Chapter VI

SUMMARY

Multidrug resistant enteropathogenic *Escherichia coli* associated with urinary tract infections
Escherichia coli are found naturally in the intestinal tract of all humans and many other species of animals. Subsets of E. coli have evolved possessing virulence properties and the association of these organisms with worldwide outbreaks of many enteric/diarrhoeal cases is well established. Pathogenic strains of E. coli have been divided into different pathotypes and each pathotype cause diseases using different combinations of the virulence factors, with different molecular pathways. Common E. coli pathotypes include Shiga toxin producing E. coli (STEC), enterohaemorrhagic E. coli (EHEC), enterotoxigenic E. coli (ETEC), enteroaggregative E. coli (EAEC), enteroinvasive E. coli (EIEC) and diffusely adherent E. coli (DAEC).

The E. coli normally present in the gastrointestinal tract, due to the proximity to the urinary tract ascends through the urinary passage to the urinary bladder and the kidneys to produce infections. Under normal circumstances the human urinary tract is able to combat with the microbial invasion. To cause UTI the organism has to evade the host defense mechanism, which is determined by the virulence determinants. But distinct pathotypes of E. coli causing urinary tract infection have not been clearly defined and commonly has been termed as uropathogenic E. coli (UPEC). Interestingly the enteropathogenic E. coli has also
been recovered time to time from extra intestinal sources like the urinary tract and incriminated as causative organism of UTI and non-diarrheal (urinary tract) hemolytic uremic syndrome.

*E. coli* are found to be the most frequent urinary pathogens isolated from 50-90% of all uncomplicated cases of urinary tract infections. Urinary tract infections (UTI) are the second most common infection of the human being affecting both the genders irrespective of age group.

This study was undertaken to determine the various enteropathogenic virulence factors in *E. coli* isolates from urinary tract infection cases using PCR amplification to have a better understanding of the uropathogenic strains and see if they really form a distinct group or have evolved from any other pathotypes. A study of the antimicrobial sensitivity and resistant pattern among the isolates was also done since widespread and most often the misuse, inadequate doses of antibiotics has been a cause of alarming raise in drug resistant strains.

Six hundred and fifty urine samples were collected from patients with clinical cases of urinary tract infection from 14 different parts of North eastern regions, who had no previous exposure to antibiotics. The samples were processed as per the technique of Edwards and Ewing (1986) for isolation and identification of *E. coli* and 405 strains of *E. coli* were isolated (62%) followed by *Klebsiella* sp.
Proteus sp. (6%), Enterococcus sp. (3%), coagulase negative Staphylococcus (2%), Staphylococcus aureus (1%) and 61 were negative for the test either due to multiple growth of organisms (15%) or no growth of any organisms (85%).

The 405 E. coli isolates were serotyped at National Escherichia and Salmonella Centre, Kasauli (H. P). Out of 405 isolates serotyped 303 were typable and belonged to 28 different serotypes, 60 isolates were refractory to typing and 42 were rough. Serotypes isolated were O25(38), O1(31), O117(23), O6(18), O8(18), O171(18), O20(12), O11(12), O41(12), O24(11), O9(10), O42(9), O89(9), O15(8), O21(7), O143(7), O147(5), O153(5), O5(5), O62(5), O86(5), O111(5), O130(5), O131(5), O140(5), O141(5), O153(5) and O5(5).

Antimicrobial susceptibility testing of the isolates was performed by the disk diffusion method against 24 common antimicrobial agents (Hi-Media, Mumbai) viz. Imipenem, Nitrofurantoin, Pipercillin Tazobactum, Amikacin, Levofloxacin, Ciprofloxacin, Ofloxacin, Netilmicin, Norfloxacin, Amoxycillin/Clavulanic acid, Nalidixic Acid, Cefepime, Gentamicin, Tobramycin, Cefazoline, Chloramphenicol, Ticarcillin/Clavulanic Acid, Ceftazidime, Ampicillin, Ticarcillin, Tetracycline, Cefuroxime, Co-Trimoxazole and Pipercillin on Muller Hinton agar (Hi-Media, Mumbai). Results were interpreted as per cent sensitive (%S) and per cent resistant (%R) using WHO

95
break points. The highest incidence of drug resistance engaged to Pipercillin, Co-
Trimoxazole, Cefuroxime, Tetracycline, Ticarcillin, Ampicillin, Ceftazidime, 
Ticarcillin/Clavulanic Acid, Chloramphenicol, Cefazoline, Tobramycin whereas 
fifty percent or more of isolates, on the other hand, were sensitive to Gentamicin, 
Cefepime, Nalidixic Acid, Amoxyclov, Norfloxacin, Netilmycin, Ofloxacin, 
Ciprofloxacin, Levofloxacin Amikacin, Pipercillin Tazobactum, Nitrofurantoin 
and Imipenem.

*E. coli* is developing resistant to multiple antimicrobial agents very 
rapidly. Our study revealed 50 isolates of *E. coli* to be resistant to 13 antimicrobial 
agents, followed by 62 isolates to 10, 34 isolates to 9, 21 isolates to 7 and 203 
isolates to 5 agents and 35 were sensitive to all the 24 antimicrobial agents. As our 
study was concerned with multidrug resistant *E. coli* so 370 multidrug resistant 
strains were selected excluding the 35 antimicrobial sensitive isolates from our 
study.

PCR analysis for the detection of the five virulence genes, viz. estI, 
elI, stx1, stx2 and hlyA was carried out on 370 isolates as per method described 
by Osek *et al.*(1999) and Khan *et al.*(2000) which showed the presence of *est* gene 
in 21 isolates belonging to serotypes O25(7) and O171(7) and 7 isolates were 
refractory to typing. Among the other genes tested, *hlyA*, and *stx2* genes were 
detected in 7 isolates each belonging to serotype O41 and O5.
The association of serotypes and enterotoxin is well established but their ability to cause UTI is not well defined. Given that enterotoxigenic genes have been detected in *E. coli* from UTI cases as this study suggests, the *E. coli* strains recovered from patient’s urine originated in their gastrointestinal tract. Assuming that *E. coli* strains causing UTIs are not a distinctly different pathotype, widely called the uropathogenic *E. coli*, but any other enteropathogenic pathotypes (ETEC, STEC, EHEC) can cause UTI by evolving mechanism to invade and colonize the urinary tract and cause infections after reaching the urinary tract from the gastrointestinal system.