7. Summary

Urinary tract infections are one of the most common bacterial infection and accounts for significant morbidity. It affects all age groups and has serious impact on the socioeconomic life of individuals as well as the society.

This study was conducted with the patients of Government and private hospitals of Namakkal district, TamilNadu, India for a period of one year to assess the etiology of UTI.

Four hundred and ninety eight mid stream urine was collected from the cases of UTI and transported to the Microbiology processing center immediately. Gender wise and age wise categorization was done to assess the incidence of UTI and urinary pathogens.

More number of urine samples were collected from female inpatients (64%), which indicated that female were more susceptible to UTI.

Age wise sample collected were grouped in to 4 categories. Maximum incidences (41%) were noted among elderly age groups (61-80). Low incidence was noted among 41-60 age groups (12%).

Gram staining of urine sample showed that all the samples were in bacteriuria condition out of which fourty two percentages of samples showed only bacteria, 34% showed bacteria and pus and 24% of samples indicated the presence of bacteria, pus and inflammatory cells. These results indicated the bacteruria condition of all the samples.
From 498 urine samples collected, 769 uropathogens were isolated with the help of different selective cum differential media. Among these 51% of urinary isolates belonging to *Escherichia coli* followed by 17% of *Enterococcus* sp., 16% *Klebsiella* sp., 10% *Pseudomonas* sp., 3% each of *Proteus* sp., and *Staphylococcus* sp.

The pure *E. coli* isolates (127) were subjected to antibiotic sensitivity assay. All the isolates were found to be multiple drug resistant, however, the UPEC isolates showed 35% sensitivity against cephalosporins. This report revealed the alarming situation about UTI and need for the development of a new strategy for the better UTI treatment and management. Antibiotic resistance profile and pattern of multidrug resistance revealed that, out of 127 UPEC isolates 100% of isolates were resistant to Ceftriaxone followed by 95.2% to Tetracycline, 92.1% to Amikacin, 86.5% to Erythromycin, 76.2 % to Gentamycin, 74.7 % to Ciprofloxacin, 74.6% to Nalidixic acid, 69.9% to Cotrimaxazole and 61.9% to Cephodaxime. None of the organisms were susceptible to all the antibiotics tested.

Among the 127 *E. coli* isolates, 11 antibiotic resistant groups were detected. These organisms were subjected for virulence assessment.

Biofilm and β lactamase production ability is the major virulence determinant of uropathogens. Among the 11 isolates six isolates possess ESBL ability (E3, E7, E8, E16, E33 and E64). Biofilm formation is one of the major virulence factors of urinary pathogen, except E1 and E32 all the other strains possessed biofilm producing ability.
RAPD pattern of uropathogenic *E. coli* revealed that the all 11 strains were categorized into 10 different clones and were transmitted not from a single source. All the UPEC isolates showed variable pattern of RAPD with different primers. Six different variability was noted between 11 strains. Similarity index of *E. coli* population revealed that none of the isolates were 100% similar with their genetic relatedness. In the similar way, all the strains were more than 50% in its RAPD pattern.

Results of amplification with the resistance gene revealed that TEM gene was found in E3, E7, E8 and E16. CtxM gene was found in all the isolates except E7 isolate while SHV gene was harboured by only two isolates E8 and E64, whereas none of the organism showed the availability of OXA gene.

In this study 100% of isolates had plasmids, strain E3, E8 and E64 harboured 2 plasmids bands and the remaining isolates had one band. The plasmid size ranges from 3530bp to above 4973 bp. The lowest weight of the plasmid band was observed in 2\textsuperscript{nd} isolate, which plasmid size was 1904bp.

Among different plants studied for antimicrobial activities of UPEC, *Phyllanthus emblica* extracts revealed effective antimicrobial pattern. Alkaloids like phyllanthinde and phyllantidine are prevalent in this plant. Hence these two phytochemicals were selected and subjected for molecular docking studies.

Antibiotic resistance of the bacteria could be due to specific genes like TEM, SHV, OXA, CTXM. These genes were found in most of the isolates. After plasmid curing, pure fresh isolates were subjected for the amplification of resistance
gene. Results of amplification of resistance gene revealed that none of the resistance gene was found in all the UPEC strains. It was also indicated that all the isolates becomes ESBL negative.

Study of plasmid mediated antibiotic resistance genes showed that 47.8% strains showed the presence of TEM, 43.4% SHV and 30.4% CTX-M gene. This also indicated that the strains of the study belong to ESBL category.

Plasmid profile indicated that 3 plasmids were found in four isolates, whereas two plasmids were detected in 18 isolates and one strain showed only one plasmid. All the plasmids were of high molecular weight in nature.

The 16srRNA analysis revealed that the strain E8 showed 99% similarity with E. coli.

This study revealed that uropathogenic E. coli possess highly potent pathogenic islands, able to cause severe UTI and was difficult to treat using regular antibiotics.

Antibiotic resistance becomes serious threat to the human society. To overcome the problems of antibiotic resistance, people have turned towards the benefits of Traditional System of medicine. Medicinal plants are effective against the pathogens even if they are multidrug resistance. The antimicrobial activities were determined for the aqueous extracts of Mangifera indica seed, Phyllanthus emblica fruit, Catharanthes roseus leaves and Aegle marmelos leaves by making use of disc diffusion method.

Bacterial proteins are the ultimate target to inhibit their growth since these are the executers of many cellular functions. The protein intimin is responsible for host bacterial interaction and leads to inflammatory diseases like cystitis and pyelonephritis. Phyllantidine and Phyllanthine from Phyllanthus emblica were
assessed for its interaction with Eae *intimin (E. coli)* protein. The molecular docking results clearly showed that the binding values of *Phyllanthine* with Eae *intimin (E. coli)* is (-173.44). *Phyllantidine* with Eae shows a binding value of -149.99. *Phyllanthine* with Eae *intimin (E. coli)* shows negatively high binding values when compared to the existing drug *Gentamicins*. *Phyllanthine* is a potential inhibitor for Eae *intimin (E. coli)* pathogenic protein structure.

All the extracts of plant revealed the presence of phenolic compounds, flavonoids and tannins in both the extracts tested. Tannins normally inhibited the growth of bacteria by protein precipitating activity. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane bound enzymes such as the ATPase and phospholipase. Hence the presences of these compounds in the test plant confirm the antimicrobial potential against dreadful UTI pathogens.