# STUDY OF VIRULENCE FACTORS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF UROPATHOGENIC ESCHERICHIA COLI IN A TERTIARY CARE HOSPITAL


## ABSTRACT

### INTRODUCTION

Escherichia coli is the major causative agent of urinary tract infections. UPEC possess a variety of virulence factors that enable it to colonize, invade and persist within the urinary tract, leading to both complicated and uncomplicated UTIs. Knowledge of virulence factors of Escherichia coli is responsible for pathogenesis of UTIs and their antimicrobial susceptibility pattern will help in better understanding of the treatment of UTI. Emergence and spread of multi drug resistant strains of Escherichia coli have raised considerable interest in understanding their diversity and epidemiology of infections in humans.

### AIM

To determine the virulence factors and antimicrobial susceptibility pattern of Uro Pathogenic Escherichia coli.

### MATERIALS AND METHODS

A prospective study is done on urine samples over a period of 3 months from May 2025 to July 2025 in a tertiary care hospital to detect virulence factors like Haemolysis, Hemagglutination and gelatin hydrolysis.

Antibiotic susceptibility test was done by Kirby Baur disc diffusion method as per the CLSI guidelines.

### RESULTS

Out of total 350 urine samples tested 114 were Escherichia coli isolates. Out of the 114 Escherichia coli isolates 69 were females and 45 males. Among the 114 isolates -43 isolates showed haemolysis virulence factor, 31 isolates showed hemagglutination factor- 22 were mannose resistant and 9 mannose sensitive hemagglutination and, 11 isolates showed gelatin hydrolysis and 29 isolates did not show any of the virulence factor.

Antibiotic susceptibility testing revealed that UPEC strains showed maximum resistance to **Ampicillin (55.2%)**, followed by **Cotrimoxazole (48.2%) and Norfloxacin (44.7%)**. Most isolates were sensitive to **Meropenem (82.4%)**, Amikacin (79.8%) and followed by **Nitrofurantoin (74.5%)**.

### CONCLUSION

Detection of virulence factors of Uro-pathogenic Escherichia coli shows a strong association to urinary tract infection. And presence of multiple virulence factors leads to drug resistance.



### KEY WORDS

Urinary tract infection, Uropathogenic Escherichia coli, Virulence factors, Haemolysis, Hemagglutination, Gelatin hydrolysis

## INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span. They can be symptomatic or asymptomatic. Escherichia coli is the most common cause of UTIs, accounting for about 85% of community acquired and 50% of hospital-acquired infections, it predominates strongly at most ages. UPEC is the main cause of community-acquired UTIs (about80-90%).

Escherichia coli is a commensal in the human intestinal tract, when enters into unnatural sites, it can cause a variety of infections, e.g., UTIs, sepsis, pyelonephritis etc. Serotypes which lead to UTIs are designated as uropathogenic Escherichia coli (UPEC)[1].It has been known that certain serotypes of E.coli are consistently associated with uro-pathogenicity and are designated as uropathogenicE.coli that expresses chromosomally encoded virulence markers[3]. Uropathogenic strains account for 90% of all UTIs among ambulatory patients and upto 50% of all nosocomial UTIs[2].

The common virulence factors include surface hydrophobicity, colonization factor, capsule, serum resistance, resistance to phagocytosis, hemolysin production, enterotoxin and siderophore, fimbriae and hemagglutination. The ability of *E. coli* to adhere to the uroepithelium is mediated by fimbriae, thereby resisting elimination by the flow of urine. Adhesion is therefore measured to be important step in the pathogenesis of UTI[4].

During UTIs, UPEC pathogenesis includes:

(a) UPEC colonization of the periurethral and vaginal areas with colonization of the urethra;

(b) ascending into the bladder lumen and growth as plantktonic cells in urine;

(c) adherence to the surface and interaction with the bladder epithelium defense system (see below);

(d) biofilm formation;

(e) invasion and replication by forming bladder Intracellular Bacterial Communities (IBCs) where quiescent intracellular reservoirs (QIRs) form and reside in the underlying urothelium;

(f) kidney colonization and host tissue damage with increased risk for bacteremia/septicemia.

Considering the high degree of morbidity and mortality due to UTIs caused by uropathogenic E. coli and also the drug resistance among strains has further aggravated the problem of UTI's. Therefore, the present study was carried out with aim to know the prevalence of various virulence factors and the antimicrobial susceptibility pattern in UPEC[5].

## MATERIALS AND METHODS

This is a prospective study which was conducted in the Department of Microbiology in a tertiary care hospital over a period of 3 months i.e from May 2025-July 2025. Patients of all age group were included. A total of 350 urine samples were tested out of which 114 were Escherichia coli were isolates.The samples were processed immediately as per the standard guidelines in the lab. The isolates were taken for the detection of virulence factors.

The virulence factors tested were

1.Haemolysin production:

2.Haemagglutination

3.Gelatin hydrolysis

1.Haemolysin production

The Escherichia coli isolates were inoculated onto 5 %sheep blood agar and incubated overnight at 37degree Celsius and observed for a zone of complete lysis around the colony. Escherichia coli ATCC 25922 was used as a negative control.

Presence of clear zone of complete hemolysis indicates hemolysin production.

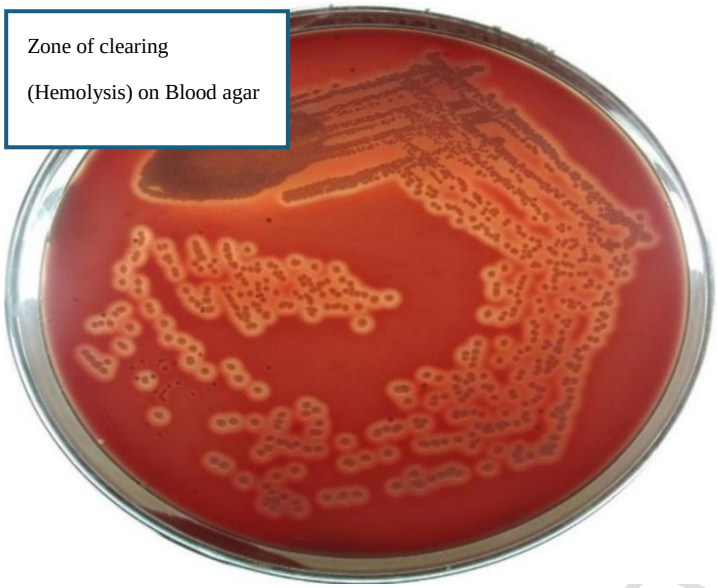


FIG 1: HEMOLYSIS

2.HAEMAGGLUTINATION

The test was carried out as per the direct bacterial hemagglutination test-slide method. One drop of red blood cell (RBC) suspension was added to a drop of broth culture and the slide was rocked at room temperature for 5 min. Presence of clumping was taken as positive for hemagglutination. Mannose-sensitive hemagglutination was detected by the absence of hemagglutination in a parallel set of test in which a drop of 2% W/V D-mannose was added to the red cells and a drop of broth culture. Mannose resistant hemagglutinating (MRHA) was detected by the presence of hemagglutination of 3% 'O' blood group human RBCs in the presence of 2% W/V D-mannose.

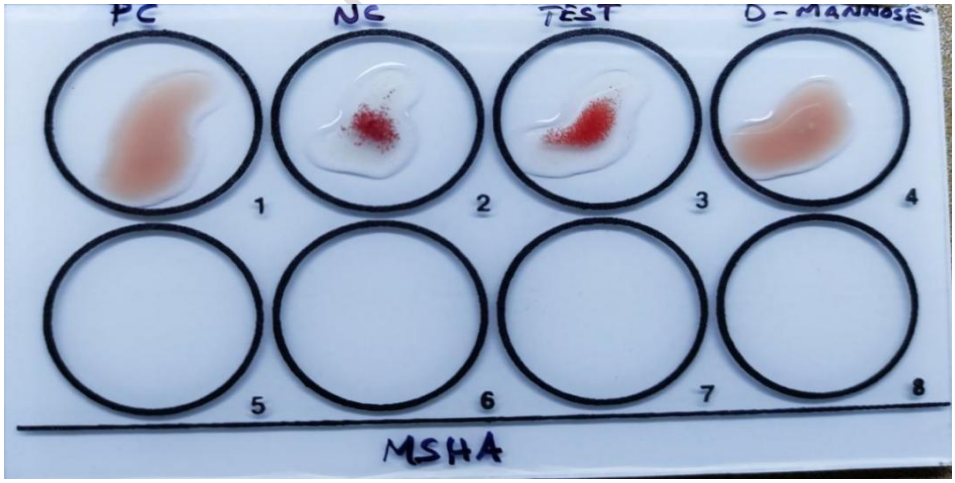


FIGURE 2: MANNOSE SENSITIVE HAEMAGGLUTINATION

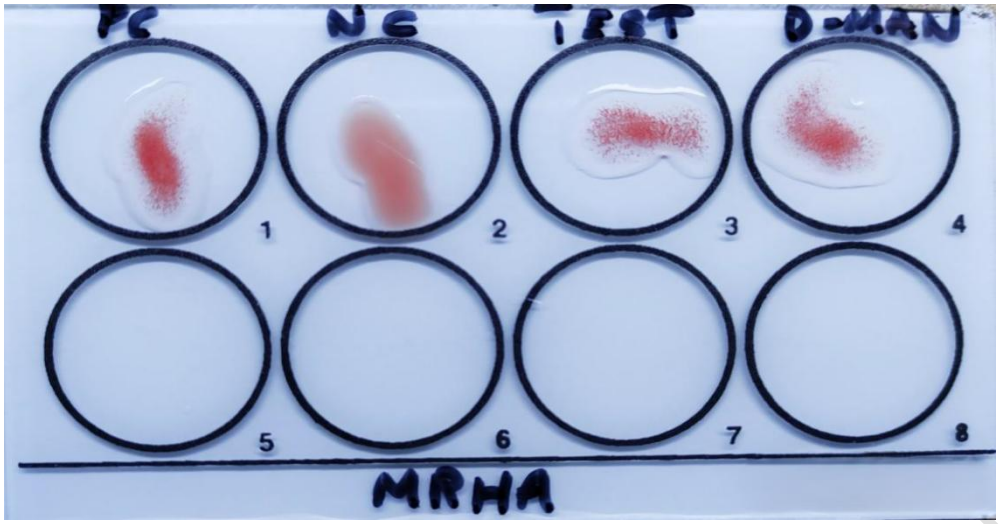


FIGURE 3: MANNOSE RESISTANT HAEMAGGLUTINATION

3.GELATIN HYDROLYSIS.

Gelatinase production was tested using gelatin agar. Escherichia coli isolated was inoculated on gelatin agar and incubated overnight at 37 degrees celsius for 24 hrs. Later the gelatin agar plate was flooded with 1%tannic acid. Development of opacity around colonies shows gelatinase production.

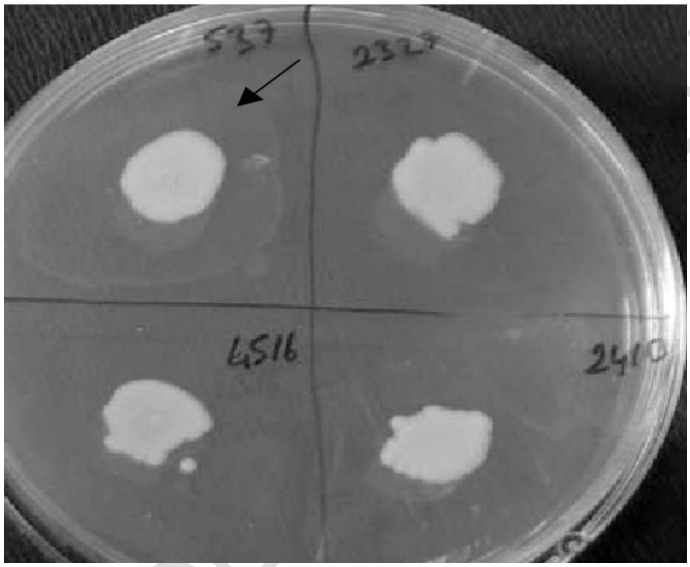

FIGURE 4: GELATIN HYDROLYSIS

4. Antimicrobial susceptibility

Antimicrobial susceptibility testing was done on Mueller Hinton Agar by Kirby Bauer disc diffusion method as per CLSI guidelines.

## RESULTS

A total of 350 urine samples received from symptomatic cases of urinary tract infection with significant bacteriuria were taken. Out of these, 114 Escherichia coli isolates were processed and studied for the virulence factors and their antibiotic susceptibility pattern. Out of the 114 Escherichia coli isolated samples, 60.5% were from females and 39.47% were from males. Among 114 isolates, 37.7% showed haemolysis, 19.3% showed mannose resistant haemagglutination, 7.9% showed mannose sensitive haemagglutination, 9.7% showed gelatin hydrolysis and 25.4% did not show any of the virulence factors.

Antibiotic susceptibility testing revealed that UPEC strains showed maximum resistance to Ampicillin (55.2%)**,** followed by Cotrimoxazole (48.2%) and Norfloxacin (44.7%). Most isolates were sensitive to Meropenem (82.4%), Amikacin (79.8%) and followed by Nitrofurantoin (74.5%).

TABLE 1: GENDER WISE DISTRIBUTION OF ESCHERICHIA COLI ISOLATES

| GENDER | TOTAL | PERCENTAGE |
|---|---|---|
| FEMALES | 69 | 60.5% |
| MALES | 45 | 39.4% |
| TOTAL | 114 | 100% |

TABLE 2: VIRULENCE FACTORS AMONG THE UROPATHOGENIC ESCHERICHIA COLI ISOLATES(n=114)

| VIRULENCE FACTOR | NUMBER | PERCENTAGE |
|---|---|---|
| HAEMOLYSIS | 43 | 37.7% |
| HEMAGGLUTINATION | (31) | |
| MANNOSE RESISTANT HEMAGGLUTINATION | 22 | 19.3% |
| MANNOSE SENSITIVE HEMAGGLUTINATION | 9 | 7.9% |
| GELATIN HYDROLYSIS | 11 | 9.7% |

TABLE 3: ANTIBIOTIC SUSCEPTIBILITY PATTERN OF UPEC ISOLATES

| ANTIBIOTIC | SENSITIVE | PERCENTAGE | RESISTANT | PERCENTAGE |
|---|---|---|---|---|
| AMPICILLIN | 51 | 44.8% | 63 | 55.2% |
| TRIMETHOPRIM SULFAMETHOXAZOLE | 59 | 51.8% | 55 | 48.2% |
| NORFLOXACIN | 63 | 55.3% | 51 | 44.7% |
| CEFTRIAXONE | 67 | 58.8% | 47 | 41.2% |
| MEROPENEM | 94 | 82.4% | 20 | 17.6% |
| AMIKACIN | 91 | 79.8% | 23 | 20.2% |
| NITROFURANTOIN | 85 | 74.5% | 29 | 25.5% |

## DISCUSSION

UPEC are the most important group of micro-organisms responsible for UTI. UPEC differ from non-pathogenic E. coli by the production of specific virulence factors which enable the bacteria to adhere to uroepithelial cells and to establish UTI[5].UTIs which are not properly treated from their onset can become a renal threat in time, finally leading to renal failure[8].

These virulence factors enable some members of the normal flora to elicit an infection by overcoming the host defence mechanisms[1].

The capacity of E. coli to produce many virulence factors contributes to its pathogenicity[1,7].

Incidence of UTI was more common in females 60.5% than in males in our study. Piatti et al[5,6]. also reported a higher prevalence of UTI in female (77%). The reasons for the high prevalence of the UTIs in females can be due to the anatomical structure of the urogenital tract having short urethra, presence of normal flora in vagina, menstrual cycle and pregnancy[5].

Priscilla et al[1] also reported a higher incidence of UTI was more common in females (66.72%) than in males (33.27%) in our study.

Our study also correlates with Sanjay Singh Kaira et al [4] who reported 56.09%, Mittal et al[1,8] (53.3%) and Chhaya et al[8,9](53%).

Hemolysin production is associated with human pathogenic strains of E. coli, especially those causing more clinically severe forms of UTI[5,10]. It is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury and impaired host defenses[1,11]. In the present study, 37.7% E. coli isolates produced hemolysin. In other studies conducted by Raksha et al[5,12], Siegfried et al[5,13] Hughes et al[5,14], Shruthi et al [1,15]hemolysin production was detected in 41.36% and 59.6%, 59.7% and 41.9% isolates respectively.

The role of bacterial adherence in the pathogenesis of urinary tract infection is that colonization of the urogenital epithelium of susceptible individuals by specific bacteria is associated with successful microbial invasion of the urinary tract[16] and lead to UTIs. Thus, possession of MRHA by UPEC can be considered as one of the important virulence factor in the pathogenesis of UTIs. My study correlates with Seigfried et al [1,13], Vagarali et al [13] , Raksha et al[1,12], Kauser et al [3,16] have reported the incidence of MRHA E.coli isolates as 23%, 25%, 30.9%, 30% respectively. In the present study also the rate of MRHA positive E. coli isolates was 19.3 %

Gelatinase, an important virulence factor which is capable of hydrolyzinggelatin, collagen, and is associated with inflammation. Shetty et al [17,18] observed that gelatinase is not an important virulence factor. While Mittal et al [5] observed that gelatinase producing strains were multidrug resistant. In the present study Gelatinase production was seen in 25.4 % of isolates which is similar to Mittal et al [5] where it was 67.5%. But Vaish et al [18,19]& Jayanthi et al [18,20] showed lesser production of gelatinase which was 2% & 6% respectively.

Antibiotic susceptibility pattern was studied for all E. coli isolates. These isolates were most commonly resistant to Ampicillin, Cotrimoxazole and Norfloxacin. And the maximum sensitivity was shown to Meropenem, Amikacinand then followed by Nitrofurantoin. The present study has shown the production of various virulent factors and developing drug resistance in UPEC.

The antibiotic susceptibility pattern observed in our study correlates with Tabasi et al [8,21], Karam et al [8,22] and Chhaya et al[8,9].

The present study also correlates with Kauser Y et al [16,18], Vaish et al [18,19]& Jayanthi et al [18,20].

Antibiotic resistance may provide a substantial advantage to the survival of the pathogen. The drug resistance among UPEC is on rise therefore the selection of appropriate antibiotics after antibiotic susceptibility testing is must for proper treatment of patients and to avoid emergence of drug resistance.

Resistance to commonly used antibiotics is because of excessive use and misuse of the antibiotics by the healthcare personnel and dissemination of multidrug resistance among hospital strains[18,23,24].

## CONCLUSION

Detection of virulence factors of Uro-pathogenic Escherichia coli shows a strong association to urinary tract infection. And presence of multiple virulence factors leads to drug resistance. So this helps in better understanding and treatment of Urinary tract infection.Since most Urovirulent strains express multiple virulent factors simultaneously, further studies at molecular level are necessary.

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