

## **STUDIES ON THE PREVALENCE OF *ESCHERICHIA COLI* IN THE URINARY TRACT INFECTION OF NORMAL AND PREGNANT WOMEN.**

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### **ABSTRACT**

Some strains of *Escherichia coli* produce cytotoxins (verotoxin 1 and 2 or shiga-toxin 1 and 2 and shiga toxin variants). Verotoxins (VT) or shiga toxins (ST) produced by some *E. coli* strains damage endothelial cells in both kidney and brain, causing renal failure and neurological complications. The present study deals with the presence of pathogenic verotoxin producing multiple drug resistance *Escherichia coli* causing Urinary tract infection. In the present investigation urine samples positive for urinary tract infections from both normal and pregnant women were screened for the presence of *E.coli*. The urine samples were collected from different hospitals and pathology labs in Bangalore. The samples were plated on Nutrient agar and checked for

Gram negative bacilli which were further plated on Eosin methylene Blue (EMB) agar for the characterization of metallic green sheen producing *E.coli* from other organisms. The present study revealed that of the 153 samples taken 71 showed positive for *E.coli*. The ratio of pregnant to non pregnant women positive to *E.coli* infection was found to be 1:3 with 52 and 17 positive metallic green colonies showing Gram –ve bacilli. The assessment provides a potential for sourcing novel antibiotic substances for chemotherapy against numerous resistant pathogenic strains of *Escherichia coli*.

**Keywords:** *Escherichia coli*, Multidrug resistant, Verotoxin, Urinary tract infection.

### **INTRODUCTION**

*Escherichia coli* are one of the most common uro-pathogens, accounting for approximately 90% of UTI (Urinary Tract Infection). The causative uropathogens included *Escherichia coli*

(86%), *Staphylococcus saprophyticus* (4%), *Proteus* species (3%), *Klebsiella* species (3%), *Enterobacter* species (1.4%), *Citrobacter* species (0.8%), *Enterococcus* species (0.5%) and other less frequent isolates in aggregate causing 1.3% of infections. *Escherichia coli* causes up to ~80% of uncomplicated urinary tract infection and 7000 people die from *Escherichia coli* related infections annually. In most cases of UTI the bacteria originates from the patient's own intestinal flora and spread via the perineum to the vagina. Depending on the well known virulence factors but less understood host factors, the bacteria ascend via the urethra to the bladder where they infect the bladder epithelium causing UTI. It can present with a range of symptoms or may be totally asymptomatic and diagnosed only on routine dip testing. The presenting symptoms will vary with age and sex of the patient and also with the severity and site of the infection.

Almost half of all women report at least one UTI sometime during their lifetime, and after an initial UTI, 20-30% of women experience a recurrence. Antibiotic use changes the vaginal flora and promotes colonization of the genital tract with E Coli resulting in subsequent increased risk of UTI.<sup>[1, 2]</sup> Different types of UTI include urethritis (infection of the urethra), cystitis (infection of the bladder) and pyelonephritis (infection of the kidneys). Although everyone is susceptible to UTI, there are specific subpopulations that are at increased risk of UTI. That includes: Women- vulnerable in part because the urethra is only 4 centimeters long and bacteria have only this short distance to travel from outside to the inside of the bladder, People with Urinary Catheters – such as critically ill who cannot empty their own bladder, People with Diabetes – changes to the immune system makes a person with diabetes more vulnerable to infection, Men with Prostate Problem – such as an enlarged prostate gland that can cause urinary retention and incontinence, Babies – especially those born with physical problems (congenital abnormalities) of the urinary system and Pregnant women.<sup>[3]</sup>

Management of the infection is becoming progressively complicated by the ongoing increase in resistance to antibiotics manifested by UTI causing organisms. A remarkable increase in antibiotic resistance among the UTI causing E. coli isolates has been observed during the last few years. Resistance has emerged even to newer, more potent antimicrobial agents.<sup>[4]</sup> Multiple antibiotic resistance in bacteria has been ascribed to the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype.<sup>[5-8]</sup> Since a plasmid or transposon can carry several resistance indexes, resistance to several antimicrobial agents may be acquired simultaneously and results in multiple drug

resistant (MDR) organisms. <sup>[9-12]</sup> MDR organisms and their associated emerging complications make the treatment more challenging and many even threaten the respective patient's lives.

It has been demonstrated that certain *E. coli* isolates produce a closely related group of toxins, which were initially called verotoxins. This family of toxins were subsequently called Shiga-like toxins (SLT), and more recently Shiga toxins (Stx), because of the close relation to the Stx of *Shigella dysenteriae* type 1. <sup>[13]</sup> These pathogenic strains of *E. coli* produce specific virulence factors that facilitate their interactions with the target host: colonization of the epithelial surfaces, crossing of the mucosal barriers, invasion of the blood stream and internal organs, and/or production of toxins causing cellular and tissue damages leading to organ dysfunction, clinical signs, symptoms and diseases. They are grouped in so-called "pathotypes" (or "virulotypes") on the basis of four criteria: the clinical syndrome, the target species, the adherence factors, and/or the production of exotoxins.

Vero toxin producing *E. coli* are also called Entero hemorrhagic *E. coli* or Shiga toxin producing *E. coli* strains. These strains are related bacteriophage-encoded cytotoxins that block protein synthesis and induce host cell death. <sup>[14]</sup> These toxins are neurotoxic, cytotoxic and enterotoxic and are very effective against the vascular endothelium found in the digestive tract, glomerulus of the kidneys, lungs and heart. Several sites of the central nervous system are affected as well. Strains that produce shiga toxins can cause disease of varying severity, including watery diarrhea, bloody diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome (HUS) and death.

The two most important toxins, Stx1 and Stx2 (for shiga-toxin) are found on such islands and are composed of 5 B subunits surrounding an active A component, which is transported into the cell after the B subunits recognize and bind to a specific glycolipid receptor, Gb3. Vero toxins bind to this specific receptor identified as the glycosphingolipid globotriaosylceramide (Gb3) on leukocytes and endothelial cells of target organs, and cell susceptibility to Vero toxin damage is a function of Gb<sub>3</sub> expression on cell membranes. <sup>[15,16]</sup>

## MATERIALS & METHODS

### Sampling

The likelihood of detecting a UTI by urine culture is highest if urine is collected on arising. This sample is likely to be most concentrated and bacteria in the bladder will have had time to multiply overnight.

The present study was conducted on verotoxin producing *Escherichia coli* isolated from urine samples of normal and pregnant women from various hospitals and diagnostic centers. The samples were transported in ice packs and refrigerated until further use in the lab. A total of 153 urine samples were collected by clean catch midstream from female patients among which 90 were pregnant women samples and 63 were non-pregnant.

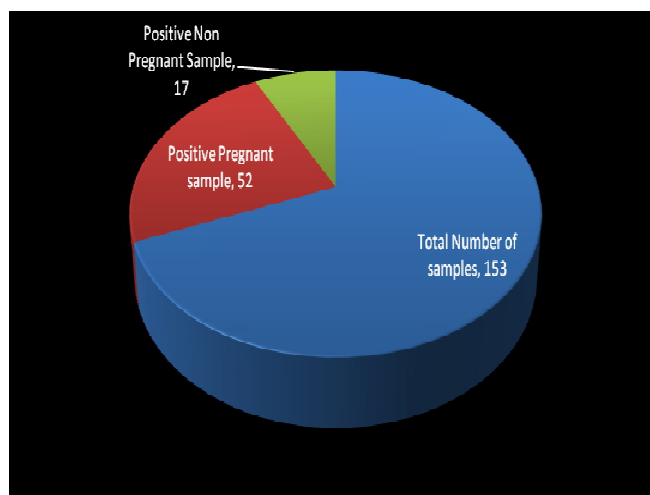
### Plating

Samples for urine cultures were tested within half an hour of sampling or refrigerated. Samples were inoculated on Blood agar as well as Mac Conkey's agar and incubated at 37° C for 24 hours, and for 48 hours in negative cases. A specimen was considered positive for UTI in the light of the number of yielded colonies ( $\geq 10^5$  cfu/ml) and the cytology of urine through microscopic detection of bacteriuria and PMNS ( $\geq 8$  leukocytes/mm<sup>3</sup>) and also colony morphology on Mac Conkey's agar. It is further confirmed by inoculating on EMB agar and looking for metallic sheen. Bacterial identification was based on standard colony characteristics and biochemical properties of isolates.

## RESULTS

### Enumeration

Of the 153 urine samples investigated 90 pregnant women and 63 non-pregnant women samples analyzed showed 52 and 17 positive cases of UTI respectively. The results were analyzed based on the CFU's formed on blood agar plates and also brick red colonies which gave precipitating margins were considered positive for *Escherichia coli*. Metallic green sheen colonies on EMB agar further confirmed the presence of *Escherichia coli*. Gram's staining yielded slender Gram negative rods. Of the uropathogens isolated as expected *Escherichia coli* was the most frequent isolate accounting to 80%, followed by *Staphylococcus* sps, *Proteus* sps, *Klebsiella* sps, *Pseudomonas* sps, *Streptococcus* sps, *Enterobacter* sps, *Enterococcus* sps, *Candida* sps making up the rest 20%. The study revealed that Gram negative bacteria were responsible for 92% of UTI while Gram positive bacteria amounted for the remaining 8%.



**Fig-1 Graphical percentage representation of positive and negative samples**

**Table-1 Tabular representation of positive and negative samples**

Sample no.	Total positive for E.coli	Pregnant	Non pregnant
1	0	0	0
2 -9	4	1	3
10-13	0	0	0
14-30	10	4	6
31- 65	15	6	9
66 -100	8	2	6
101- 04	1	0	1
105 -116	4	0	4
117- 125	5	1	4
126 -129	2	0	2
130 - 136	3	0	3
137 - 146	8	1	7
147 -153	9	2	7

## DISCUSSION

Urinary tract infections (UTI) are the infection of the urinary bladder (cystitis) - lower GI tract or of the kidneys (pyelonephritis)- upper GI tract. Almost 95% of UTIs are caused by bacterial infections both gram positive or gram negative bacteria. *Escherichia coli* are one of the most common uropathogens, accounting for ~80-90% of UTIs (Urinary Tract Infections). In the present study among all the organisms isolated from positive UTI urine samples, *Escherichia coli* was found to be the most frequent isolate. *Escherichia coli* amounted to ~80% of the total uropathogens screened. The ratio of pregnant to non pregnant women positive to *E.coli* infection was found to be 1:3 with 52 and 17 positive metallic green colonies showing Gram –ve bacilli. Majority of pathogens were isolated from pregnant

women as they are found to be more susceptible to UTI infection in comparison to adult women population. The risk of UTI in women increases during pregnancy and after menopause.

This will give The present study will be an innovative step towards the development and discovery present an insight into understanding the pathogenicity and acquisition of virulence traits in *Escherichia coli* and also give us an understanding for new methodology to control resistant *Escherichia coli* causing UTI and finding out alternative therapies.

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