Chapter V

DISCUSSION

Multidrug resistant enteropathogenic Escherichia coli associated with urinary tract infections
E. coli (Escherichia coli) is one of several types of bacteria that normally inhabit the intestine of humans and animals (commensal organism). Some strains of E. coli are capable of causing disease under certain conditions when the immune system is compromised or disease may result from an environmental exposure.

Strains of E. coli that cause disease are difficult to distinguish from those that are part of the normal intestinal flora of man and animals. The detection of their toxins or toxin genes is the only approach to detect the pathogenic strains of E. coli. Toxins have been detected by using biologic (cell culture) and immunologic assays. Toxin genes have been detected by DNA hybridization and polymerase chain reaction (PCR) assays. However, the reports on the application of these assays for the detection of virulence factors of E. coli are very meagre (Rahman, 2002).

Escherichia coli is responsible for more than 80% of all UTIs and causes both asymptomatic bacteriuria (ABU) and symptomatic urinary tract infection (Hedlund et al., 2001; Svanborg and Godaly, 1991). These infections are typically caused by a single bacterial clone and are in effect monocultures.
Urinary tract infections are probably the most common bacterial infections. Bacteria responsible for UTI, often originate from the faecal and perineal flora (Kaper et al., 2004; Wullt, 2002). Under normal circumstances, these bacteria are cleared from the urinary system by effective protective mechanisms. If, however, they overcome these mechanisms, they can colonize the lower urinary tract. Subsequent progress is determined by the host susceptibility and bacterial virulence factors. Manifestations can vary from asymptomatic bacteriuria to symptomatic cystitis, pyelonephritis and blood stream infection.

The present study was carried with a view to isolate and identify enteropathogenic *E. coli* causing urinary tract infection and to study their serotypings from urine samples collected from North-east regions of India, study the susceptibility pattern of antimicrobial agents against the isolates, then to select multidrug resistant *Escherichia coli* and to screen the isolates for virulence determinants like *stx1, stx2, elt, est,* and *hly* genes by polymerase chain reaction.

This study reveals some interesting results regarding the occurrence of *E. coli* as a major causative agent of UTI in samples of urine collected from North-eastern states of India. It has been found that 405 of 650
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(62.30%) urine samples examined showed pure growth of \textit{E. coli}. The isolation percentage was within the generally reported isolation percentage limits of 50-90\% (Steadman and Topley, 1998) Other organisms isolated were \textit{Klebsiella sp.} (14.7\%), followed by \textit{Proteus sp.} (6.46\%), \textit{Enterococcus sp.} (3.38\%), Coagulase negative \textit{staphylococcus} (2.15\%), \textit{Staphylococcus aureus} (1.53\%) and no growth of organism (9.38\%). Urinary tract infections (UTI) are the second most common infection in the community practice and an important cause of morbidity and mortality in Indian subjects affecting all age groups (Acharya, 1992).

**Serotyping of isolates**

Out of 405 isolates serotyped 303 were typable and belonged to 28 different serotypes, 60 isolates were refractory to typing and 42 were rough. The frequency of serotypes isolated in descending order as 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). All the serotypes isolated in this study and many more have been isolated by different workers from cases of urinary tract infection across India (Kapoor and Kulshrestha, 1998, Chugh et al., 1972).


Antibiogram of *E. coli* isolates

Antimicrobial agents are the first choice of medicine for controlling many infectious diseases. For controlling such diseases in human beings, farm animals and birds, a variety of antibiotics and antimicrobial agents are used. In poultry industry and animal husbandry some antibiotics are widely used as growth promoting agents. Very often, antibiotic use is indiscriminate and unduly prolonged which ultimately leads to the development of antibiotic/drug resistance in the pathogenic bacteria and other microorganisms. This situation occurs in *E. coli* as well where resistance is plasmid mediated and occurs due to selective pressure (Aslam and Service, 2006). Drug resistant bacteria may enter human enteric system through contaminated food and water and colonize the gut. The R-factor of such pathogenic bacteria is then transferred to normal intestinal flora of human (Vidovic *et al*., 2007).

The present study revealed that only 35 isolates (8.64%) did not show resistance to any of the 24 antimicrobial agents tested, while majority (91.36%) of the isolates showed varying degrees of resistance to these antimicrobial agents. Highest degree (94.33%) of sensitivity was shown by the isolates towards Imipenem followed by Nitrofurantoin, Pipercillin

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Tazobactum, Amikacin, Levofloxacin, Ciprofloxacine, Ofloxacin, Netilmicin, Norfloxacin, Amoxycillin/clavulanic acid, Nalidixic Acid, Cefepime, Gentamicin, (80.33%, 75.31%, 74.33%, 68.65%, 68.65%, 68.65%, 64.45%, 62.25%, 61.24%, 59%, 54.57%, 53.83%).

Less than 50% of strains showed sensitive to Tobramycin, Cefazoline, Chloramphenicol, Ticarcillin/Clavulanic Acid, Ceftazidime, Ampicillin, Ticarcillin, Tetracycline, Cefuroxime, Co-Trimoxazole and Pipercillin (49.63%, 45.67%, 43.46 %, 39.26%, 37%, 36%, 31.36%, 30.62%, 28.65%, 24% and 23%)

In general, present finding agree with those reported in the past. Antibiotic resistance pattern of *E. coli* isolated from different sources has been reported by several workers (Rahman *et al.*, 1986; Singh *et al.*, 1995; Gowda *et al.*, 1996; Goswami *et al.*, 2002; Tabatabei and Nasirian, 2003; Aslam and Service, 2006). The variations observed in the sensitivity to different antimicrobial agents may be due to difference in the method used and it is also possible that strains present in different region may become resistant to antimicrobial agents used in that particular region.
Multiple antimicrobial resistant patterns

Out of 405 isolates, 370 isolates were found to be multidrug resistant and so selected for the present study. Fifty strains (13.51%) exhibited resistant to 13 antimicrobials (54.17%) out of 24 tested, followed by 16.75%, 9.10%, 5.65% and 54.86% of isolates to 41.67%, 37.5%, 29.16% and 20.83% respectively. Similar results were reported by Zakaria for sensitivity and resistance pattern (Zakaria, 2005).

The highest incidence of drug resistance engaged to Pipercillin, Co-Trimoxazole, Cefuroxime, Tetracycline, Ticarcillin, Ampicillin, Ceftazidime, Ticarcillin/Clavulanic Acid, Chloramphenicol, Cefazoline, Tobramycin may be due to the fact that these preparations are more extensively and indiscriminately used for various ailments both in man and animals. Fifty percent or more of isolates, on the other hand, were sensitive to Gentamicin, Cefepime, Nalidixic Acid, Amoxycillin/Clavulanic acid, Norfloxacin, Netilmicin, Ofloxacin, Ciprofloxacin, Levofloxacin, Amikacin, Pipercillin Tazobactum, Nitrofurantoin and Imipenem. This may be due to their lesser usages in the treatment. Thus, periodical antimicrobial testing is essential to modify the treatment/therapy according to sensitivity/resistance patterns of the isolates. It must be emphasized that changes evolving in increasing drug resistance in bacterial pathogens has
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become a true hinderance to successful therapy of UTI and require important reassessment of local empirical choices for managing urinary tract infection.

Virulence genes of E. coli:

The pathogenic process of E. coli are controlled by an array of virulence factors like adhesins, colonising factors, enterotoxins, etc. that act in tandem and ultimately manifest in the typical symptoms of infection (Rahman, 2002). The expression of these is controlled by virulent genes present usually in plasmids and sometimes in chromosomal pathogenicity islands (PI). These factors include production as well as action of heat labile (LT) and heat stable (ST) enterotoxins, entero-haemolysin (Hly), Shiga toxin (Stx) and intimin (Eae) which are encoded by est, elt, hly, stx, and eae genes, respectively.

Polymerase chain reaction amplification of 370 isolates using specific primers used to detect the prevalence of 5 different virulence genes viz., stx1, stx2, est, elt and hlyA, showed the presence of est gene in 21 isolates belonging to serotypes O25(7) and O171(7) and 7 isolates were refractory to typing (Table 8). Kapoor and Kulshrestha (1994) detected ST and LT positive strains of E. coli from UTI cases in animal cell culture system and infant mouse. Saxena and Yadav (1985) also reported similar observations. Among the other genes tested, hlyA and stx2 genes were
detected in seven isolates each belonging to serotype O41 and O5, respectively. Among E. coli strains causing UTI, the production of hemolysin is often associated with other factors assumed to contribute to virulence. Although E. coli isolates from UTI cases positive for hlyA gene has been reported in many other studies (Opal, et al., 1990, Johnson et al., 1991, Kerenyi et al., 2005) but very few reports suggest the incrimination of Shiga toxin (Stx) producing E. coli in cases of UTI. Rathore et al. (2003) reported that 44.4% of E. coli isolated from UTI cases belonging to 4 different serotypes (O9, O5, 100, O172) could produce Shiga toxins (Stx). Tarr et al (1995) described a case with nondiarrheal haemolytic uremic syndrome (HUS) and acute pyelonephritis caused by STEC (shiga toxin producing E. coli) O103. Starr et al (1998) reported a case of UTI with an O5 STEC positive for stx1, stx2 and hly genes detected by DNA hybridization was detected as the causative organism. E. coli serotype O91 positive for stx2 gene was found responsible for UTI infection followed by haemolytic uremic syndrome (HUS), (Kater et al, 2000). Hacker et al (1990) attributed the emergence of uropathogenic STEC in one of two ways: from an inherently uropathogenic strain of E. coli, with the ability to colonize and replicate in the urinary tract, that became lysogenized with one or more Stx-encoding or from an EHEC strain that acquired elements, such as P-pili, that would
enhance its ability to colonize and was probably of fecal origin and not primarily a uropathogen of urinary tract.

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, it is highly likely that 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. Future research effort should emphasize in studies to trace the natural history and the evolutionary pattern of the organism, its mechanism of transmission, the impact of enteropathogenic *E. coli* strains to the urinary tract and evolving changes in drug resistance, should they become frequent pathogens of the urinary tract in near future as these strains are not the natural inhabitants of the urinary tracts.

The study, therefore, clearly indicates the isolation of multiple antimicrobial agent resistant *E. coli* harbouring virulence genes from urine samples to be the reservoir and source of infection of these pathogenic strains. Evaluation of pathogenic *E. coli* to a clinical novelty during the past
decade has become a serious public health concern. Dissemination of these multiple drug resistant pathogenic *E. coli* into the environment with urine, even in low count may cause contamination of water bodies and as a result would lead to contamination of foods and then to serious public health problems. *As E. coli* has the ability to cause broad spectrum of diseases in man, animals and birds, so the concern is mostly related to the potentially serious clinical outcomes of the infections and possible transmission to human from different sources.

Therefore, more studies on the antibiotic resistant enterotoxin harbouring human disease causing *E. coli* strains are needed. There is no record available in the systematic survey of the enteropathogenic *E. coli* associated with human illness from the North Eastern region. This study with isolation of enterotoxigenic *E. coli* from UTI patients that are multidrug resistant would help the health authority to take necessary steps to prevent the spread of diseases and also rendering necessary treatment of the sufferers.

Thus it will usher in a new era in the treatment and prevention of diseases.