 30S ribosomal protein RpsL [5].

Another resistance mechanism involves reducing the accumulation of antibiotics by reducing drug permeability or by vigorous pumping out. An example is AcfAB-TolC [5] that could actively pump out multiple antibiotics with targets located inside cells. Furthermore, susceptibility of bacteria to antibiotics is highly related to the bacterial stage. Non-growing cells after the log exponential phase are relatively more tolerant to antibiotic treatments. The underlying mechanism is still largely unknown, but might be caused by the absence of active targets for antibiotics in non-growth cells.

![Mechanism of drug resistance](image.png)

Fig. 1.2. Mechanism of drug resistance [5]
1.1.2.1 Efflux of antibiotics from bacteria

Efflux pumping have a significant input in antibiotic resistance and also serve other functions in bacteria such as the intake of ions and essential nutrients, Expulsion of metabolic end products and harmful substances. As well as helps the cell communication with environment [6]. Bacteria such as Escherichia coli and Staph. aureus, frequently accomplish drug resistance by rising expression of efflux pumps [7, 8]. This mechanism consists of a special type of protein that works to get rid of antibiotics from the cytoplasm of bacteria by draining them back into the extracellular environment [8, 9]. Generally gram negative organisms and many eukaryotes have antimicrobial extrusion proteins. Mostly the action of efflux pumps is against one specific group of antimicrobials with some exceptions. [9]. The five main types of efflux pump transporters are 1) Major Facilitator (MF) 2) Multidrug and toxic efflux (MATE) 3) Resistance-nodulation division (RND), 4) Small multidrug resistance (SMR), and 5) ATP binding cassette (ABC). The major energy source used by these efflux pumps transporters to eliminate antimicrobials from cytoplasm are proton motive force and hydrolysis of ATP [9, 10].

Fig. 1.3. Bacterial cells employ multiple mechanisms to overcome antibiotics [5].
1.1.2.2 Outer membrane (OM) permeability

The entry of both hydrophobic and hydrophilic compounds into gram negative bacteria is difficult because of the outer membrane permeability. The outer membrane functions as a selective barrier with the aid of a highly hydrophobic lipid bilayer with pore forming proteins with specific size-exclusion properties. The decreased permeability of OM to antimicrobials significantly affect the efficiency of drugs as they primarily acts on intra cellular processes. Hydrophilic antibiotics which are of relatively small size like, Beta-lactam drugs gains access to the cell interior through the pore forming proteins (water filled channel proteins integrated in the outer membrane, e.g., OmpF in *E. coli* and OprD in *Pseudomonas aeruginosa*). By diffusing across the lipid bilayer macrolides and other hydrophobic antibiotics gain access. The prevalence of more antibiotic resistant strains visualise the role of OM barrier in acquiring resistance [11].

1.1.2.3 Target modifications

This system depends on changes of bacterial locales that are focused by anti-infection agents and along these lines keeping the anti-infection from binding to its site of activity. For example fluoroquinolone resistance is attributed to mutations within the drug target (DNA gyrase and topoisomerase) [12].

1.1.2.4 Enzymatic modification of the antibiotic

Enzymes that modify antibacterial antibiotics are divided into two general classes: a) Beta-lactamase that degrade antibiotics and b) others (including the macrolide and aminoglycoside-modifying proteins) that perform chemical transformations to render the antibiotic inefficient [12].

1.1.2.5 The acquisition and spread of antibiotic resistance in bacteria

The spread age of antibiotic resistant strains of certain bacteria have reached to such extend that they can resist any antibiotics available in market. In some instances the Antibiotic resistance in bacteria a due to the genetic trait of that organism which makes it naturally resistant. In some cases the resistance is acquired by mutation in its DNA or by acquiring resistant DNA from external environment [13].
Inherent (natural) resistance

Microscopic organisms might be inalienably impervious to an anti-microbial. For instance, a living being does not have a vehicle framework for an anti-toxin; or a living being does not have the objective of the anti-microbial particle; or, as on account of gram-negative microorganisms, the cell divider is secured with an external film that builds up a penetrability boundary against the antibiotic.

Vertical gene transfer

The spontaneous mutation can lead to formation of antibiotic resistant genes. The passing of resistant gene directly to next generation of bacteria during DNA replication is called as vertical gene transfer.

Horizontal gene transfer

Sidelong or level quality exchange (HGT) is a procedure where small fragments or parts of DNA are exchanged among microorganisms of same or different species. Transduction, transformation and conjugation changes are examples for such transfer. Conjugation is a plasmid mediated transfer through direct cell to cell contact of bacteria and is considered as leading type of HGT.

Transformation

This is a mechanism by which a bacteria receive DNA fragments usually remains of dead or lysed bacterial fragments from its nearby environment.

Transduction

The transferring of DNA among related species of bacteria through bacteria-specific viruses (bacteriophages) [13].
Fig. 1.4. Mechanism of drug resistance by horizontal transfer [13].

1.1.2.6 Beta lactam

In 1928, Alexander Fleming observed that culture plate on which *Staphylococci* were being grown had become contaminated with a mold of the genus Penicillium and the growth of bacteria near the fungus had been restricted. Fleming isolated the fungus in pure culture and demonstrated that it produced an antibacterial substance Penicillin (Abraham and Chain 1940). Abraham and Chain (1940) have shown that certain bacteria produce an enzyme called penicillinase, which destroy penicillin. *Staphylococcus aureus* strains capable of producing penicillinase began to appear in community with in few years after the introduction of penicillin into clinical use. Penicillinase resistant penicillins were introduced to treat such infections. Afterward broad spectrum penicillins were introduced followed by first generation cephalosporins. For more than twenty years until beta lactamase producing gram negative bacteria emerged as an increased threat these antibiotics were considered as the first line treatment option. [14]. Six new antibiotics within a time period of seven to eight years which includes carbapenems and monobactams were introduced by industry to overcome the new threat of beta lactamase producing gram negative bacteria. Emergence of new lactamases continued in significant numbers [15].

*Beta lactam* antimicrobials are the most common treatment option for gram positive, gram negative and anaerobic bacterial infection [16]. Beta lactams are a family of antimicrobial agents consisting of four major groups: penicillins, cephalosporins, monobactams and carbapenems.
All of these groups have a beta lactam ring. (Fig. 1.5) The beta lactamase enzymes are capable of hydrolysing the beta lactam ring. The major characteristic feature that distinguishes each group are the difference in additional rings. e.g. Thiazolidine ring (penicillin), Cephem nucleus (cephalosporins) [17]. They act on bacteria by two mechanisms: first, they integrate into cell wall of bacteria and obstruct the action of transpeptidase which is essential for cell wall synthesis. The Second mechanism is by getting attached to the penicillin binding proteins (PBP's), which suppresses cell wall hydrolases. This will increase the presence of more cell wall hydrolases that can lyse cell wall of bacteria. In order to overcome such antimicrobial mechanisms bacteria synthesise group of enzymes called beta lactamases which are capable of inactivating beta lactam drugs [18].

![Beta lactam ring](image)

Fig. 1.5. Showing beta lactam ring destruction by beta lactamases enzyme

1.1.2.7 Beta lactamase enzyme

Beta lactamases enzymes are able to catalyse the hydrolysis of beta lactam ring. Currently 500 or more types of different beta lactamases have been discovered in nature. Both gram positive and gram negative bacteria can synthesise these enzymes [19]. Beta lactamase producing gram positive bacteria release the enzyme into the surroundings medium. But Gram negative bacteria release the enzyme into the periplasmic space. So, this is called group protection for gram positive bacteria and individual protection for gram negative bacteria [20].
1.1.2.8 Extended spectrum cephalosporins (ESCs)

Cephalosporins of first generation are primarily effective in treating gram positive cocci. New groups of cephalosporins (second, third and fourth generations) were manufactured with extended activity against the gram negative rods, e.g. ceftazidime, cefotaxime and ceftriaxone [17].

1.1.2.9 Extended spectrum beta lactamase (ESBLs)

The Extended spectrum beta lactamases (ESBLs) are the group of enzymes responsible for the resistance to the oxyimino-cephalosporins like cefotaxime, ceftazidime, ceftriaxone, cefuroxime and the monobactams like aztreonam, as well as to other older beta lactam drugs [21].

Organism responsible for ESBL

The organisms that most commonly producing ESBLs are *E. coli* and *Klebsiella pneumoniae*, less frequently by *Enterobacter, Proteus, Pseudomonas aeruginosa* and *Morganella morganii* [22].

1.1.2.10 Molecular classification

Nucleotide and amino acid sequences in these enzymes forms the basis of molecular classification. The four different classes are A, B, C and D. Action of classes A, C, and
D action is a serine based mechanism, while class B or metallo beta lactamases action depends on zinc.

*Carbapenemases*

Stability of carbapenems to AmpC -lactamases and extended-spectrum- lactamases are well known. Carbapenemases consist of a wide range of β-lactamases that can effectively act against the oxyimino-cephalosporins, carbapenems and cephamycins.

**β-Lactamases**

- **Class A**
  - SHV
  - TEM
  - CTX-M
  - KPC

- **Class D**
  - OXA

- **Class B**
  - IMP
  - VIM
  - NDM-1

- **Class C**
  - AmpC
  - CMY
  - FOX
  - MOX
  - ...

**Metallo-β-Lactamases (MBLs)**

**AmpC β-Lactamases**

---

**Fig. 1.7. Molecular classification of Carbapenemases**

**Fig. 1.8. Shows Hodge test for CRE (Carbapenem Resistant Enterobacteriaceae)**
1.1.3 Risk group

Patients with neutropenia, transplant recipients, premature neonates are considered to be at high risk in acquiring infection with ESBL. Screening for ESBL while admission of these patients groups are highly recommended.

1.1.3.1 High risk units include

Hematology, oncology and intensive care units are often sources of ESBL. Organ Transplantation units and chronic care facilities are also considered as risky areas.

1.1.4 Treatment

The nearness of ESBLs muddles the choice of antimicrobials, especially in patients with genuine contaminations, for example, bacteremia. The explanation behind this is ESBL producing microbes are regularly multi impervious to different antimicrobials, and CTX-M delivering confines are co-impervious to the fluoroquinolones. Antimicrobial agents that are consistently utilized for observational treatment of genuine group onset contaminations, for example, the third-era cephalosporins are not effective against ESBL-delivering microorganisms. The spread of multiple drug resistant bacteria made choosing antibiotics for empirical therapy a complicated one. Empirical therapy is necessary after a clinical diagnosis of infection because susceptibility results from lab often requires more than one day. Choice of drug against ESBL on such instance is mostly Carbapenems eg imipenem, meropenem [20].

1.1.4.1 Quinolones

Ciprofloxacin, a fluoroquinolone, is an intense and expansive range anti-infection, and has great antibacterial movement against most gram negative bacteria and gram positive cocci. Ciprofloxacin has a better capacity than infiltrate most tissues contrasted with different antibiotics, aggregates in macrophages and neutrophils and is bactericidal in low pH environment.

1.1.4.2 Aminoglycosides

Unlike gentamicin and tobramycin effectiveness of amikacin seems to be preserved and remains as a choice in empiric therapy [20]. Aminoglycosides are also considered as antibiotic of choice to treat infections caused by ESBL producing pathogens. These antibiotics are effective against both gram-positive and gram-negative organisms [23,
Aminoglycosides acts by inhibiting bacterial protein synthesis and disrupting the translocation [25]. Examples of aminoglycoside antibiotics are tobramycin and amikacin.

1.1.4.3 Tigecycline

Tigecycline is a novel, potent, broad spectrum antibiotic. They act by inhibiting translation of protein in bacteria. Tigecycline bind to the 30S ribosomal subunit and restricting the entry of aminoaecyl to RNA molecules into the A site of the ribosome [26]. Guideline to interpret susceptibility testing of tigecycline are still not available. Data of many lab studies suggest tigecycline as an alternative to carbapenems for treating infections with ESBL-producing Enterobacteriaceae.

1.1.4.4 Colistin

Once considered as a poisonous anti-microbial, clinicians have now swung to colistin if all else fails operator for the treatment of diseases brought on by multidrug resistant gram negative microscopic organisms. The antimicrobial focus of colistin is the bacterial cell membrane, where the polycationic peptide ring associates with the lipopolysaccharides, permitting infiltration across the external film by uprooting Ca²⁺ and Mg²⁺. Interaction of phospholipids with the cytoplasmic membrane affects membrane integrity and causes bacterial cell lysis [26]. KPC mediated carbapenem resistance is increasing and this made usage of colistin in empirical therapy a necessity. The cephamycins which includes cefoxitin and cefotetan, are generally unaffected to hydrolysis by ESBL producing Enterobacteriaceae strains. During treatment some isolates may reduce the expression of outer membrane proteins, which can lead to resistance. Legitimate utilization of antimicrobials is critical for different reasons. It lessens superfluous costs, diminishes rise of imperviousness to helpful and lifesaving anti-infection agents, and minimizes numerous symptoms. India does not have any methodical program for considering the antimicrobial resistance design. Appropriate utilization of anti-infection agents is guaranteed by planning an anti-infection strategy. ESBL screening as a standard test has not yet been polished in numerous piece of India. ESBL happens at a disturbing rate among Enterobacteriaceae detaches among the hospitalized patients which can bring about a flare-up in the group that might be hard to treat So, the present study was attempted to see commonness of ESBL in a tertiary care doctor's facility by various phenotypic strategies, which gave a benchmark
review of medication resistance design in Nagercoil (India) and to see the powerlessness example of ESBL creating strains.

1.2 Prevalence of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) in a Tertiary care hospital and laboratory detection of these important organism

*Staphylococcus aureus* is an important pathogen that can cause serious infections. Before the invention of antibiotics, invasive *S. aureus* disease was a major cause of mortality. *S. aureus* produce many toxins and enzymes. The more dangerous forms of *S. aureus* produce such toxins which are source of host tissue damages, hindering phagocytosis and causing disease symptoms. In systemic infections, it causes osteomyelitis, mastitis, wound infection & occasionally toxic shock syndrome [27].

1.2.1 *S. aureus* virulence factors

The *S. aureus* can produce proteins commonly known as MSCRAMMS (microbial-surface parts perceiving cement lattice particles) to tie fibrinogen, fibronectin, laminin, vitronectin, collagen, elastin and thrombospondin to elevate adherence and connection to endothelial cells and storm cellar films. *S. aureus* while in stationary phase can synthesise large numbers of membrane-damaging exotoxins and proteases which promote damage of tissue. Proteases, nuleases, lipases and staphylokinase, a fibrin-specific thrombolytic enzyme also mediate tissue invasion. [28].Some strains of *S. aureus* create Toxins like toxic shock syndrome toxin 1 (TSST-1), which leads to the activation of large numbers of T cells and results in proliferation and cytokine release [29]. Virulence factors of *S. aureus* is shown in Fig. 1.9.
1.2.2 *Staphylococcus aureus*: A Nosocomial Pathogen

MRSA remains for methicillin safe *Staphylococcus aureus*. Methicillin-Resistant *Staphylococcus aureus*, otherwise called the "superbug," is a wellspring of significant sympathy toward general wellbeing, a pathogenic microorganisms that causes numerous confounded diseases in people. Most staph germs are spread by skin-to-skin contact (touching). A specialist, nurture, other human services supplier or guests to a healing center may have staph germs on their body that can spread to a patient. Once the staph enters the body, it can spread to bones, joints, blood or any organ, for example, the lungs, heart or cerebrum. *Staphylococcus aureus* can produce diseases ranging from skin infections to fetal diseases [31]. Infections like cellulitis, abscesses, impetigo, folliculitis and furunculosis are some of examples. Beta-lactam drugs can be useful in treatment of general non- MRSA *Staphylococcus aureus* infections. Glycopeptides are drug of choice for infections with MRSA.

1.2.3 Hospital- acquired MRSA infection

Various studies pointed the increased incidence of infections with *S. aureus* that leads to varying level of morbidity in the hospitalised patients. Studies by Fraunholz and Sinha in 2012, have shown the way by which *S. aureus* infects a person and its survival within the victim [32]. Post-surgical infections accounts for approximately one-fourth of all hospital acquired infections. Many risk factors for *S. aureus* infection
are linked with patients stay in a surgical intensive care unit (SICU) in hospitals, i.e. care procedure numbers, nearness of infected patients, surgical wounds and foreign bodies’ presence and long-term antibiotic treatment. Throughout the world, particularly in developing countries burn wounds remains an important problem of public health associated with diseases, long-term disability and mortality [33, 34]. The burned dead moist tissues, along with damaged tissues, provide a nutrient medium which will favour the growth of a many types of bacteria. In a definite part of burns this type of bacteria causes septicaemia and death.

1.2.4 Community-associated MRSA disease

The dramatic rise of MRSA isolation rates in hospitals that nearing 50% of S. aureus infections makes Community in a high risk. Unlike MRSA found in hospitals CA-MRSA are susceptible to most antibiotics other than β-lactams [35, 36]. The presence of type IV SCCmec element encoding resistance to all β-lactam antibiotics [37, 38]. The toxin genes such as Panton-Valentine leukocidin (PVL) and other enterotoxins are also present in CA-MRSA [39]. They are not related to genotypes endemic in the hospital [40].

1.2.5 Staphylococcal Chromosome Cassette mec (SCCmec)

HA-MRSA and CA-MRSA produces β-lactamase and a modified target PBP (penicillin binding protein) to inactivate β-lactam antibiotics. The transpeptidase PBP2a encoded by mecA, is responsible for broad spectrum of resistance to all beta lactam drugs [41]. Staphylococcal chromosome cassette mec (SCCmec) harbours mecA which is transferred horizontally among staphylococcal species. HA-MRSA carries type I-III SCCmec; these genetic materials contains many resistance genes for non-β-lactam antibiotics. However CA-MRSA almost always carry IV SCCmec element (21 to 24 kb), which is free of non-β-lactam resistance determinants [37, 38].

1.2.6 Antimicrobial resistance and MRSA in Tamil Nadu

In developing countries like India MRSA infection has become a major disease in hospitals and they have been a cause of increasing cost, disease rate and deaths associated with surgical operations. For methicillin resistance the responsible gene mecA exists on the chromosomes. The resistance mechanism is related to lack of inaccessibility of certain penicillin-binding proteins (PBPs) in the organisms. In this
study. Isolation, Identification and Molecular characterization of indigenous MRSA strains was done from urine, sputum, and blood, various surgical and accidental wounds. A total of 1750 Clinical specimens were collected from different wards of tertiary care hospital and processed into Blood Agar, Chocolate agar for isolation and also on CHROMagar’ for MRSA. 122 Out of 1750 isolates were identified by coagulase and Dnase test. MRSA isolates were confirmed by latex agglutination test for the presence of Penicillin-Binding Protein 2a (PBP2a). All positive MRSA were molecularly characterized using Gene Xpert Cepheid PCR. All MRSA isolates showed 100% resistance against Amoxicillin-Clavulanic acid, Ampicillin, Amoxicillin and Ciprofloxacin but sensitive to Vancomycin and Linezolid. Vancomycin (98%) was the most effective drug followed by Linezolid (90%). Present study conclude that high prevalence of MRSA was found in hospitals, were more prone to MRSA infection. All MRSA isolates were resistant to commonly used antibiotics only Vancomycin was the drug resistant against MRSA hence considered as the most effective drug Study of Antimicrobial Susceptibility pattern of MRSA against commonly used antibiotics. Isolation, Identification and Molecular characterization of indigenous MRSA strains from different sites. Study of Antimicrobial Susceptibility pattern of MRSA against commonly used antibiotics.

1.3 Preparation, characterization and in vitro activity of liposome incorporated antibiotics against drug resistant bacteria.

Antimicrobials have dependably been demonstrated of restricted application in medicinal services because of their poisonous quality or feeble bio circulation and pharmacokinetics. Despite the fact that such antimicrobials have potential action, yet they are just utilized as last possibility of treatment where danger of reactions is high. Antibiotics of various classes, for example, quinolones, aminoglycosides, beta-lactams, cephalosporins, retroviral, macrolides and polypeptides are connected with the weaknesses of medication toxicities, bring down bioavailability and in addition bacterial resistance. A legitimate medication conveyance framework can conquer these downsides. The liposome can end up being a major walk towards abolishment of these disadvantages. Liposomes are a standout amongst the most tried and adaptable frameworks among all the lipid-based nanotechnologies for medication conveyance.
1.3.1 Liposome

The name liposome evolved from Greek words: "Lipos" signifying "fat" and "Soma" signifying 'body'. In 1961 Alec D. Bangham described phospholipids for the first time. The liposomes are a round, modest intracellular vesicle having no less than one lipid bilayer, made with an indistinguishable substance from a cell layer. Liposomes are useful in transportation of particles, for example, supplements and medications (i) into the cell, (ii) out of the cell, and (iii) between various parts of a cell.

1.3.2 Liposomal structure

Liposomes are concentric vessels with an aqueous volume forms a core surrounded by a membranous lipid bilayer, composed of phospholipids. These phospholipids are molecules with a hydrophilic head and a hydrophobic tail group [42, 43]. The hydrophilic part is attracted and the hydrophobic tail, is repelled by water [44, 45]. Upon disruption of membrane phospholipids, they tend to rearrange itself into tiny vesicles with monolayer or bilayer. The monolayer structures results in micelles and bilayer forms liposomes.

![Fig. 1.10. Structure of liposome [42].](image)

1.3.2.1 Structural components of liposome

The main components of liposomes are Phospholipids and Cholesterol.
Phospholipids

Phospholipids are major structural components of biological membrane such as cell membrane. The most common phospholipid used is phosphatidylcholine (PC). The molecules of PC are insoluble in water. Phosphatidylcholine can be obtained from both natural as well as synthetic sources. Natural phospholipids are phosphatidylcholine, phosphatidylethanolamine (PE) and phosphatidylyserine (PS) whereas synthetic phospholipids include Dioleoylphosphatidylcholine (DOPC), Disteroylphosphatidylcholine (DSPC) and Dioleoylphosphatidylethanolamine (DOPE).

Cholesterol

The introduction of cholesterol in liposome bilayer significantly changed creation of membranes. Cholesterol is not a component of bilayer structure but used in formulation as fluidity buffer i.e. below the phase transition temperature, it makes the membrane of increased permeability and above phase transition temperature, it makes the membrane of higher stability. Cholesterol is often integrated into phospholipid membrane at a concentration of 1:1 or 1:2 molar ratios of cholesterol to PC.

1.3.3 Classification of liposomes

Liposomes can be classified on basis of size and number of lamellae. Size of Liposomes vesicles often ranges 0.025 μm to 2.5 μm. Single or bilayer membranes are seen on Liposomes. Circulation half-life of liposomes can be calculated based on vesicle size. Drug encapsulated in the liposomes are influenced by size and number of bilayers. Based on size and number of bilayers liposomes are divided into two groups, multilamellar vesicles (MLV) and unilamellar vesicles. Unilamellar vesicles are subdivided into (a) Large Unilamellar Vesicles (LUV) and (b) small unilamellar vesicles (SUV) [46]. Vesicle in the unilamellar liposomes consist of a single phospholipid bilayer sphere covering the aqueous solution. Vesicle in multilamellar liposomes are onion shaped. Many unilamellar vesicles will form on the inside of the other with lesser size, forming a multilamellar structure of concentric phospholipid spheres which are separated by layers of water [47].
1.3.4 Types of liposomal drug delivery platforms

The ability of liposomal drug delivery systems to carry both lipophilic and hydrophilic particles make it possible to encapsulate many drugs. By introducing water resistant molecules into the bilayer membrane, molecules which are hydrophilic can be captured into the aqueous interior [48-51]. Biocompatible lipid exterior and large aqueous interior permits the of delivery macromolecules, such as deoxyribonucleic acid, proteins and imaging agents [52, 53]. Production of liposomes are based on properties like size, charge, number of lamellae, lipid composition, surface modification with polymers and ligands—these factors contribute to the stability in vitro and in vivo [51, 53]. Antibiotics encapsulated within liposomes are protected from inactivation, degradation and getting diluted in the circulation [52]. Four major liposomal delivery systems in practise are a) conventional liposomes b) sterically - stabilized liposomes c) ligand-targeted liposomes d) combination of the above.

1.3.4.1 Conventional liposomes

This category of liposomes are made up of a lipid bilayer consist of cationic, anionic, neutral lipids and cholesterol around an aqueous volume (Figure 1.3.4). Conventional liposomal delivery system improves the therapeutic index of encapsulated drugs like doxorubicin and amphotericin [48-51, 54]. Decreased toxicity and enhanced biodistribution are also achieved. Quick expelling from the bloodstream is one of the main disadvantage with the conventional liposomes [51, 55, 56].

1.3.4.2 Sterically - Stabilized liposomes or PEGylated liposomes

Sterically-Stabilized liposome with improved circulation time were introduced to overcome the shortcoming of conventional liposomes. Using polyethylene glycol (PEG), helped to achieve a steric barrier which in turn improved the activity of encapsulated drugs by decreasing the opsonisation with serum components, and the reduced uptake by the RES [57-59]. Steric stabilization also manage the pharmacokinetics of liposomes [60].

1.3.4.3 Ligand-targeted liposomes

Ligand-targeted liposomes provides a site specific delivery of drugs to designated cells or organs, with the aid of specific ligands. Commonly used ligands are antibodies, peptides and carbohydrates (Fig. 1.11) [61, 62]. Stability and higher binding avidity
because of the presence of two binding sites on the molecule makes monoclonal antibodies a perfect candidate. Reduced pharmacokinetics and immunogenicity caused a poor performance in vivo (74). To overcome these shortcomings, latest generations of liposomes used a combination of the above design and improved liposomal targeting and delivery of drugs.

Fig. 1.11. Schematic representation of the different types of liposomal drug delivery systems [61].

1.3.5 Biological challenges facing liposomal drug delivery systems

Like any antigen that get into the body, liposomes are also met with many defence mechanisms that recognize, neutralize and eliminate. The main defence mechanisms are RES, opsonization and immunogenicity [63].

1.3.5.1 The reticuloendothelial system (RES) and liposome clearance

The main site of liposome accumulation following their systemic administration is RES [64, 65]. The body parts included in RES are liver, spleen, kidney, lungs, bone marrow, and lymph nodes [65]. The ability of the RES to absorb liposomes circulating in the body is due to the fenestrations present in microvasculature. Large pores in these capillaries absorb the liposomes carrying the drugs and later remove them from circulation. Resident macrophages in the RES clear liposomes through direct
interactions [66]. The adsorption of proteins like immunoglobulins, fibronectin, lipoproteins to the phospholipid membrane is known as vesicle opsonization [59, 66].

Integration of PEG polymers to the liposomal membrane help to improve circulation time and prevents removal through RES mediated by steric stabilization [67, 68]. A local surface concentration is achieved by PEGylation process, which can sterically stop electrostatic and hydrophobic reactions with plasma proteins or cells, reducing liposomal absorption of the RES [68]. The usage of PEG reduces, but does not totally stop the absorption of liposomes by the RES.

1.3.5.2 Opsonins and vesicle destabilization

The interaction between liposomal drug delivery systems and plasma proteins is significant in the determination of nanocarrier biodistribution, efficiency and toxic effect [51]. The importance of Plasma proteins in liposomal clearance by opsonization and vesicular destabilization is significant [69]. Major factors that contribute in opsonization of liposomes by serum proteins are surface charge, size and stability [68, 69]. The rate of interaction is reduced with smaller liposome size that measures from 200 to 800 nm in diameter. The opsonic activity is not supported by smaller liposomes [66]. The elimination of large unmodified liposomes are faster than small, neutral, or positively charged liposomes [52, 67, 70]. The high electrostatic charge promotes liposomes interaction with biomolecules which act as opsonins [68, 70]. Various studies shows that big, charged liposomes are removed by the liver in few minutes whereas spleen clear them in an hour [65, 66]. The integration of cholesterol can increase liposome stability by minimizing the phospholipid exchange [63]. Integration of cholesterol into the liposomal membrane reduce the lipid exchange with the red blood cells and lipoproteins which causes the reduction of high phase transition temperature lipids which are replaced by less physiologically stable particles [63, 71, 72]. Integration of cholesterol into small (about 100 nm), neutral liposomes increases the circulation time several hours [73].

1.3.5.3 The enhanced permeability and retention (EPR) effect

The Liposomes that overcome the RES and opsonization have to face the EPR effect [74, 75]. EPR effect is the increase in permeability of the vasculature which supplies pathological tissues like tumors and tissues with inflammation. These sites are often associated with deregulations in angiogenesis or the increased expression of vascular
permeability factors, [75] which create fenestrations ranging from 300 to 4700 nm. This leads to the aggregation of liposomes by passive targeting [76]. The tight junctional regions between the endothelial cells are considered to have a width of 12 to 20 nm [77]. When exposed to inflammatory mediators, permeability of the microvasculature increases forming gaps of size up to 1 μm [78].

1.3.5.4 The accelerated blood clearance (ABC) phenomenon

Synthetic alterations of liposomes in order to improve their utility as a drug delivery vehicles induces human body to produce various antibody against them. Administration of PEGylated liposomes repeatedly can lead to the loss of their long circulating properties resulting in quicker clearance from the blood [79-81]. This phenomenon is called as accelerated blood clearance (ABC). The ABC is a big concern when multiple dosing PEGylated formulations are required in treatment [79]. Studies shows a maximum clearance of liposomes 4 to 7 days after the first dosage in rats and ten days in mice [80, 81]. The factors affecting this phenomenon are lipid dose, PEG surface density, and the interval between the first and consecutive injections [82]. Injection empty PEGylated liposomes repeatedly in rats increased marked anti-PEG IgM production [81]. Spleen plays major role in this immune response. The quantity of anti-PEG IgM production decreased in splenectomized rats [83].

1.3.5.5 Complement Activation–Related Pseudoallergy (CARPA)

Some liposomal systems activate the innate immune response, which is followed by the activation of complement system (a part of innate immune system), leads to acute hypersensitivity syndrome known as complement activation–related pseudoallergy (CARPA) [83]. Two to four percent of patients develop infusion-related hypersensitivity reactions against liposomal drug therapy. (e.g., Doxil R, Ambisome, and DaunoXome R ) [84, 85]. CARPA is an immediate, non-IgE mediated hypersensitivity reaction. the major symptoms are anaphylaxis, facial swelling, headache, and cardiopulmonary distress [84, 86]. Desensitization techniques using empty liposomes and pre-administration of complement inhibitors are found to be effective in preventing CARPA [86].

Neutral small unilamellar vesicles are found to be the least reactogenic of the liposomal platforms [86]. The immunogenic reactions against liposomal therapies
can cause change in pharmacokinetics, loss of efficacy, and the rise of potentially serious toxicities like anaphylaxis [85].

1.3.6 **Drug release from liposomes**

The ability of lipid bilayer in the liposome to fuse with other bilayers like cell membrane allows them to deliver the contents it carries. Liposome are expected to deliver it contents rapidly with minimal side effects to the cells as they are made up of biodegradable inert substances. The less toxicity, non-antigenic, easily modifiable surface and many other properties makes liposomes an ideal vehicle for drug delivery. Liposomes are preferred over systems of transport like microemulsions and nanoparticles [87, 88]. The aqueous core of liposome are surrounded by a hydrophobic membrane which obstructs dissolved hydrophilic solutes to move through the lipids while hydrophobic chemicals are dissolved into the membrane. Liposomes are helpful to transport both hydrophobic and hydrophilic molecules. The delivery of drugs to a targeted site by liposomes is achieved by the fusing of the lipid bilayer with other bilayers like cell membrane. The drugs are either filled into the aqueous core or integrated to the lipids in the membrane. The liposomes size varies from 1 µm to 4-8 nm.

An antimicrobial agent needs to be encapsulated in liposomes because of many advantages. First it protects the encapsulated drug from enzymatic hydrolysis. As an illustration, the penicillin and cephalosporins are prone to the degradative action of Beta-lactamase that is generated by some infective agents. The second being, lipid nature of these formulation, it can easily permeate cell membrane of microorganisms and increase cellular concentration, that eventually reduces the required dose and toxicity as found in the case of liposomal formulation of amphotericin B [89, 90].

1.3.6.1 Factors affecting release of drug

- Solvents
- PH
- Temperature
- Agitation
- Enzymes
- Cell Culture
1.3.7 Clinically approved liposomal-based therapeutics

Many liposome based drugs are commercially available and many are under development. Doxorubicin is the first FDA approved nano drug. Doxorubicin uses PEG conjugated liposomes [91]. PLD along with other drugs treats a wide range of cancers, which includes AIDS-associated Kaposi’s sarcoma, leukemia, ovarian, breast, bone, lung, and brain cancers. FDA has also approved many traditional cationic based liposomal drugs. Liposomal amphotericin B, for antifungal prophylaxis [92], daunorubicin for the management of leukemia patients and solid tumors [93], verteporfin effective against mascular degeneration [93], cytosine arabinoside for patients with neoplastic meningitis or lymphomatous meningitis [93], and morphine sulfate for pain management are commercially available. Clinical studies are still going on to check the therapeutic efficacy and outcome of dose escalation [93]. An example of such study is the prospective Phase 2 trial on liposomal amphotericin B which focus on risk and tolerance of high dose [94]. Major benefits of these commercially available medications are decreased toxicity by raised vasculature permeability/accumulation at tissue of target and able to encapsulate medications with a range of lipophilicities and prevention from bio-degradation is a b [93, 95, 96].

1.3.8 Benefits with liposomes

Liposomes are able to deliver hydrophobic, hydrophilic, amphipathic drugs and agents. Biocompatibility, flexibility, non-toxicity and total biodegradation are some of the major benefits. Whether systemic or non-systemic administration they are non-immunogenic. Liposome's are able to provide a lipophilic condition and aqueous "milieu interne" in same system. Protection of encapsulated drug from outer enviornment and functioning as a sustained releasing depot. (Cyclosporin, Propranolol) are important features of Liposomes. A variety of preperations can be achieved with Liposomes ranging from suspension of aerosols, gels, lotions, or vesicular powder form .Routes of administration are also flexible. Topical, intravenous, intramuscular, ocular, nasal are few examples of possible routes of administration. Big molecules like interleukin2, haemoglobin and small molecules are equally possible to be encapsulated with Liposomes. Higher efficiency, stability, therapeutic index and low level of toxicity of entrapped molecule through
Encapsulation is achieved with Liposomes. (Amphotericin B, Taxol). Sensitive tissues are less exposed to drug toxicity and ability to alter the pharmacokinetic and pharmacodynamic property of drugs (increased circulation life time, reduced elimination), the potential of pairing with site-specific ligands and achieving active targeting, controlled release are some the other benefits with Liposomes.

1.3.9 Liposomes as delivery systems for antibiotics

Encapsulated drugs in lipid vesicles has created new dimensions in designing the desired pharmacokinetic and pharmacodynamic properties [97, 98]. Improvement in pharmacokinetics and biodistribution significantly decreased toxic side effects, broadened bacterial effectiveness by destroying intra cellular pathogens. Delivering drug to specific target and improved action by circumventing bacterial resistance are also of significance. The liposomal carriers provides adequate and sustained supply of content while circulating in the body, which results in maintaining an effective drug concentration for a lengthier duration which is contrary to free antibiotics. Drug encapsulated in liposomal vesicles are protected from the deactivating immunologic, chemical and enzymatic reactions [99]. Application of liposomes in treating intracellular infections are very effective as drugs retention on tissues are improved. Isoniazid, rifampin, and clarithromycin significantly enhanced antibacterial efficacy in liposomal form in comparison with the conventional medications for management of tuberculosis [100, 101].

Effectiveness of Liposomal encapsulated medications has been extensively investigated for the elimination of intracellular and nonobligatory intracellular pathogenic bacteria [102]. The earliest researches with liposomal medicines for elimination of intracellular organisms has been designed with traditional liposomes carrying gentamicin for the management of brucellosis [97]. On-going has proved the chances to aim liposomes to specific tissues, organs, or even bacteria itself [103]. As vehicles for medicines liposomes are also helpful to prevent formation of biofilm. Studies has been conducted to find specificity and affinity of immunoliposomes to Streptococcus oralis biofilms [104]. The effective lipid formulation, drug distribution and bacterial-vesicle interactions that can leads to enhanced antibacterial drug activity against bacteria, such as P. aeruginosa, K. pneumoniae, E. coli, Acinetobacter sp, and S. aureus have been published [105].
1.3.10 Stability of liposomes

A major complication associated with Liposome application is to maintain stability and this is primarily related to the physicochemical characteristics of the lipid membrane [106]. Integration of cholesterols normally increases the stability of Liposomes [107], particularly while using unsaturated phospholipids in formulation of liposomes. The three factors that contribute to liposome stability are physical, chemical and biological. The physical stability of the liposomes are related to temperature, its size distribution due to aggregation/fusion of liposome bilayers or leakage of encapsulated material [108]. Confirming the chemical stability of liposomes includes evaluating the oxidation of unsaturated fatty acid chains and the hydrolysis of the lipids [108, 109]. Addition of cholesterol and antioxidants to the liposome medications can prevent the oxidation [107]. Hydrolysis of phospholipids detaches the hydrophobic chains of ester bonds, which might increase permeability of the phospholipids [52, 110]. The Layering of liposome using inert hydrophilic polymers can provide biological stability [111]. Other major factors that affects stability of liposomes are net surface charge, hydrophobicity and fluidity [106]. Improved storage life for Liposomes can be achieved by optimisation of size distribution, pH and lyophilization.

1.3.11 Liposomal toxicity

The toxicity associated with Liposomal drugs are a major area of concern. Toxicity can be resulted from poor penetration, solubility and stability of the drug after uptake [112]. The characteristics of charged lipids and the net charge of lipids also contribute to the toxicity of liposomal medicines [113]. An example is usage of cationic lipids in dioleoylphosphatidylethanolamine (DOPE) and dimethyldioctadecylammonium bromide (DDAB), the cell proliferation would be affected [114, 115]. Cationic liposomes can also be used as vehicle for pulmonary delivery of an anionic substance and successfully used to inject DNA into mammalian cells [114]. The liposomes with negative charge have a decreased half-life in the blood than neutral liposomes. Positively charged liposomes are recognised as more toxic and were quickly expelled from circulation [113]. Considering all these factors together, the right choice of lipid and charged particles in liposome formulation can considerably decrease the liposomal toxicity [116].
1.3.12 Antimicrobial activity of liposome–encapsulated drugs

The conventional method of detecting direct effectiveness of antimicrobial agent on microbes is either by disc diffusion or by dilution. Managing resistant pathogen is one of the biggest and difficult challenge for physicians. High level of resistance to Penicillin by organisms such as *Staphylococcus aureus* have caused the exclusion of Penicillin from list of therapeutic choices against this organism. Recent trends shows an increase in infections caused by Methicillin resistant *Staphylococcus aureus* and highly resistant strains of *Pseudomonas aeruginosa* However, Nacucchio et al. 1985, reported the antibacterial activity of piperacillin against *S. aureus* is enhanced by liposome encapsulation of the drug [117]. The study demonstrated a higher growth inhibition when Piperacillin was encapsulated into liposomes at a 50% MIC of Piperacillin. The enhanced action of liposome-encapsulated Piperacillin or Gentamicin against *P. aeruginosa* and *Escherichia coli* strains resistant to these antibiotics has been reported [118]. Similarly strains of *Pseudomonas aeruginosa* earlier resistant to Tobramycin and Ticarcillin shown susceptibility to antibiotics encapsulated in Liposomes. The effectiveness of liposome-encapsulated antibiotics against the β-lactamase producing strains and the non-β-lactamase–producing strains are found to be same.

Fluidosomes® fluid vesicles composed of DPPC/DMPG 18:1 has been shown to be integrated with the bacterial cell membrane of *P. aeruginosa* and this make it possible to directly deliver Tobramycin into the periplasmic space resulting in achieving a raised therapeutic index of tobramycin and higher antibacterial effect with a minimal drug concentration. For the eradication of drug resistant *P. aeruginosa* strains, a direct interaction between liposome and bacterial cell can be promising. Effective efflux system and decreased permeability of bacterial cell membrane are the major factors that contribute in development of bacterial resistance. The application of antibiotics in liposomal vesicles could possibly overcome bacterial resistance mechanisms [119]. A DMPC/CHOL in molar ratio 2:1 containing gentamicin formulation has shown improvement in bactericidal activity [30]. Similar MIC reduction are also achieved with DPPC/CHOL liposomes of molar ratio 2:1 comprised of amikacin, gentamicin, and tobramycin. Such experiments have also been performed with other antibiotics encapsulated in the identical cholesterol vesicle system. [120]. Four to sixteen time reduced MIC level are achieved with liposomal polymyxin B in
comparison with the free antibiotics. The usage of lipid vesicles of polymyxin B can decrease the ill effects like nephrotoxicity resulting from the systemic consumption of these medicines. Occurrence of Ototoxicity, and neuromuscular blockade are also eliminated. Liposomal form of this drug also have an enhanced antibacterial activity.

Reports using liposomes encapsulating antimicrobial agents are selected for the treatment of bacterial infections [121-124]. The successful usage of liposomes as vehicle of antibacterial molecules depends on many factors like dimension, and charge. The membrane fluidity and biodegradability of liposomes are also significant [123, 125-127].

Mulling over of the above elements, our study was intended to see the viability of liposome-exemplified antimicrobial (amikacin, ciprofloxacin, cloxacillin and vancomycin) plans against clinical disengage of ESBL positive E. coli, ESBL positive Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus and MRSA. ATCC Strains of gram negative bacilli and gram positive cocci developed in culture media. For that multilamellar liposomes were prepared and encapsulated inside the liposomes and was coated with PEG. Impact of liposomal drug activity depends on physicochemical properties of antibiotic and its targeted locus in microbial cell. Antibacterial activities of free antibiotics, liposomal and PEG coated liposomal antibiotics against 10 clinical isolates and 2 laboratory strain of E. coli ATCC 25922 and Staphylococcus aureus ATCC 29213 were selected to perform. Liposomal details were described by determination of drug loading efficiency, UV-spectrophotometer, FTIR, AFM and TEM. Besides, antimicrobial bactericidal studies were finished by plate well diffusion technique by measuring the zone size of clinical strain and ATCC strain, and looking at the action of liposomal amikacin, ciprofloxacin, cloxacillin and vancomycin with comparable centralizations of non-captured amikacin, ciprofloxacin, cloxacillin and vancomycin. Our liposomal formulation can significantly increase the zone of inhibition against resistant strain used in this study due to (i) a greater drug penetration within bacterial cells and (ii) protection against unfavourable environmental conditions. Other body sites can be reached by modulating liposome size, lipid composition, and surface characteristics.

The main objective is to develop a non-traditional delivery system of antibiotics for treatment of multidrug resistant bacterial infection and focuses on ways
to increase the efficacy of antimicrobial treatment against bacterial infection caused by drug resistant bacteria.

- To determine the prevalence of extended spectrum beta lactamase (ESBL) among *Escherichia coli* and *Klebsiella pneumoniae* and MRSA in a tertiary care hospital and to detect the in vitro antibacterial activity of Liposome containing antibiotics against resistant bacteria.
- To screen *Escherichia coli* and *Klebsiella pneumoniae* resistant to 3rd generation cephalosporins and *Staphylococcus aureus* resistant to Cefoxitin by disc diffusion method, *Staphylococcus aureus* resistant to oxacillin by Etest (gradient diffusion method).
- To confirm ESBL by double disc diffusion test, minimum inhibitory concentration (MIC) breakpoints by VITEK 2 automated instrument, and by gradient diffusion method (Etest).
- To compare different double disc diffusion methods - e.g. Ceftazidime (CAZ) + Clavulanic acid; Cefotaxime (CTX) +Clavulanic acid; Cefepime (CPM) and Ceftazidime with Augmentin [2].
- To detect resistant genes VIM, IMP, KPC, OXA48 and NDM by Cepheid Gene Xpert PCR for Carbapenemase producing *E.coli* and *Klebsiella pneumoniae*.
- To confirm MRSA by VITEK 2.
- To detect resistant genes mecA (MRSA), nuc (*S. aureus*), by Cepheid Gene Xpert PCR.
- To prepare liposome from hen’s egg yolk.
- For liposomal characterization UV, FTIR, AFM, TEM used and encapsulation efficiency were also included in this study.
- To prepare liposomal drugs (amikacin, gentamycin, cloxacillin and vancomycin) for antimicrobial studies.
- To prepare PEG coated antibiotic loaded liposomes and to detect the antimicrobial activity against drug resistant bacteria with the encapsulation efficiency.
Infection due to ESBL producers range from uncomplicated urinary tract infection to life threatening sepsis. ESBL producers are associated with increased mortality and morbidity. Organisms producing ESBLs are clinically relevant and remain an important cause for failure of therapy with Cephalosporins. MRSA isolates is a serious public health concern and its ever-increasing rate is exerting pressure on the healthcare system. At present, more than 20% of clinical S. aureus isolates in tertiary care hospitals are methicillin resistant. The application of liposomes to assist drug delivery has already had a major impact on many biomedical areas. They have been shown to be beneficial for stabilizing therapeutic compounds, overcoming obstacles to cellular and tissue uptake, and improving bio distribution of compounds to target sites in vivo. Taken together, using liposomes as drug delivery vehicles may pave the way towards developing more advanced and powerful therapeutic measures against infectious diseases especially against multidrug resistant bacteria (MDR).