Chapter I

INTRODUCTION

Multidrug resistant enteropathogenic *Escherichia coli* associated with urinary tract infections
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*Escherichia coli* is the most common colonic flora of warm-blooded animals including humans. *E. coli* is important to human health because it is a source of vitamin B₁₂ and K, which it manufactures in the large intestine from the undigested foods. It is a single species of bacteria, whose different varieties (called strains) exists, each possessing different characteristics like some are beneficial and the others detrimental that can cause potentially deadly diseases. This is the case with *E. coli O157: H7*, which is considered a dangerous pathogen that can infect humans.

*E. coli* are the gram negative, facultative anaerobe of the intestine, which usually remains harmlessly confined to the intestinal lumen. But highly adapted *E. coli* clones have evolved having the ability to cause a broad spectrum of disease in man and animals assuming worldwide public health importance (Sarkar and Panigrahi, 2007). The concern is mostly related to the potentially serious clinical outcomes of the infections and possible transmission to human from different sources.

Some pathogenic strains cause gastrointestinal illness ranging from mild diarrhoea to potentially fatal complications such as haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura. Infection due to *E. coli* is usually called collibacillosis which may be
systemic or enteric. Other diseases caused by *E. coli* are dysentery, neonatal, meningitis, neonatal diarrhoea, traveller’s diarrhoea, etc. There are at least six types of pathogenic *E. coli*, of which four are implicated in food borne illness. The pathogenicity of any microorganism depends on the expression of an array of virulence factors controlled by virulence genes present in the genome. In *E. coli* virulence genes are located in virulence encoded plasmids and chromosomal pathogenicity islands (PI). One of the major virulence factors of *E. coli* is the Shiga toxin (Stx). Two major reactive groups of Stx exist namely Stx1 and Stx2 and their structural genes stx1 and stx2 are found to be encoded in lysogenic lamboid phages (Reissbrodt, 1998). Such genes are normally expected in *E. coli* of faecal origin. Other similar toxins produced by *E. coli* are heat labile (LT) and heat stable (ST) enterotoxins. These toxins are associated with diarrhoea and gastroenteritis and are encoded by elt and est genes, respectively.

The major problem in identifying and characterizing pathogenic strains of *E. coli* is that they predominantly resemble commensal *E. coli* in many aspects apart from producing toxins (Bettelhheim and Beutin, 2003). Therefore molecular methods like polymerase chain reaction (PCR) have been widely preferred for detection of the virulence genes to identify the enteropathogenic *E. coli* strains (Paton and Paton, 1997; Osek et al., 1999; Galane and Roux, 2001; Rahman, 2002; Murugkar et al., 2004)
due to its sensitivity and specificity. Prevalence studies of these toxigenic genes are of importance in pathogenic characterization of the isolates. A single strain of \textit{E. coli} may express any of the \textit{stx} genes or both together, any of the heat stable or labile gene or both together and also may harbor \textit{hlyA}.

Over 700 antigenic types (serotypes) of \textit{E. coli} are recognized based on O, H, and K antigens. At one time serotyping was important in distinguishing the small number of strains that actually cause disease. Thus, the serotype O157:H7 (O refers to somatic antigen; H refers to flagellar antigen) is uniquely responsible for causing HUS (hemolytic uremic syndrome). Nowadays, particularly for diarrheagenic strains (those that cause diarrhea) pathogenic \textit{E. coli} are classified based on their unique virulence factors and can only be identified by these traits. Hence, analysis for pathogenic \textit{E. coli} usually requires that the isolates first be identified as \textit{E. coli} before testing for virulence markers.

Enteric \textit{E. coli} (EC) are classified on the basis of serological characteristics and virulence properties (Todar. K., 2007). Depending upon the virulence markers (virotyping), six virotypes of \textit{E. coli} have been distinguished (Nataro and Kaper, 1998). Their description is given below (Table 1)
TABLE 1: Clinical, epidemiological features and virulence factors of various *E. coli* pathotypes
(Nataro and Kaper, 1998)

<table>
<thead>
<tr>
<th>PATHOTYPE</th>
<th>CLINICAL FEATURES</th>
<th>EPIDEMIOLOGICAL FEATURES</th>
<th>VIRULENCE FACTORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteropathogenic</td>
<td>Watery diarrhea and vomiting</td>
<td>Infants in developing countries</td>
<td>Bundle-forming pilus, attaching and effasing</td>
</tr>
<tr>
<td>Enterohemorrhagic</td>
<td>Watery diarrhea, hemorrhagic colitis, haemolytic-uremic syndrome</td>
<td>Foodborne, waterborn outbreaks in developed countries</td>
<td>Shiga toxins, attaching and effasing</td>
</tr>
<tr>
<td>Enterotoxigenic</td>
<td>Watery diarrhea</td>
<td>Childhood diarrhoea in developing countries, traveler’s diarrhea</td>
<td>Pili, heat-labile and heat-stable enterotoxins</td>
</tr>
<tr>
<td>Enteroaggregative</td>
<td>Diarrhoea with mucous</td>
<td>Childhood diarrhoea</td>
<td>Pili, cytotoxins</td>
</tr>
<tr>
<td>Enteroinvasive</td>
<td>Dysentery, watery diarrhea</td>
<td>Food born outbreaks</td>
<td>Cellular invasion, intracellular motility</td>
</tr>
<tr>
<td>Diffuse-adhering</td>
<td>Poorly characterized</td>
<td>Older children</td>
<td>-</td>
</tr>
</tbody>
</table>
Escherichia coli is the leading causative organism of the most common bacterial infection i.e. urinary tract infection (70-90%) (Steadman and Topley, 1998). Urinary tract infections (UTIs) are a serious health concern. Forty to 50% of women experience at least one UTI, leading to an estimated 8 million annual physician visits in the United States alone (Schappert, 1999; Zielske et al., 1981). E. coli which is the most frequent urinary pathogen isolated from 50 – 90% of all uncomplicated UTI that even causes morbidity and mortality (Steadman and Topley, 1998). E. coli present in the gastrointestinal tract as commensals and provide the pool for initiation of UTI. So the highly adapted E. coli clones that 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 (Acharya et al., 1992). 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 (Saxena and Yadav, 1985; Kapoor
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and Kulshrestha, 1998) and nondiarrheal (urinary tract) hemolytic uremic syndrome (Tarr PI, 1996; Kater et al., 2000).

The urinary system consists of the kidneys, ureters, bladder, and urethra. A pair of brown organ located below the ribs towards the middle of the back is the kidneys which are considered to be the main organ of the system. The kidneys remove excess liquid and wastes from the blood in the form of urine that helps to maintain a stable balance of salts and other substances in the blood, and also to produce a hormone that aids the formation of red blood cells. Narrow tubes are called the ureters that carry urine from the kidneys to the bladder, a sack-like organ in the lower abdomen and hence the urine is stored in the bladder and emptied through the urethra.

The volume of urine excreted by an individual depends on the intake of fluids and foods. An average adult approximately passes about a quarter and a half of urine each day. The volume formed at night is about half that formed in the daytime.

Normally, urine is sterile. Urine itself functions as an antiseptic, washing potentially harmful bacteria out of the body during normal urination. It is usually free of bacteria, viruses, and fungi but does contain fluids, salts,
and waste products. An infection occurs when tiny organisms, usually bacteria from the digestive tract, cling to the opening of the urethra and begin to multiply. The urethra is the tube that carries urine from the bladder to outside the body.

In many cases, bacteria first travel to the urethra. When bacteria multiply, an infection can occur. An infection limited to the urethra is called urethritis. If bacteria move to the bladder and multiply then a bladder infection is called cystitis. If the infection is not treated promptly, bacteria may then travel further up the ureters to multiply and then infect the kidneys. A kidney infection is called pyelonephritis.

The urinary system is structured in a way that helps ward off infection. The ureters and bladder normally prevent urine from backing up toward the kidneys, and the flow of urine from the bladder helps wash bacteria out of the body. In men, the prostate gland produces secretions that slow bacterial growth. In both sexes, immune defenses also prevent infection. But despite these safeguards, infections still occur.

A common source of infection is catheters, or tubes, placed in the urethra and bladder. A person who cannot void or who is unconscious or
critically ill often needs a catheter that stays in place for a long time. Some people, especially the elderly or those with nervous system disorders who lose bladder control, may need a catheter for life. Chances of bacterial invasion in the bladder in such cases can not be overlooked.

Again people with diabetes have a higher risk of a UTI because of changes in the immune system. Any other disorder that suppresses the immune system raises the risk of a urinary infection.

UTIs may occur in infants, both boys and girls, who are born with abnormalities of the urinary tract, which sometimes need to be corrected with surgery. UTIs are more rare in boys and young men. In adult women, though, the rate of UTIs gradually increases with age. Scientists are not sure why women have more urinary infections than men. One factor may be that a woman's urethra is short, allowing bacteria's quick access to the bladder. Also, a woman's urethral opening is near sources of bacteria from the anus and vagina. For many women, sexual intercourse seems to trigger an infection, although the reasons for this linkage are unclear. According to several studies, women who use a diaphragm are more likely to develop a UTI than women who use other forms of birth control. Recently studies revealed that spermicidal
foam also tends to have growth of \textit{E. coli} bacteria in the vagina. (NKUDIC, 2005).

Most of the people with urinary tract infections possess symptoms and a few remain asymptomatic. Symptoms may include a frequent urge to urinate and a painful burning feeling in the area of the bladder or urethra during urination. Very often women feel an uncomfortable pressure above the pubic bone, and some men experience fullness in the rectum. The voided urine itself may look milky or cloudy or may even reddish if blood is present. Normally, a UTI does not cause fever if it is in the bladder or urethra. A fever may mean that the infection has reached the kidneys. Other symptoms of a kidney infection include pain in the back or side below the ribs, nausea, or vomiting.

In children, symptoms of a urinary infection may be overlooked or attributed to another disorder. A child or an infant with urinary tract infection look irritable, does not eat normally and also suffers from an unexplained fever that does not go away, has incontinence or loose bowels. Unlike adults, children are more likely to have fever and no other symptoms. This can happen to both boys and girls.
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So the urinary tract infection (UTI) is a serious health problem affecting millions of people each year. It is the second most leading bacterial infection in the world. Urinary tract infection solely responsible for 10 million of cases in Europe alone per year and *Escherichia coli* is the main causative organism of UTI.

*E. coli* is very rapidly developing multiple drug resistance. Resistance develops quickly, in parallel with the use of antibiotics. An 11-year study of cancer patients at a hospital in Switzerland found that no strains of *Escherichia coli* resisted any of the fluoroquinolone antibiotics between 1983 and 1990. But between 1991 and 1993, 28 percent of the strains tested were resistant to all five of them. During the study period, the percentage of patients getting antibiotics rose from 1.4 percent to 45 percent.

Extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* are increasingly common. They are assumed to be resistant to all β-lactams and many are also resistant to other antimicrobials. Multidrug resistant organisms present therapeutic dilemmas, and treatment with intravenous agents is often recommended because of in vitro resistance to oral antimicrobials. However, in vitro inactivation of ESBLs by oral β-lactamase inhibitors makes
their use in minor infections theoretically attractive option that has not been well studied (Paterson et al., 2005 and Boyd et al., 2004).

So *Escherichia coli* infections have become problematic due to the emergence of a multiple-antibiotic resistant *E. coli* in urinary tract infections. Uropathogenic *E. coli* (UPEC) is extremely resistant to amoxicillin, nalidixic acid, ticarcillin, cefotaxime, cefoxitime, ceftriazone, mecillinam and enrofloxacin, norfloxacin. Since the description of the first TEM-derived β-lactamases conferring resistance to clavulanate, a number of these enzymes have emerged in various parts of the world (Jacoby and Bush, 1997).

The widespread and most often use of antimicrobial drugs, inappropriate prescribing of antibiotics, poor infection control strategies and inadequate administration of antimicrobial agents have led to a general rise in the emergence of resistant bacteria like *E. coli*.

This study was undertaken to determine the various enteropathogenic virulence factors in *E. coli* isolates from UTI cases using polymerase chain reaction amplification to have a better understanding of the enteropathogenic strains and also to see if they really form a distinct group or have evolved from any other pathotypes. A study of the antimicrobial
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sensitivity and resistant pattern among the isolates was also done since widespread and most often the misuse of antibiotics has been a cause of alarming raise in drug resistant strains. As *E. coli* is a common inhabitant of the intestinal tract of humans and animals, the number of bacteria varies in different animals, but an animal will excrete *E. coli* from 130 million to over 18 billion *E. coli* each day. Hence, there is a close association between *E. coli*, faecal material and, possibly, enteric pathogens.

At a first instance, although the importance of the presence of the enterotoxigenic *E. coli* in the urinary tracts of human is not realized, its release into the environment through urinary secretions along with urine, even in small numbers may result into contamination of water bodies and in turn may result into contamination of foods and then to serious public health problems. *E. coli* clones have evolved having the ability to cause a broad spectrum of diseases in man and animals assuming worldwide public health importance. The concern is mostly related to the potentially serious clinical outcomes of the infections and possible transmission to human from different sources.

Therefore, studies concerned with surveillance of antibiotic resistance among bacteria especially toxigenic strains causing human diseases are needed. There is no record of systematic survey of the enteropathogenic
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*E. coli* associated with human illness from the North Eastern region. Through this study, with an aim of accumulating data on UTI patients carrying enterotoxigenic bacteria that are multidrug resistant would inturn help the health authorities to take necessary steps to prevent spread of disease among masses. Therefore, this work has been undertaken to initiate the survey work basing on the UTI patients of North eastern region in which drug resistant *E. coli* isolates were subjected to PCR analysis for diagnosis of their enteropathogenicity.

So, the present study was taken up with following objectives:

1. To collect and process urine samples from north eastern region.
2. To isolate and identify *Escherichia coli* causing UTI.
3. To study serotypings of isolated *Escherichia coli*.
4. To study the antibiogram of *E. coli*.
5. To select multidrug resistant strains of *E. coli*.
6. To study the virulence factors like stx1, stx2, elt, est, and hly genes by polymerase chain reaction amplifications to detect the association of enteropathogenic *E. coli* strains with UTI.