INTRODUCTION

Many infectious diseases have been brought under control around the world yet microbial infections remains the leading cause of death in the world. The microbes responsible for these infections are often antibiotic resistant pathogens. The problem assessed during the present work is to carry out studies on screening of multiple drug resistant strains of bacteria causing complicated and uncomplicated bacterial infections. The current techniques useful for the functional analysis of infectious pathogens and several standard methods for screening multiple resistance as well as ESBLs have to be used.

Irrelevant prescriptions of antibiotic, incomplete dosage completion by patient are the majority of social issues making the problem more vigorous. Regardless of the drug chosen, it is important that the patient must complete the antibiotic regimen prescribed by the health care provider. In addition, the bacteria in the infected system can develop resistance to the antibiotic and can spread the resistant bacteria to members of community. If no other antibiotics are available to fight the bacteria, the consequences can be deadly for the infected individuals.

Whenever antibiotics wage war on microorganisms, a few of the enemy are able to survive the drug. Because microbes are always mutating, some random mutation eventually will protect against the drug. Antibiotics should be used only when needed and as directed should not be prescribed by the physicians unless the disease absolutely requires it. In view to come up with the problem conventional and non conventional remedies must be used together.
1. URINARY TRACT INFECTIONS (UTI)

UTIs are among the most common infections seen by physicians in the community and hospital setting affecting children and adults. 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 cultures (Dusé and Klugman, 1993). UTI are most commonly caused by various species of *Escherichia, Klebsiella, Bacillus, Enterobacter* and *Pseudomonas*. Although other organisms involved are *Enterococcus faecalis, Candida albicans* etc.

1.1. Occurrence and characterization of UTI

The infection may be community acquired or nosocomial, often as a consequence of urethral catheterization and is more prevalent in women (Farahan et al., 2004). Inadequate sanitation, catheterization, urinary tract obstruction, diabetes, pregnancy, weakened immunity etc. are the causes of acquisition of UTI and drug resistance related to it. Being the burning health issue, since 1996, FDA has actively addressed antimicrobial resistance. The chemotherapy for UTI, for uropathogens is recommended by CLSI also called as NCCLS guidelines (NCCLS guidelines 2002). Institute recommends testing of pathogen must be done as per the antibiogram using the above guidelines.
1.2. ETIOLOGY OF UTIS:

Enterobacteriaceae are endogenous to the gastrointestinal tract and are often implicated in majority of all UTIs. In hospitalised patients, the most common causative agents of UTI include opportunistic bacteria like *E. coli*, *Enterobacter*, *Klebsiella*, *Proteus*, *Serratia* and *Pseudomonas* and are also known to be resistant to most common antibacterial agents (Abigail and Dixie, 2005).

Infrequent causative agents of UTIs include bacteria such as *Staphylococcus aureus*, *Gardnerella vaginalis*, *Corynebacterium* and *Lactobacilli*, yeasts such as *Candida* sp. and viruses such as Adenovirus type 2 (associated with acute haemorrhagic cystitis in children) (Dusé and 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).

1.2.1. The Enterobacteriaceae:

The Enterobacteriaceae are a large family of bacteria, including many of the more familiar pathogens, such as *Salmonella* and *Escherichia coli*. Genetic studies place them among the *Proteobacteria*, and they are given their own order (*Enterobacterials*), though this is sometimes taken to include some related environmental samples. Members of the Enterobacteriaceae are rod-shaped, and are typically 1-5 μm in length (Ananthanarayan and Panikar, 2009). Like other *Proteobacteria* they have Gram-negative stains, and they are facultative anaerobes, fermenting sugars to produce lactic acid and various other end products. Most have many flagella used to move about, but a few genera are non-motile.
Many members of this family are a normal part of the gut flora found in the intestines of humans and other animals, while others are found in water or soil, or are parasites on a variety of different animals and plants. *Escherichia coli*, better known as *E. coli*, is one of the most important model organisms and its genetics and biochemistry have been closely studied. Most members of *Enterobacteriaceae* have peritrichous Type I fimbriae involved in the adhesion of the bacterial cells to their hosts (Cruickshank, 1978)

### 1.2.2. Genus *Escherichia:*

*Escherichia* is a genus of Gram-negative, non-spore forming, facultative anaerobic, rod-shaped bacteria from the family *Enterobacteriaceae*. *Escherichia coli* are the most numerous aerobic commensally inhabitants of the large intestine and gastrointestinal tracts of warm-blooded animals. A capsule or microcapsule is often present and a few strains produce profuse polysaccharide slime. *E. coli* was first isolated by “Theobald Escherich” in 1885 from faeces of infants. The major sources of infection are undercooked ground beef, consumption of non pasteurized milk and juice, raw sprouts, contact with infected live animals, waterborne transmission through swimming in contaminated lakes, pools, or drinking inadequately treated water and also the person to person transmission (Arya, 2005).

#### 1.2.3. Pathogenesis:

A variety of microorganisms have been implicated in UTI. Symptoms of UTI depend on whether the infection is in the lower UT (urethritis and cystitis) or in the upper UT (acute non-obstructive pyelonephritis) and are characterized by a rapid onset of dysuria, urgency and frequent micturition.
The *E. coli* serotypes commonly responsible for UTI are those normally found in faces and generally only one serotype is isolated from infected urine at a time, though recurrence may be due to different serotypes. K and O are the antigenic structures recognised for *E. coli* which decides its serotype whether an antibiotic is prescribed to treat a UTI and the choice of antibiotic will depend on several factors. These include the site of the infection (upper tract versus lower tract), the bacterium causing the infection, and any allergies the individual may have to antibiotics. Other considerations include the severity of the infection (complicated versus uncomplicated), the antibiotics used to treat previous infections, and the doctor's or health care provider's knowledge of any apparent antibiotic resistance. Most Gram-negative bacilli that causes UTI originate in the colon contaminate the urethra ascend into the bladder and most of the time migrate, to the kidney or prostate. Although the exact pathogenesis of UTI and host predispositions are not clearly understood (Murray *et al*., 1998), factors potentially contributing to the development of UTI include anatomic abnormalities, metabolic factors, hospitalization, gender and genetic factors in women (Dusé and Klugman, 1993). Populations at risk include the elderly, young adults, children and the newborn (Richmond, 1981; Sanders *et al*., 1988; Neu, 1992).

The pathogenesis of uncomplicated UTI is complex and influenced by many host biological and behavioural factors and by properties of the infecting uropathogens. Most uncomplicated UTIs in women are associated with underlying functional or anatomical abnormalities of the urinary tract, whereas sexual intercourse, spermicide use, a history of recurrent UTI and recent antimicrobial
chemotherapy are important risk factors. In some young healthy women, especially those with 'low UTI risk' behaviour, features of pelvic anatomy appear to be associated with UTI risk. In postmenopausal women, anatomical and functional characteristics of the genitourinary tract are more strongly associated with UTI risk than in younger women. A genetic predisposition to recurrent UTI is suggested by the association of recurrent UTI in certain age groups with the ABH blood group non-secretor phenotype, a maternal history of UTI and early age at onset of UTI. Virulence determinants of uropathogens are much more important in the normal host than in the host who has a functional or anatomical abnormality of the genitourinary tract (Hooton, 2000). *E. coli* O157:H7 was first recognized as a pathogen as a result of an outbreak of unusual gastrointestinal illness in 1982.

The outbreak was traced to contaminated hamburgers, and the illness was similar to other incidents in the United States and Japan. *E. coli* O157:H7 serotypes are closely related, descended from a common ancestor, divergent in plasmid content more than chromosomal content, and are no more related to other shiga toxin producing strains than any other randomly chosen *E. coli* serotype. *E. coli* O55:H7 and *E. coli* O157:H7 are most closely related and diverged from a common pathogenic ancestor that possessed the ability to form attaching and effacing lesions. *E. coli* O157:H7 serotypes apparently arose as a result of horizontal gene transfer of virulence factors. Various toxins produced by *E. coli* are CFAT, LT, ST, SLT and VT which are involved in various types of infections of *E. coli*.
1.2.4. Treatment and curative measures:

   a. Precautionary measures:

   Some precautions can be taken in order to prevent the infection spread. Extended Spectrum Beta Lactamases (ESBL) producing *E. coli* infections may arise from germs in the person’s own bowel, and will not transmit directly to cause a urinary tract infection in others. So the key to reducing the opportunity for *E. coli* ESBL to make people ill is to reduce the circulation of this germ in the healthy community by everyday ‘clever cleaning’.

   These can be for all patients, at all times regardless of setting e.g. rest home, clinics, inpatient areas, primary care areas etc. this prevents the transmission from patient to staff, from staff to patient and covers possibility of unrecognised carriage with a multi-resistant organism. Hand hygiene like use of soap & water or alcohol gel/rub before & after direct patient contact, after removing gloves, improved adherence with hand hygiene has reduced the transmission. Gloves, aprons, eye protection units etc. must be used while handling non-intact skin, mucous membranes, blood & body fluids. Re-usable equipment decontamination and environmental cleaning must be done.

   Devices like catheters must be detached after recommended use to minimize duration of exposure and catheter-care protocols must be followed. Antibiotics use must be done wisely to treat infection not colonisation.
**b. Drugs / antibiotic classes used to treat UTI:**

1. **Aminoglycosides:** Amikacin Sulfate Injection (Amikin), Gentamicin Sulfate Injection (Garamycin Injection), Tobramycin Sulfate Injection (Nebcin), Nitrofurantoin


3. **Quinolones:** Ciprofloxacin Injection (Cipro Injection), Ciprofloxacin XR (Cipro XR), Ciprofloxacin tablets (Cipro), Gatifloxacin Injection (Tequin IV), Gatifloxacin tablets (Tequin tablets), Levofloxacin Injection (Levaquin Injection) Levofloxacin Tablets (Levaquin), Ofloxacin (Flxin), Ofloxacin Injection (Flxin IV)

4. **Sulfonamides and Related Compounds:** Sulfamethoxazole (Gantanol), Sulfamethoxazole and Trimethoprim (Bactrim, Bactrim DS, Bethaprim, Co-trimoxazole, Cotrim, Cotrim DS, Septra, Septra DS, Sulfatrim), Septra Injection, TMP-SMX Injection, Co-trimoxazole injection), Sulfamethoxazole; Trimethoprim, SMX-TMP Oral Suspension (Co-Trimoxazole Oral Susp, Septra Oral Susp, Sulfatrim Oral Susp) , Trimethoprim: Trimethoprim (Primsol, Proloprim, Trimpex)
1.3. Isolation and identification of clinical isolate:

Since microbes are present everywhere, the unwanted microorganisms may be introduced into the culture media (in petri plates and test tubes) through the air or by direct contact with contaminated surfaces. The aseptic transfer of microorganisms involves the transfer of microbes from one container to another container without contamination. The urine culturing for the confirmation of UTI, isolation of pathogen and the determination of invitro Resistogram are the most recommended practices by NCCLS (NCCLS., 1997- 2010).

Importance of isolation:

1. This technique helps to separate the organism on a given nutrient medium.
2. The organism can be studied well after isolation.
3. The isolated organism can be studied further by their biochemical reactions on various biochemical properties exhibited by them.

Culturing microorganisms: Most bacteria can be cultured artificially on culture media containing required nutrients, pH and osmotic pressure. The microorganisms grow in an atmosphere and temperature most suited to their metabolic reactions. The pathogens are isolated in pure culture so that they can be identified and also tested for their sensitivity to antimicrobials. The specimens are cultured in known volumes, and the number of bacterial colonies appearing after incubation can be counted. Operation room requirements and blood from blood bank frequently checked for sterility by using pure culture methods.
1.4. **Antibiotics:**

An antibiotic is a chemical compound that inhibits or abolishes the growth of microorganisms, such as bacteria, fungi, or protozoans. The original meaning of antibiotic includes any agent with biological activity against living organisms; however, the term is commonly used to refer to substances with anti-bacterial, anti-fungal, or anti-parasitical activity.

1.4.1. **Definition:**

The first antibiotic compounds used in modern medicine were produced and isolated from living organisms, for example, the penicillin class produced by fungi in the genus *Penicillium*, or *streptomycin* from the genus *Streptomyces*. With the advent of organic chemistry many antibiotics are now also obtained by chemical synthesis, such as the sulfa drugs. Many antibiotics are relatively small molecules with a molecular weight less than 2000 Da.

1.4.2. **The Basic Characteristics of Antibiotics:**

Today, there are about 4000 compounds with antibiotic properties. Antibiotics are used to treat and prevent infections. Antibiotics are derived from three sources: moulds or fungi; bacteria; or synthetic or semi-synthetic compounds. They can be used either internally or topically, and their function is to either inhibit the growth of pathogens or to kill them. Antibiotics can thus be divided into Bacteriostatic drugs, which merely inhibit the growth of the pathogen, and Bactericidal drugs, which actually kill the bacteria where distinction depends on the drug concentration, the bacterial species, and the phase of growth. Antibiotics are more effective against actively growing bacteria. Antibiotics can also be divided into
broad-spectrum and narrow-spectrum antibiotics. Antibiotics fight against bacteria by inhibiting certain vital processes of bacterial cells or metabolism (Dzidic et al. 2008).

1.4.3. Classification of antibiotics as per their mechanism of action:

1. Cell wall inhibitors, such as Penicillin and Vancomycin.
2. Inhibitors of nucleic acid synthesis, as Fluoroquinolines as DNA synthesis inhibition and Rifampin as RNA synthesis inhibition.
3. Protein synthesis inhibitors, such as Aminoglycoside.
4. Anti-metabolites, such as the Sulfa drugs.
5. Antibiotics that can damage the cell membrane, such as Polymyxin B and Gramicidin.

1.4.4. Antibiotic resistance:

Inappropriate, incomplete or misuse of antibiotics may result in the development of antibiotic resistance by the infecting organisms, similar to the development of pesticide resistance in insects. Evolutionary theory of genetic selection requires that as close as possible to 100% of the infecting organisms be killed off to avoid selection of resistance; if a small subset of the population survives the treatment and is allowed to multiply, the average susceptibility of this new population to the compound will be much less than that of the original population, since they have descended from those few organisms which survived the original treatment. This survival often results from an inheritable resistance to the compound which was infrequent in the original population but is now much more frequent in the descendants thus selected entirely from those originally infrequent resistant organisms.
1.4.5. Beta Lactam Antibiotics:

β-lactams belong to a family of antibiotics which is characterized by a β-lactam ring. Penicillins, cephalosporins, clavams (or oxapenams), cephemycins and carbapenems are members of this family. The integrity of the β-lactam ring is necessary for the activity which results in the inactivation of a set of transpeptidases that catalyze the final cross-linking reactions of peptidoglycan synthesis. The beta-lactams get their name from the characteristic ring structure — shown here in blue — that they all share. (The green arrow shows the bond that is broken by the beta-lactamases that are synthesized by many penicillin-resistant bacteria.)

1. **Penicillins:**

Penicillin G (a natural product produced by the fungus *P. chrysogenum*), Ampicillin (a semi-synthetic) and Amoxicillin (a semi-synthetic)

2. **Cephalosporins**

There are over two dozen of them in current use. Most are semi-synthetics derived from the secretion of the fungal sp. *Cephalosporium*. Some examples: Cephalexin (e.g., Keflex), cefixime (e.g., Suprax) Carbapenems such as Meropenem (Merrem®), Ertapenem (Invanz®)
1.4.6. **Molecular mechanism of antimicrobial activity:**

The beta-lactams all work by interfering with the synthesis of the bacterial cell wall — a structure that is not found in eukaryotes. The walls of bacteria are made of a complex polymeric material called peptidoglycan. It contains both amino acids and amino sugars. The amino sugars are of two kinds

1. N-acetylglucosamine (NAG) and its close relative

2. N-acetylmuramic acid (NAM).

These two form a linear polymer of NAG alternating with NAM. They are linked by a **glycosidic bond** between the #1 and #4 carbons (this is the linkage attacked by lysozyme) and are oriented in the same way they are in cellulose. Side chains containing 4 or 5 amino acids are attached to each NAM. These form covalent bonds with amino acids in adjacent chains. The bonds may be direct to the next chain or include additional **peptide cross bridges** (e.g., 5 glycine residues) which extend to chains in the same plane (shown here) as well as to chains above and below. This elaborate, covalently cross-linked structure provides the great strength of the cell wall.

The beta-lactam antibiotics bind to and inhibit enzymes, transpeptidases (penicillin binding proteins PNBs), needed for the synthesis of the peptidoglycan wall. In this stage the linear glycan strands are cross linked via their peptide chains to the cell wall. The transpeptidase enzymes are located on the outer face of the cytoplasmic membrane. They first remove the terminal D-alanine residue from each pentapeptide on the linear glycan. This reaction involves breakage of the peptide between the two D-alanine residues on the linear glycan. In *E. coli* this acceptor is the free amino group
on meso-diaminopimelic acid. Thus the beta lactam antibiotics effectively inhibit the transpeptidase by acting as alternative substrates. They mimic the D-alanyl-D-alanine residues and react with the transpeptidases.

The beta lactam bond is broken (instead of the equivalent peptide bond joining the alanine residues) but the remaining ring system in the beta lactam (a thiazolidine in penicillins) is not released. Instead, the transpeptidase remains linked to the hydrolysed antibiotic with a half life of 10-15 minutes. Whilst bound to the beta lactam, the transpeptidase cannot participate in further rounds of peptidoglycan cross linking reaction by reaction with its true substrate. Thus penicillins have little effect on resting bacteria; they are lethal to dividing bacteria as defective walls cannot protect the organism form bursting in hypotonic surroundings (Hugo and Russel, 1984).

1.4.7. Resistance to beta lactam antibiotics:

In Multiple Antibiotic Resistance, transmissible antibacterial resistance by means of plasmids, transposons or integrons as horizontal and vertical gene transfer, is the major cause of concern as it can lead to the rapid spread of antibiotic resistance in pathogen and has proven difficult to eradicate (Belkum et al. 2001; Roy 1993; Salem 2010). Mainly the plasmids are responsible for drug resistance carriage. They are R plasmids which carry gens for resistance to antibiotics. Other plasmids enhancing the pathogenicity of MDR organism are F plasmid (genetic transfer by conjugation), Col plasmid (colicin production) and virulence plasmids (Ti and Ri). Many plasmids are conjugative or transmissible as all F plasmids,
many R plasmids and some Col plasmids. Though some R plasmids are non transferable but are mobilized with a conjugative plasmids as “nic bom” site dependency (Singh, 2009). This may be visualized as being constructed from modular DNA segments viz. replication module, sex factors, R-determinative module, Col module, modules specifying restriction modification systems, I S elements etc. The multidrug resistance of the bacteria either inherent or opted by plasmid collectively contributes to their survival and virulence. The virulence factors associated with multidrug resistance includes serum resistance, atypical biochemical properties, production of colicin (Harnett and Gyles, 1984), haemolysin and aerobactin etc. The findings that virulence factor attainment can be encoded by antibiotic resistant plasmid are consistent with the increasing incidence of antibiotic resistance in pathogenic bacteria (Arya, 2005).

Resistance to β-lactams in clinical isolates is primarily due to the hydrolysis of the antibiotic by a β-lactamase (penicillanase in case of degradation of antibiotic penicillin). Mutational events resulting in the modification of PBPs (penicillin binding proteins) or cellular permeability can also lead to β-lactam resistance. β-lactamases constitute a heterogenous group of enzymes. Several classification schemes have been proposed according to their hydrolytic spectrum, susceptibility to inhibitors, genetic localisation (plasmid or chromosomal), gene or amino-acid protein sequence.
1.5. **Antimicrobial Susceptibility testings:**

**Agar diffusion method/ Disk diffusion method:**

Also called as Kirby- Bauer method, uses filter paper discs impregnated with antimicrobial agents. A petri plate is inoculated with a test bacterium. Discs impregnated with known quantities of antimicrobial agents, are placed on inoculated agar plate surface. Drug from the disc diffuses through the agar medium. When the plate has been inoculated long enough for bacteria to produce a confluent lawn of growth, results can be interpreted. Some of the drug discs will have clear halo. That’s where the bacterial growth is inhibited by the antimicrobial agent. The more sensitive the organism is the larger the halo will be, so the size of each halo will be measured and compared to susceptibility standards for each drug. Based on these comparisons, the microorganism is considered to be sensitive, intermediate or resistant to each drug (Baker and Breach 1980).

These results can be extremely useful. Knowing whether an organism is sensitive, intermediate or resistant to various antimicrobial agents is almost always enough information to choose the proper drug and its dosage. More ever, disc- diffusion tests are technically easy and relatively inexpensive to perform. It is by far the most common type of susceptibility test used in clinical medicine.
1.6. Functional analysis of Multiple Drug Resistant Pathogens

1.6.1. Virulence factors contributing the pathogenicity:

As *Escherichia coli*, causes majority of UTI its subsets which are identifiable using O, K and H antigens have increased ability to cause symptomatic urinary infections. Recent studies confirm that uropathogenic *E. coli* have several attributes that are lacking in the commensal *E. coli*. They carry chromosomal gene clusters on ‘pathogenicity islands’, encoding adhesins and other virulence factors.

These virulence factors may act independently or their actions may be complementary to each other (Mishra *et al.*, 2001; Taylor, 1983). The findings that serum resistance could be encoded by antibiotic resistant plasmid is consistent with the increasing incidence of antibiotic resistance in pathogenic bacteria and the tendency for genetic determinant of a variety of pathogenicity functions to be plasmid born. There are associated virulence factors are there as Colicinogeny, Haemolysin production, production of heat labile (LT) and heat stable (ST) enterotoxin (Arya, 2005) resistance to normal Human serum, Haemagglutination (Srikanth and Makaden, 2003), Hbp production, Aerobactin production etc.

The most important amongst these, probably, are the adhesins that help them to adhere to uroepithelium and this property was recognized decades ago. These include type 1, S and P fimbriae, and adhesins like Dr1. The type 1 fimbriae are widely prevalent and are probably involved in colonization of lower urinary tract. Mannose-sensitive haemagglutination (MSHA) denotes presence of these fimbriae.
The role of P fimbriae in upper UTI are encoded by the pap operon and are present in faecal and pyelonephritis causing \textit{E. coli} isolates. It is shown that some pap positive isolates, especially those isolated from asymptomatic infections, do not express P fimbriae. Phenotypic expression of P fimbriae can be detected by mannose-resistant haemagglutination (MRHA) of human erythrocytes. Attachment of P fimbriae is also associated with increased host inflammatory response.

Other factors associated with uropathogenic \textit{E. coli} include production of haemolysin, serum resistance and release of aerobactin. Haemolysin provides \textit{E. coli} with possible selective advantage by releasing iron from lysed erythrocytes and enhances pathogenicity by destroying phagocytic and epithelial cells. The cytolytic protein toxin secreted by most haemolytic \textit{E. coli} strains is alpha-haemolysin. \textit{E. coli} also produces cell associated lysin on blood agar plates. In the present study, though the nature of haemolysin was not further characterized it can be considered as cytotoxic necrotising factor (haemolysin) (Raksha 2003). Measuring a phenotype in vitro does not always correlate with in vivo expression and may underestimate the presence of a virulence factor in vivo. Identifying a genotype, on the other hand, does not mean that it is expressed in the body. However, MRHA can be used for presumptive identification of virulence factors in \textit{E. coli} (Katouli et al., 2005). The distribution of virulence properties can also vary depending on host characteristics and type of infection. There are however, very few reports in the literature, where, phenotypic expression of virulence factors in \textit{E.coli} and antibiogram has been compared in isolates from different patient groups (Moreno et al.2005). The present study was therefore undertaken to determine differences if any, in the presence of
phenotypically expressed virulence factors like P-fimbriae, type 1 fimbriae and haemolysin among *E. coli* causing urinary infections in three different groups of patients. Antibiogram was also recorded to determine differences, if any, between the groups. (Naveen and Mathai, 2005)

Endotoxin production was very common in case of *E. coli*. The drug resistance and endotoxin production can exaggerate the bacterial virulence and the total consequences of UTI. Endotoxin is used to detect or quantify bacterial endotoxin that may be present in or on sample of articles to which the test is applied. It uses Limulus Amebocyte Lysate (LAL) obtained from the horse shoe crab (*Limulus polyphemus* or *Tachyppus trientatus*) which has been prepared and charcterized for use as an LAL reagent.

LaL reagent (contains bivalent ions, procloting enzyme system and clottablle protein) when reacts with endotoxin gives gel formation, turbidity or precipitation. The gel formation indicates the presence of endotoxin in the sample. The rate of reaction depends upon the concentration of endotoxin.

Some strains of *E. coli* produce a substance called colicin that kills competing strains. The producing strains are immune to the action of this chemical but reproduce at a reduced rate because some of their metabolic energy is devoted to its production. Serum resistance or Serum killing power could be mediated through O-antigen polysaccharides and outer membrane lipoproteins. Bacterial resistance to lethal activity of serum is thought to contribute the pathogenicity of some invasive bacteria. The resistance to the bactericidal activity is considered to be an important virulence factor of proteus sp. In particularly critical cases, a serum killing power test
may be done. Some of the patients own drug containing blood is withdrawn and tested to see if it kills the infecting microorganisms.

1.6.2. Atypical properties exhibited by the pathogens:

As the degree of acquisition of drug resistance increases the chances of occurrence of such mobile elements will be there in the bacterial cell and hence the more will be the versatility in their biochemical, physiological, habitual and cellular properties (Avison et al., 2004). Atypical biochemical behaviour of *E. coli* strains isolated from diseased conditions has been reported. Often these characters were found transmissible along with drug resistance, as reports indicate. (Arya, 2005), Hence it is possible to use this unusual biochemical behaviour as an epidemiological tool in characterizing the isolates from disease outbreaks. Some atypical biochemical characters and can be listed as Production of H$_2$S, Production of Urease, Ability to utilize citrate, Adonitol and Raffinose etc.

1.7. Minimum Inhibitory concentration:

MIC (Minimum Inhibitory concentration): MIC, in microbiology, is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism.
1.7.1. Types of MIC:

MICs can be determined by agar or broth dilution methods usually following the guidelines of a reference body such as the CLSI, BSAC or EUCAST. There are several commercial methods available including the well established E test strips and the recently launched Oxoid MIC Evaluator method. The E test system comprises a predefined and continuous concentration gradient of different antimicrobial agents, which when applied to inoculated agar plates and incubated, create ellipses of microbial inhibition. The MIC is determined where the ellipse of inhibition intersects the strip, and is easily read off the MIC reading scale on the strip. Clinically, the minimum inhibitory concentrations are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents.

1.7.2. Agar plate method:

After determining the Total Viable Count was made using various dilutions of pure cultures in sterile saline, the dilution which gives countable colonies was selected. Wells were made using the vacuum based well maker on nutrient agar plate already seeded with the selected dilution of the clinical isolate and the variable concentrations of antibiotics were added into the well. Each plate was incubated at $37^\circ$ C for 24 hrs and then examined for zones of inhibition. The lowest concentration of each antibiotic, which inhibited growth, was taken as the MIC.

1.7.3. Broth Microdilution method:

The Broth Microdilution method is more complicated and expensive than disc diffusion method, but it yields more precise results for critically ill patients. This method tests the drugs ability to
prevent the growth of bacteria in liquid medium. More information can be obtained by taking bacteria from clear tubes and attempting to grow them on a drug free petri plates. If organism does grow on the petri plate the tube from which the bacteria were taken contained a bacteriostatic concentration of the drug. The tube with the lowest bactericidal concentration contains the minimum Bactericidal concentration (MBC) of the drug.

Knowing the MIC and MBC gives the valuable information to the clinician. The MIC and MBC can be compared to serum drug levels to see the inhibitory or bactericidal concentrations are being reached in the patient’s body. In addition, relative values of MIC and MBC provide the information about the drug itself. If the MBC is much higher than the MIC, the drug is bacteriostatic. But if both the values are about the same, the drug is bactericidal. Many infections can be treated effectively by the bacteriostatic drugs because they tip the balance in the favour of patient’s immune system, but a person with a weak immune system may need a bactericidal drug.

1.8. **Extended Spectrum Beta Lactamases (ESBLs):**

Beta-lactamases are bacterial enzymes that inactivate beta-lactam antibiotics. Beta-lactamases that inactivate all the penicillins and cephalosporins including the extended spectrum cephalosporins are termed Extended Spectrum Beta-Lactamases, abbreviated as ESBLs. Beta-lactamases are bacterial enzymes that inactivate beta-lactam antibiotics. Beta-lactamases that inactivate all the penicillins and cephalosporins including the extended spectrum cephalosporins are termed Extended Spectrum Beta-Lactamases, abbreviated as ESBLs. They gives the structural and resistotypical relation of any antibiotic for the particular pathogen and give the idea about the
resistotyping of pathogen and the relative sensitivity or resistance prediction probably shown by the pathogen.

ESBL testing helps the physician to prescribe the perfect medication for the pathogen and thus to avoid the unnecessary exposure of other antibiotics to whom the pathogen is relatively resistant (Kumarasamy et al., 2010). There are over 150 different ESBLs described all of which are mutations of the classical broad-spectrum beta lactamase enzymes that were initially named TEM and SHV (TEM-1, TEM-2, SHV-1). ESBL’s are named TEM, -4 etc., SHV-2, -3 etc., CTXM-1, -2 etc., OXA-1, -2 etc. ESBLs hydrolyze penicillins, cephalosporins and the monobactam, conferring resistance to all of these drug classes. They do not hydrolyse the Cephamycin antibiotics (i.e. Cefoxitin), which are close relatives to the cephalosporins. ESBL’s are also inhibited by beta-lactamase inhibitors such as clavulanate, sulbactam and tazobactam. ESBLs are generally inactive against the carbapenem antibiotics (Imipenem, Meropenem, Ertapenem).

The isolates showing resistance to one of the three C’s (Ceftazidime, Ceftriaxone, Cephotaxime) were screened for ESBL Detection using the kit provided by Himedia Ltd. Using ceftazidime and ceftazidime Clavulanic acid and cefotaxime and cefotaxime Clavulanic acid. The test was done as per the manufacturer’s protocol. TDEM, Disk Potentiation test was done to confirm the ESBL nature of the clinical isolate using ceftazidime, cefotaxime and their combinations with Clavulanic acid. That can be also detected by oxide disc method (Carter et al. 2000), Etest ESBL and the BD Phoenix, VITEK 1, and VITEK 2 Automated Instruments (Maurine et al.2002)
1.8.1. Occurrence of ESBLs

Members of the family Enterobacteriaceae commonly express plasmid-encoded β-lactamases i.e. Extended spectrum beta lactamase (ESBL) (e.g., TEM-1, TEM-2, and SHV-1) which confer resistance to penicillins but not to expanded-spectrum cephalosporins. In the mid-1980s a new group of enzymes, the extended-spectrum β-lactamases (ESBLs), was detected (Chaudhary and Aggarwal, 2004). ESBLs are beta-lactamases that hydrolyze extended-spectrum cephalosporins with an oxyimino side chain. These cephalosporins include cefotaxime, ceftriaxone, and ceftazidime, as well as the oxyimino-monobactam aztreonam. ESBLs confer resistance to expanded-spectrum cephalosporins (e.g. ceftriaxone, cefotaxime, and ceftazidime), aztreonam, and related oxyimino-beta lactams. Typically, they derive from genes for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these β-lactamases.

This extends the spectrum of β-lactam antibiotics susceptible to hydrolysis by these enzymes. An increasing number of ESBLs not of TEM or SHV lineage have recently been described. The ESBLs are frequently plasmid encoded. Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes as aminoglycosides. Therefore, antibiotic options in the treatment of ESBL-producing organisms are extremely limited. Carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms, yet carbapenem-resistant isolates have recently been reported. ESBL-producing organisms may appear susceptible to some extended-spectrum cephalosporins. However, treatment with such antibiotics has been associated with high failure rates.
1.8.2. Treatment of ESBLs

Generally, an isolate is suspected to be an ESBL producer when it shows in vitro susceptibility to the second generation cephalosporins (Cefoxitin, Cefotetan) but resistance to the third generation cephalosporins and to aztreonam. Moreover, one should suspect these strains when treatment with these agents for Gram negative infections fails despite reported in vitro susceptibility. Once an ESBL producing strain is detected, the laboratory should report it as “resistant” to all penicillins, cephalosporins, and aztreonam, even if they test as susceptible. Associated resistance to Aminoglycosides and Trimethoprim-sulfamethoxazole as well as high frequency of co-existence of Fluoroquinolone resistance creates problems. Beta-lactamase inhibitors such as clavulanate, Sulbactum or Tazobactam in vitro inhibit most ESBLs, but the clinical effectiveness of beta-lactam antibiotic /beta-lactamase inhibitor combinations cannot be relied on consistently for therapy. Currently, carbapenems are generally regarded as the preferred agent for treatment of infections due to ESBL-producing organisms. Carbapenems are resistant to ESBL-mediated hydrolysis.

1.8.3. Inducible nature of ESBLs:

Inducible expression of the AmpC gene occur when the enzyme is produced at a high level when the organism is exposed to inducing agents, such as cephamycins (i.e. cefoxitin), ampicillin and carbapenems (i.e. Imipenem, Meropenem, Ertapenem). Induction is temporary and may be reversed when the antibiotic inducer is removed. In some organisms, mutations occur that cause the ampC gene to become permanently expressed at high levels. Plasmid-
mediated AmpC beta-lactamases can be found in organisms that do not carry the chromosomal AmpC. Plasmid-mediated AmpC’s have been detected in organisms such as *E. coli, Klebsiella sp, Proteus sp* and *Salmonella sp*.

1.8.4. Methods to determine ESBLs

An ESBL confirmatory test involves testing cefotaxime and ceftazidime alone and in combination with clavulanate. Clavulanate inhibits the activity of the ESBL enzyme and makes the organisms appear more sensitive to drug + clavulante combinations.

This “greater sensitivity” with clavulanate can be demonstrated when the disk containing clavulanate has a zone diameter that is ≥ 5 mm larger that the zone diameter of the drug tested alone, or when a zone of enhanced sensitivity is observed when a cephalosporin antibiotic is placed in close proximity to a clavulanic containing disk. This is commonly referred to as the “keyhole phenomenon.”

Various phenotypic methods for determining ESBL includes Standard Disk Diffusion Method (SDDM)/ Disc Approximation Method (DAM), Three-Dimensional Extract Method (TDEM), Phenotypic Confirmatory Disk Diffusion Method (PCDDM), Disk Potentiation method: (DPM) etc. Computer based methods like BD-Phoenix are also available.

1.8.5. Classification of ESBLs:

The ESBLs are frequently plasmid encoded. Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes (for example, Aminoglycosides). Therefore, antibiotic options in the treatment of ESBL-producing
organisms are extremely limited. Carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms, yet carbapenem-resistant isolates have recently been reported. ESBL-producing organisms may appear susceptible to some extended-spectrum cephalosporins (Ramainoane, 2005). However, treatment with such antibiotics has been associated with high failure rates.

1. Class A/ I type of beta lactamases:

   a. TEM beta-lactamases (class A)

   TEM-1 is the most commonly encountered beta-lactamase in gram-negative bacteria. Opening the active site to beta-lactam substrates also typically enhances the susceptibility of the enzyme to b-lactamase inhibitors, such as Clavulanic acid.

   b. SHV beta-lactamases (class A)

   The SHV-1 beta-lactamase is responsible for up to 20% of the plasmid-mediated Ampicillin resistance in this species. ESBLs in this family also have amino acid changes around the active site, most commonly at positions 238 or 238 and 240. (Hæggman et al.)

   c. CTX-M beta-lactamases (class A)

   These enzymes were named for their greater activity against cefotaxime than other oxyimino-beta-lactam substrates (eg, ceftazidime). Rather than arising by mutation, they represent examples of plasmid acquisition of beta-lactamase genes normally found on the chromosome of Kluyvera species, a group of rarely pathogenic commensally growing organisms.
2. Metallo beta lactamases:

These are the zinc dependent beta lactamases which are observed in various genetic configurations. The recent threat of ESBL pandemic was caused by this class of beta lactamases and the gene was named as NMD1. (Kumarasamy, 2010).

   a. IMP-type (Imipenam resistant eta-lo-beta-lactamases): Plasmid mediated IMP-type carbapenemases, 17 varieties of which are currently known, became established in Japan in the 1990s in both enteric gram-negative organisms and in Pseudomonas and Acinetobacter species. IMP enzymes spread slowly to other countries in the Far East, were reported from Europe in 1997, and have been found in Canada and Brazil.

   b. VIM (Verona integron-encoded metallo-b-lactamase): A second growing family of carbapenemases, the VIM family, was reported from Italy in 1999 and now includes 10 members, which have a wide geographic distribution in Europe, South America, and the Far East and have been found in the United States.

3. Amp C beta lactamases (Class III):

   Amongst the mechanisms of resistance to third generation cephalosporins, production of ESBLs and AmpC b-lactamases are the most common. AmpC b-lactamases are clinically important because they confer resistance to narrow-, expanded-, and broad-spectrum cephalosporins, beta lactam- b-lactamase inhibitor combinations and aztreonam. AmpC beta-lactamases differ from ESBLs in that they are cephalosporinase and are resistant to beta-lactamase inhibitors. They hydrolyze the cephemycins (eg. Cefoxitin) but not the 4th generation
cephalosporins (eg. Cefepime). AmpC is normally produced in low levels by many organisms and is not associated with resistance, but it can be produced at high levels and cause resistance. High-level production of AmpC usually causes resistance to all beta-lactams, except carbapenems and 4th generation cephalosporins. These enzymes are typically associated with multiple antibiotic resistances, leaving a few therapeutic options (Taneja et al., 2008). AmpC β-lactamases, in contrast to ESBLs, hydrolyse broad and extended-spectrum cephalosporins (cephamycins as well as to oxyimino-β-lactams) but are not inhibited by β-lactamase inhibitors such as Clavulanic acid.

4. **OXA beta-lactamases (class D)**

OXA beta-lactamases were long recognized as a less common but also plasmid-mediated beta-lactamase variety that could hydrolyze oxacillin and related anti-staphylococcal penicillins. These beta-lactamases differ from the TEM and SHV enzymes in that they belong to molecular class D and functional group 2d. The OXA-type beta-lactamases confer resistance to Ampicillin and Cephalothin and are characterized by their high hydrolytic activity against oxacillin and Cloxacillin and the fact that they are poorly inhibited by Clavulanic acid. Amino acid substitutions in OXA enzymes can also give the ESBL phenotype. While most ESBLs have been found in *E. coli*, *K. pneumoniae*, and other Enterobacteriaceae, the OXA-type ESBLs have been found mainly in *P. aeruginosa*.

1.9. **Enzyme beta lactamases:**

Beta lactamases are the enzymes (EC 3.5.2.6) are produced by some bacteria. They are responsible for their resistance to Beta
Lactam antibiotics like Penicillins, Cephalosporins, Cephamycins and Carbapenams (Smith and Hamilton, 1979). The classification initially introduced by Ambler (1980) and based on the amino-acid sequence recognizes four molecular classes designated A to D. Classes A, C, and D gather evolutionarily distinct groups of serine enzymes, and class B the zinc-dependent ("EDTA-inhibited") enzymes.

This antibiotic resistance is expressed mainly by coding Specific or ESBLs. ESBLs are enzymes that mediate resistance to extended spectrum Penicillins, Cephalosporins and Monobactams, Cephalosporins, Cephamycins etc. These antibiotics have a common element in their molecular structure: a four-atom ring known as a beta-lactam. The lactamase enzyme breaks that ring to open, deactivating the molecule's antibacterial properties. Beta-lactam antibiotics are typically used to treat a broad spectrum of gram positive and gram-negative bacteria. Beta-lactamases produced by gram-positive organisms are usually secreted.

1.9.1. The enzyme Penicillinase:

Penicillinase is a specific type of β lactamase, showing specificity for penicillins, again by hydrolysing the beta-lactam ring. Molecular weights of the various penicillinases tend to cluster near 50,000. Penicillinase was the first β-lactamase to be identified: it was first isolated by Abraham and Chain in 1940 from gram-negative E. coli even before penicillin entered clinical use but penicillinase production quickly spread to bacteria that previously did not produce it or only produced it rarely.

Efflux pumps are transport proteins involved in the extrusion of toxic substrates (including all classes of antibiotics) from cells into
the external environment. In many cases, efflux pump genes are part of an operon, with a regulatory gene controlling expression. Increased expression is associated with resistance to the substrates (Webber and Piddock, 2003). Bio membranes constitute efficient barriers towards hydrophilic molecules, most of which can penetrate cells only by specific inward transport systems or find their entry restricted to the endocytic pathway. There are many devised mechanisms to protect cells from the disordered invasion by amphiphilic molecules, many of which are endowed with biological activities leading to potentially harmful effects.

A major mechanism in this respect is constituted by active outward transport. Although efflux systems have been known for many years, their importance, both in terms of number and variety of substrates, has become clearly recognized only very recently. Drugs are often amphiphilic, whether by selection or by design, ensuring their wide tissue distribution and/or their penetration into membrane-protected compartments. Therefore, it comes as no surprise that many drugs should fall into this category of exogenous compounds for which efflux mechanisms, globally referred to as ‘drug efflux pumps,’ are numerous and fairly active.

Drug efflux, indeed, decreases the load on enzyme-mediated detoxification systems, thereby avoiding their saturation, while chemical modifications by the enzyme-based systems, which usually increase the amphiphilicity of drugs, provide drug pumps with better substrates. Most drug efflux pumps have broad substrate specificity and, therefore, may deal with a wide range of drugs of completely unrelated pharmacological classes. (Bambeke et al., 2000)
2.0. Molecular characterization of ESBL producing *E. coli* Y3:

The gram negative bacteria have been showing slow but steady accumulation and dissemination of multiple antibiotic resistances are due to plasmids, transposons and integrons. Many R genes in Enterobacteriaceae are on large, transferable extrachromosomal DNA elements, plasmids on which may be other mobile elements, transposons code for single resistance character. Integrons contain one or more antibiotic resistance gene which is present as a mobile gene cassette, inserted in various arrangements between two conserved DNA regions (Roy, 1995.)

Many bacteria become resistant to antibiotics by acquiring genes from plasmids or transposons via horizontal gene transfer. The combined effects of fast growth rates, high concentrations of cells, genetic processes of mutation and selection, ability to exchange genes mainly account for the extraordinary rates of adaptation (acquiring resistance) and rapid evolution that can be observed in the bacteria. Plasmids and transposons coding multiple drug resistance often possess another genetic element, i.e. an integron that contain one or more antibiotic resistance genes, which are present as a mobile gene cassette (Paul et al., 1995). The existence of integron and integron-associated genes explains how plasmids may accumulate a diversity of resistance genes.

The ability of plasmids to transfer genes from one cell to another was first discovered in the 1950s by Joshua Lederberg and Edward Tatum in studies on a particular *E. coli* plasmid. Large plasmids such as resistance transfer factor (80kb) can often mediate their own transfer from one cell to another by a process called
conjugation. Conjugative plasmids that encode resistance to one or more antibiotics are of particular significance for understanding the genetic basis of antibiotic resistance. Strains of *K. pneumoniae* and *E. coli* resistant to newer β-lactam antibiotics where mechanism of resistance involved is due to a new transferable, plasmid mediated β-lactamases, have been described.

At elevated temperature the 3D structure of both proteins and nucleic acids were distorted. A macromolecule in a disrupted state in which the molecules are in a nearly random coil confirmation is said to be denatured. The ordered state which is presumably originally present in nature is called native. A transition from native to denatured state is called denaturation. When DNA (ds) or native DNA is heated the bonding factors between the strands are disrupted and two strands are separated thus denatured DNA is single stranded. A great deal of information about structure and stabilizing interaction has been obtained by studying nucleic acid denaturation. This denaturation of DNA can be brought about in solution by physical/chemical treatment such as titration with acid or alkali, biological such as using helix destabilizing or melting proteins and by physical methods as heat and mechanical shearing.

DNA denaturation can be accomplished by heating a DNA solution so that a graph called as melting curve is obtained. A melting curve is obtained by heating DNA solution at different temperature and measuring the absorbance of heat treated DNA molecule at 260 nm. The temperature at which the rise in absorbance at 260 nm is half complete is known as melting temperature and designated as Tm.
3.0. Putative Herbs and herbal analysis:

From earliest times itself, plants were used for treatment of disease without knowledge about the compounds present and their mode of action. Over the centuries societies around the world have developed their own tradition to make sense of medicinal plants and their uses. The widespread use of herbal remedies and health care preparations obtained from commonly used traditional herbs and medicinal plants have been raised due to the occurrence of natural products with medicinal properties. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has also increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections occur in hospitals resulting in high mortality. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant.

The increasing interest on traditional ethno-medicine may lead to discovery of novel therapeutic agents. Medicinal plants are finding their way into pharmaceuticals, nutraceuticals, cosmetics and food supplements. The World Health Organization (WHO, 2000) estimated that 80% of the population of developing countries still
relies on traditional medicines, mostly plant drugs, for their primary health care needs (Ramachandran et al., 2007). In last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. Availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of single drugs and formulations, quality control parameters, importance and challenges of conducting clinical research in herbal drugs, simple bioassays for biological standardization, pharmacological and toxicological evaluation, toxic herbal drugs in use, various animal models for toxicity and safety evaluation are the imp aspects (World Health Organization, 2002).

As the crops are indigenous to this environment, need less care in their development and survival (Patel et al. 2010). These medicinally important plants are the cash crops and the large scale cultivation of these plants which are indigenous to the region can be done in order to increase the per hectar revenue of the land.

4.0. Bioinformatics and the problem:

Docking study for prediction of mechanism of action of Gallic acid

Drug and ligand binding depends upon their interactions because of the structural confirmation. It is possible to simulate the protein ligand interaction using softwares such as LeadIT (BioSolve IT, GmbH). In this experiment crystal structure of beta lactamase (3S0Z) was retrieved from RCSB Protein Data Bank. This enzyme was docked with penicillin to find out the actual binding site and interaction with molecules.

FlexX was developed at the Institute for Algorithms and Scientific Computing at the German National Research Center for Computer Science in Sankt Augustin, Germany. The basic procedure is to break the ligand into fragments, then repeatedly place an anchor fragment and incrementally build the entire ligand in place. For each atom in the ligand and receptor, a set of interaction surfaces is generated and stored. The interaction surfaces represent ideal locations for atoms of the other molecule to form some stabilizing interaction. The shape, size, and location of each surface depends on the type of interaction--hydrogen bonding, electrostatic (ionic), aromatic, or lipophilic (hydrophobic).

The ligand is broken into fragments, separated by rotatable bonds, and a base fragment is chosen. The base fragment is placed by aligning a triangle formed by three of its atoms with interaction surfaces of receptor atoms, using a technique called pose clustering (Rarey et al., 1996). The choice of base fragment is critical, because a fragment with insufficient interaction surfaces will
provide too little guidance for its initial placement. For each sufficiently distinct placement of the base fragment, additional fragments are added in such a way as to maximize interactions and optimize the scoring function. FlexX uses a variant of the SCORE1 scoring function developed by Hans-Joachim Boehm for the de novo enzyme inhibitor design package LUDI (Boehm, 1992). The structure files for Amoxicillin, Clavulanic acid and Gallic acid were obtained from Pubchem in sdf format and explicit hydrogen atoms were added and 3-D structure were generated using Marvin suite 5.7.1 and saved as Tripos Mol2 format (Fig. XVI2). These three molecules were docked to defined binding site on β-lactamase.

**Properties of docked confirmations:**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Top Pose Binding energy (kJ/mol)</th>
<th>Hydrophobic interactions</th>
<th>Hydrogen bonds</th>
<th>Ligand-receptor pose</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF-7</td>
<td>N.D.</td>
<td>Leu293</td>
<td>Lys290</td>
<td>Fig. Xxx1</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>-35.3125</td>
<td>Tyr150, Leu293, Lys 290, Ala292</td>
<td>Lys290, Agr148, Ile291, Ser64</td>
<td>Fig.Xxx3</td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td>-27.776</td>
<td>Lys290, Tyr150, Thr316</td>
<td>Ala292, Arg148, Lys315</td>
<td>Fig.Xxx4</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>-30.3564</td>
<td>Lys290, Tyr150</td>
<td>Lys290, Arg148, Ala292</td>
<td>Fig.Xxx6</td>
</tr>
</tbody>
</table>
INTRODUCTION TO THE WORK

In the present investigation work has been carried out in two phases, to study the multiple antibiotic resistant uropathogenic gram negative bacteria with respect to various molecular and functional aspects which contribute for their virulence, atypicalness and multiple antibiotic resistant nature especially for beta lactam antibiotics by analysis of Extended Spectrum Beta Lactamases i.e. ESBLs. The isolated clinical isolates were identified for their geographical and gender based epidemiology, resistotyped for CLSI recommended antibiotics and screened for higher degree of drug resistance. Occurrence of ESBL character was analysed.

Further screened isolates were studied for various virulence factors and atypical characters. The uroisolates showing higher degree of atypicalness and majority of positive virulence factors were investigated for production, purification and characterization of beta lactamase enzyme. Nine herbs were used to evaluate their antimicrobial and enzyme inhibition activity. The potential extract was investigated using various wet lab tests for knowing the possible bioactive phytochemical extract. The bioactive extract was formulated as Cream and Ointment to obtain it as bioactive formulation.
OBJECTIVES

1. Isolation of bacteria causing urinary tract infection.

2. Screening of bacterial strains showing multiple antibiotic resistance.

3. Determination of virulence factors and its correlation with antibiotic resistance exhibited by them.

4. Optimization of MIC of the antibiotics up to efficient concentration.

5. Screening of isolates for plasmid encoded Extended Spectrum Beta Lactamases (ESBLs).

6. Isolation, purification and molecular characterization of Plasmid DNA.

7. Acquisition of resistance to ESBLs by conjugation or transformation.

8. Production, purification and enzyme kinetic study of ESBLs.

9. To analyse the Herbal/ Ayurvedic drugs in various forms for resistotyping with MAR strains.

10. In silico analysis in order to tackle the above mentioned problem.