6. Summary and Conclusion

The investigation was carried out at Kalaburagi district; isolates were isolated from Local Gulbarga District hospital and other Diagnostic centres of Kalaburagi between December, 2012 to June, 2013.

A total of 150 *E. coli* strains were isolated from 170 clinical samples, the distribution and isolation rate of *E. coli* from different clinical samples were analysed and highest numbers of strains was isolated from urine and least from blood sample.

The profiles of epidemiological studies for antibiotic resistance were done by using 10 antibiotics. Total 150 isolates were characterized by standard methods and antibiotic susceptibility profiling was performed. The investigation showed 120 strains i.e., 80% isolates were MDR strains. The results reveal that more than half of the strains isolated from clinical samples are MDR.

Our study indicates that first three generation of cephalosporins antibiotic and fluroquinolones have limiting effect over MDR *E. Coli*. But most of strains were susceptible to imepenem, gentamycin and to some extent amoxicillin/ clavulinic acid.

Isolates were tested for resistance against commonly used third generation broad spectrum antibiotics like Cefipime (30 μg), Cephotaxime (30 μg), Cefpodoxime (30 μg), Ceftazidime (30 μg), Ceftriaxone (30 μg). Out of 150 isolates of *E. coli* around 120 (80%) isolates were resistant to third generation antibiotic.
The Combined disc diffusion test for ESBL detection was carried out using cefotaxime (30 μg), ceftazidime (30 μg) and cefotaxime/clavulanic acid (30/10μg) and ceftazidime/clavulanic acid (30/10 μg) discs. In this phenotypic confirmatory test for ESBL detection using cephalosporin/clavulanate combination discs, 110 (92%) strains showed enhanced susceptibility to ceftazidime and cefotaxime in the presence of clavulanic acid, thus indicating ESBL production in them. Among the 150 strains 120 resistant strain were ESBL positive i.e., 80% strains.

MIC for cephotaxime (0.0001 to 240 μg), ceftazidime (0.0001 to 240 μg) was performed to confirm the presence of ESBL enzymes in the resistance strains. MICs of ESBL producing isolates ranged from 8 to > 240 μg/ml for both ceftazidime and cefotaxime, majority of the isolates (80%) had MIC > 32 μg/ml and few strains (40%) had MICs of >240 μg/ml.

ESBL producing *E.coli* isolates selected for genomic DNA isolation. The presence of genomic and plasmid DNA confirmed by the banding pattern.

A randomly chosen ESBL positive strain of *E.coli* isolates were confirmed with partial genotype analysis of 16S rDNA, PCR amplification was done by using eubacterial universal primers. The BLAST analysis of sequence was matching with existing *E.coli* sequence in database, subsequently phylogenetic tree was constructed.

The genetic diversity among ESBL producing cephalosporin resistant 33 clinical *E. coli* isolates was successfully recovered by ERIC-
PCR. Clustering of isolates based on ERIC-PCR profiles revealed that presence of diverse clonal groups indicated transmission among members of different groups.

ESBL positive strains were used for the amplification of $CTX-M$ gene. All the strains were positive for the amplification studies with specific primers used as mentioned earlier. The PCR amplification of cefotaximase gene was confirmed by agarose gel electrophoresis.

Previously sequenced of $CTX-M$ gene from ESBL positive strain, was used to identify protein sequence by searching ORF, our amino-acid residue protein sequence was considered for the prediction of 3D structure.

The pair wise analysis was performed to find similar sequences in various sequence databases towards our query amino-acid residues cefotaximase protein. The EBI EMBOSS- Needle sequence alignment program was used for pair wise alignment. The query amino acid sequence of CTX-M 9 protein sequence was aligned with 3HLW, 5KMT and 5KMU from PDB sequences.

After analysis of pair wise sequence alignment with 3HLW, 5KMT and 5KMU. PDB sequences, the 3HLW showed maximum homology towards our protein sequence. Hence, PDB structure with PDB ID (ID-Q9L5C8) 3HLW selected as a template structure for homology modeling.

To design 3D structure of CTX-M 9 protein which was responsible for antibiotic resistance in *E.coli*, the SWISS-PDB software was used. The 3HLW structure was downloaded from protein data bank in a PDB file format, using file option opened the file loaded on to the software, after
displaying the 3HLW structure on the software, using the option open Swiss model, load the raw sequence on to the software. Open the option magic fit, it was used for the super imposition of raw amino acid sequence with 3HLW structure and lastly the option update threading display was used and this option were present at SWISS model option for the designing of 3D model. The designed 3D structure was further analyzed by Rasmol visualization software and verification of 3d model was done. For this present study Hex 6.3 advance version was used for docking studies with CTX-M9 structure (ligand) with Cefotaxime and inhibitor, the software Marvin Sketch was used for the sketching of the antibiotic and inhibitor structure.

Docking studies were done initially by taking original structures of CTX-M 9 protein and Cefotaxime. Both structures were loaded on to the Hex 6.3 software and by using the option file-load receptor-ligand and the option controls-docking have to be clicked. The Cefotaxime antibiotic finds the binding cavity in the CTX-M 9 protein and automatically binds (docks) with the receptor molecule (CTX-M 9). The window will show docking energy. The docking energy is in negative form indicates, the efficiency to binding of Cefotaxime antibiotic to the protein (CTX-M 9) was high. Similarly, the ligand (Inhibitor) was docked into an enzyme structure. Further, the software displays binding energy in the form of E-total (Glide Energy) for CTX-M 9 protein and inhibitor and calculates in terms of K.cal/mole. The current study revealed the glide energy crucial to ‘CTX-M-drug’ and ‘CTX-M-inhibitor’ interactions using different bioinformatics
tools which would be useful for the development of a different antibiotic/Inhibitor combination against ESBL producing MDR *E.coli*.

**Conclusion**

The *E.coli* is a major problem in medical care, due to increased drug resistance to most of the traditional antibiotics, including major class of cephalosporins β-lactams. The growing threat from resistance strains calls for development of precise diagnostic methods and effective treatment strategies. Studies have focused on molecular mechanisms of antibiotic resistance to get a better insight on spread of antibiotic resistance genes among the species. To treat *E.coli* infections in proper way there is a need of alternative novel approach to find better drugs in the field of pharmaceuticals. In recent years the pharmaceutical companies are trying to develop alternative drugs instead of conventional antibiotics/drugs.

*In silico* drug designing is an alternative approach to design specific drug for ESBL producing and Multi drug resistance *E. coli* infections. In our studies preliminary understanding of *in silico* studies with reference to structure based drug design which help to understand the real molecular interaction between drug and its target molecule. Due to the advancement in the field of Molecular biology, Structural biology, Bioinformatics and Computational Biology we can address molecular interaction of drug of target and finding their better alternative treatment of *E. coli* and related infections.

In our investigations the epidemiological survey of occurrence of *E.coli* infection in Kalaburagi District was undertaken. Through molecular
characterization we have identified the most resistance \textit{E.coli} organism to third generation cephalosporins antibiotic. The \textit{CTX-M 9} gene was amplified because the product of the \textit{CTX-M 9} gene was cefotaximase protein, which was receptor molecule for the interaction of \(\beta\)-lactam antibiotics and development of resistance in \textit{MDR E.coli}. Through the biocomputational approaches we have predicted 3D structure for cefotaximase protein. Homology modeling of the sequences of \textit{CTX-M-9} submitted to database was done. Docking of drugs (cefotaxime) as well as inhibitors (clavulanate) with these modeled enzyme-structures was performed. In silico efficacies of \(\beta\)-lactamase antibiotic and \(\beta\)-lactamase inhibitors against \textit{CTX-M 9} protein on the basis of interaction or glide energies was carried out. These interaction or glide energy values can use design new antibiotic/inhibitor combination or their analogs.