ABSTRACT

*Escherichia coli* are the predominant colonic microorganisms of all warm blooded animals including human beings which usually remain harmless and confined to the intestinal lumen. But certain *E. coli* clones have acquired 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. Infections due to pathogenic *E. coli* may be limited to the mucosal surfaces or can disseminate throughout the body. Multiple virulence factors like the ability to adhere, proliferate and colonize the small intestine as well as the capacity to produce toxins like enterotoxins contribute to the pathogenicity of these organisms. Urinary tract infections, sepsis/meningitis and enteric/diarrhoeal diseases are the main clinical symptoms of *E. coli* infection.

*Escherichia coli* are the most frequently isolated pathogen (70-90%) from cases of urinary tract infection (UTI). The 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. Women are
especially prone to UTIs for reasons that are not yet well understood. *E. coli* present in the gastrointestinal tract as commensals 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. 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 nondiarrheal (urinary tract) hemolytic uremic syndrome.

So, the present study was carried out with an aim to ascertain the association of enteropathogenic *E. coli* with urinary tract infection. A total of 650 urine samples from patients with clinical symptoms of UTI were collected from 14 different places from northeastern region of the country for the isolation of *E. coli*. Out of 650 samples processed, 405 number of *E. coli* strains (62.3%) were retrieved followed by *Klebsiella* sp. (14.7%), *Proteus* sp. (6.46%), *Enterococcus*
sp. (3.38%), coagulase negative *Staphylococcus* (2.15%), *Staphylococcus aureus* (1.38%) and 61 were negative for the test either due to multiple growth of organisms (15%) or no growth of any organisms (85%) by carrying out cultural, morphological, microscopical, and biochemical tests.

A serotype constitutes a group of microorganisms, viruses, or cells that are classified together based on their cell surface antigens and the process of determination of serotype is called serotyping. Over 700 antigenic types or serotypes of *E. coli* have been recognized based on somatic (O), flagellar (H) and capsular (K) antigens.

In the present work the pure isolates of *E. coli* were subjected to serotyping. Out of 405 isolates serotyped, 303 were typable and belonged to 28 different serotypes, 60 isolates were refractory to typing (untypable) and 42 were rough. The frequency of serotypes of *E. coli* isolates was 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).

Microorganisms can be killed, eliminated or inhibited by a number of physical, chemical and other means. All these agents which act against the microbes are called 'Antimicrobial agents'. The entire process of *in-vitro* testing
of susceptibility of a particular organism against antimicrobial agents is termed as ‘Antibiogram’ that helps in selection of proper antibiotic therapy for control of infection.

Therefore antibiogram of 405 *E. coli* were studied against 24 antimicrobial agents by disc diffusion method. The highest degree of sensitivity was shown by the isolates towards Imipenem (94.33%), followed by Nitrofurantoin, Pipercillin Tazobactum, Amikacin, Levofloxacin, Ciprofloxacin, Ofloxacin, Netilmicine, Norfloxacin, Amoxicillin/Clavulanic acid, Nalidixic Acid, Cefepime and Gentamicin in the order of 80.33%, 75.31%, 74.33%, 68.65%, 68.65%, 68.65%, 64.45%, 62.25%, 61.24%, 59%, 54.57% and 53.83%, respectively. 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%) were found to be less effective against the isolates.

*E. coli* is developing multidrug resistance very rapidly. Indiscriminate use of antimicrobial agents, inadequate doses and poor infection control policy in humans during infections has resulted in the development of resistance amongst bacteria, including *E. coli*. Antibiotic usage selects for resistance not only in pathogenic bacteria but also in the endogenous flora of
exposed individuals (animals or humans) or population. So the treatment of UTI by *E. coli* is a burning problem of clinicians of today’s world. Our study revealed 370 isolates to be multidrug resistant out of total 405. Fifty isolates were found to be resistant to 13 antimicrobial agents, 62 isolates to 10, 30 isolates to 9, 21 isolates to 7 and 203 isolates to 5 agents. Antibiotic resistant pattern revealed the isolates were highly resistant to Pipercillin (77%), Co-Trimoxazole (76%), Cefuroxime (71.35%), Tetracycline (69.38%), Ticarcillin (68.64%), Ampicillin (64%), Ceftazidime (63%), Ticarcillin/Clavulanic Acid (60.74%), Chloramphenicol (56.54%), Cefazoline (54.33%), Tobramycin (50.37%), Gentamicin (46.17%), Cefepime (45.43%), Nalidixic Acid (41%), Amoxycillin/Clavulanic acid (38.76%), Norfloxacin (37.75%), Netilmicin (35.55%), Ofloxacin (31.35%), Ciprofloxacin (31.35%), Levofloxacin (31.35%), Amikacin (25.67%), Pipercillin Tazobactum (24.69%), Nitrofurantoin (19.67%) and Imipenem (5.67%).

The infections due to pathogenic *E. coli* may be limited to the mucosal surfaces or can disseminate throughout the body. Multiple virulence factors like the ability to adhere, proliferate and colonize the small intestine as well as the capacity to produce enterotoxins contribute to the pathogenicity of these organisms. Several classes of enteropathogenic *E. coli* have been recognized on the basis of the types of enterotoxins they produce. *E. coli*
producing heat labile (LT) or heat stable (ST) enterotoxins coded by the *est* and *elt* genes are termed as enterotoxigenic *E. coli* (ETEC). Both LT and ST occur in two different forms and they disrupt the host cell functions by disturbing the intercellular ion concentrations, leading to intestinal fluid accumulation resulting in osmotic diarrhoea. *E. coli* possessing the *hly* gene termed as enterohaemorrhagic *E. coli* (EHEC) produces haemolysins, which act as spore forming cytolysin on the eukaryotic cells and result in cell lysis. Shiga like toxins (Stx), which exist in two antigenically distinct forms; Stx1 and Stx2, encoded by the *stx1* and *stx2* genes associated with hemorrhagic colitis and hemorrhagic uremic syndrome in humans and inhibiting protein synthesis are elaborated by *E. coli* strains named as Shiga toxin producing *E. coli* (STEC) and are recognized as important food borne pathogens of human. Disease outbreaks caused by these enteropathogenic groups of *E. coli* are frequently reported from all over the world causing great concern.

But the major problem lies in identifying and characterizing pathogenic strains of *E. coli* as they predominantly resemble commensal *E. coli* in many aspects apart from producing toxins. Therefore, the molecular methods like polymerase chain reaction have been widely preferred for detection of the virulence genes to identify the enteropathogenic *E. coli* strains due to its
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sensitivity and specificity. Prevalence studies of these toxigenic genes are of importance in pathogenic characterization of the isolates.

Therefore, the polymerase chain reaction amplification of 370 isolates were carried out by using their specific primers that are used to detect the prevalence of 5 different virulence genes viz., stx1, stx2, est, elt and hlyA genes and result showed the presence of est gene in 21 isolates belonging to serotypes O25 (7) and O171 (7) and 7 isolates were refractory to typing (untypable). Among the other genes tested, hlyA and stx2 genes were detected in 7 isolates each belonging to serotype O41 and O5. None of the isolates were found to harbour stx1 and elt genes.

The presence of enterotoxigenic genes in E. coli isolated from UTI cases suggest that E. coli from UTI cases not necessarily belong to a completely distinct pathotype but enterotoxigenic strains can also cause UTI. However, the mechanism that enables them to infect the urinary tract need to be studied more thoroughly. Emerging resistance to most of the widely used antimicrobial agents shown by the E. coli from cases of urinary tract infections suggest incorporation of appropriate measures to check drug prescribing policies.