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Study of virulence factors of uropathogenic Escherichia coli and its antibiotic susceptibility pattern

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***Abstract*---Background**: Urinary tract infection (UTI) is one of the most common nosocomial infections, caused by Escherichia coli. 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. The aim of this study is to check the virulence factors of uropathogenic E. coli. **Methods**: The study was conducted over a period of 1 year in microbiology department, Jagannath Gupta Institute of Medical Sciences & Hospital, Kolkata. Urine samples received were processed as per standard microbiological procedures. Virulence factors such as hemolysin, hemagglutination, serum resistance, gelatinase, biofilm production and siderophore production were studied. The antimicrobial susceptibility was done as per Clinical and Laboratory Standard Institute Guidelines. **Results:** Out of 550 E. coli isolates 367 (66.72%) cases were from females and 183 (33.27%) were males. Thus female: male ratio was 1:3. Hemolysin production was seen in 53.63%,

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hemagglutination in 54.18%, serum resistance in 53.27%. **Conclusions:** UTI is more common in middle aged females and in community set-up. The knowledge of virulence factors of E.coli will help in better understanding of the organism pathogenicity and guided empirical therapy can result in better treatment outcome.

***Keywords*---**Uropathogenic Escherichia coli, Urinary tract infection, Antibiotic susceptibility, multiple drug resistance, Virulence factors.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span[1].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 hospitalacquired infections, it predominates strongly at most ages[2]. E. 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. It has been known that certain serotypes of E. coli are consistently associated with uropathogenicity and are designated as uropathogenic Escherichia coli (UPEC) [3]. For the first time in the late 1970s it was recognized that E. coli strains causing urinary tract infections typically agglutinate human erythrocytes despite the presence of mannose and this was mediated mainly by fimbriae[4]. The virulence factors of E. coli are multiple. The important virulence factors in the pathogenesis of urinary tract infection (UTI) include adhesions (P fimbriae, certain other mannose-resistant adhesins, and type 1 fimbriae), the aerobactin system, hemolysin, K capsule, and resistance to serum killing, hemolysin production and siderophore production[5]. Bacterial adherence and colonization of the urinary tract by UPEC strains are mediated by the expression of several types of fimbrial and non-fimbrial adhesins[6]. The most common fimbriae found in UPEC strains are type I and P fimbriae which enhance virulence and are involved in initial urethral colonization. These markers of UPEC are expressed with different diseases states ranging from asymptomatic bacteriuria to chronic pyelonephritis[7]. Moreover, 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 in UPEC and to study their antibiotic susceptibility profile.

Material & Method

The study was carried out on a total of 550 E. coli isolates recovered from urine samples of patients with clinically suspected UTIs of all age groups, over a period of 1 year from Sep 2021 to Aug 2022. E. coli isolates were identified by the standard microbiological procedures. The antibiotic susceptibility testing was performed using the standard antimicrobial agents (Hi Media, Mumbai) Amoxyclavulanic acid (30 µg), ceftizoxime (30 µg), cotrimoxazole (25 µg), Gatifloxacin (5 µg), Gentamicin (10 µg), Nitrofurantoin (300 µg), Norfloxacin (10 µg), as per Clinical and Laboratory Standard Institute Guidelines[8]. E. coli (ATCC 25922) was used as control strain. Virulence factors such as hemolysin,

hemagglutination, serum resistance, gelatinase test, Biofilm production and siderophore production were detected as follows.

Detection of Virulence Factors

1. Hemolysin: The E. coli isolates were inoculated on 5% sheep blood agar and incubated overnight at 37°C. The indicator of hemolysin production was the presence of a zone of complete lysis of erythrocytes around the colony and clearing of the medium[3,6,9].

2. Hemagglutination: The test was carried out as per the direct bacterial hemagglutination testslide 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 hemagglutination (MRHA) was detected by the presence of hemagglutination of 3% 'O' blood group human RBCs in the presence of 2% W/V D - mannose[10].

3. Serum resistance: Overnight culture of E. coli on blood agar plates were suspended in Hank's balanced salt solution. Equal volume of this bacterial suspension and serum (0.05 ML) were incubated at 37°C for 3 h. Then 10 µl of this mixture was inoculated on blood agar plate and incubated at 37°C for 24 h and viable count was determined. It is termed as sensitive when colony count drop to < 1% of initial value[3,9].

4. Gelatinase test: Gelatinase production was tested using gelatin agar. The plate was inoculated with test organism and incubated at 37°C for 24 h. The plate was then flooded with 1% tannic acid solution. Developments of opacity around colonies were considered as positive for gelatinase[11].

5. Bio film production: 4 – 5 colonies from overnight culture plates were inoculated in a test tube holding 10ml of trypticase soy broth added with 1% glucose. After overnight incubation at 37°C, all tubes were decanted and washed with phosphate buffered saline. After that, all the tubes were dried and stained with 0.1% safranine. Excess stain was removed and washed with deionized water. Biofilm was observed once the tubes were dried. Biofilm formation was considered as positive by appreciating visible film lines along the sides and bottom of each tube and considered as negative when there was ring formation at the interface and clearing of tubes. All the tubes were observed[9].

6. Siderophore production assay: The test was done by using chrome azurole sulfonate agar (CAS) agar diffusion assay. The CAS assay detected color change of CAS-iron complex form blue to orange after chelation of the bound iron by siderophores. A strong ligand was added to a highly colored iron dye complex, when the iron ligand complex was formed, the release of the free dye was accompanied by a color change[12].

Results

A total of 2250 urine samples received over a period of one year from symptomatic cases of urinary tract infection were processed. Out of these, 550 samples were selected for our study. 52 of these samples showed growth of two types of

organisms including E. coli, while from rest 550 samples only E. coli were isolated. These 550 E. coli isolates were studied for the possession of these virulence factors. Out of 550 patients 367 (66.72%) cases were from females and183 (33.27%) were males. Thus female: male ratio was 1:3. The samples were received from both IPD and OPD patients (Table 1).

Table 1: Gender wise distribution of patients with UTI due to E. coli.

Female n = 367 (66.72%)		**Male n = 183 (33.27%)**	
OPD	**IPD**	**OPD**	**IPD**
127	**240**	**103**	**80**

A total of 295 (53.63%) among 550 isolates showed hemolysis. Mannose resistance hemagglutination was seen in 78 (26.17%) and mannose sensitive hemagglutination in 220 (73.82%), 293 (53.27%) showed serum resistance followed by 252 (45.81%) in Siderophore production assay, 247 (44.90%) showed Biofilm production and 235 (42.72%) showed gelatinase production. (Table 2).

Table 2: Virulence factors of Uropathogenic E. coli.

Virulence factors	**Positive**	**Percentage**
Hemolysin	**295**	**53.63%**
Hemagglutination	**298**	**54.18%**
MRHA	**78**	**26.17%**
MSHA	**220**	**73.82%**
Serum resistance	**293**	**53.27%**
Gelatinase test	**235**	**42.72%**
Bio film production	**247**	**44.90%**
Siderophore production assay	**252**	**45.81%**

Among the 550 E. coli isolates, 493 (89.63%) were sensitive to Nitrofurantoin followed by Imipenem 485(88.18%), Cotrimoxazole 214 (38.90%) and Gentamicin 210 (38.18%). 203 showed ESBL and 97 MDR (Table 3).

Table 3: Antimicrobial susceptibility pattern of UPEC (n=550)

Antibiotics	**Sensitive n%**
Nitrofurantoin	**493 (89.63%)**
Imipenem	**485(88.18%)**
Cotrimoxazole	**214 (38.90%)**
Gentamicin	**210 (38.18%)**
Amoxyclavulanic acid	**143 (26%)**
Ceftazidime	**259(47.09%)**

Multiple virulence factors (MVF) (>3) were present three in 170 (30.9%), Multi virulence factors producing isolates had strong positive correlation with MDR. Serum resistance producing E. coli isolates were more MDR (P) which was statistically significant. Hemolysis and Siderophore production isolates showed a

strong positive correlation with MDR. Gelatinase E. coli isolate had correlation with MDR which is not statistically significant.

Discussion

Urinary tract infections which are not properly treated from their onset can become a renal threat in time, finally leading to renal failure. In general, the more virulence factors a strain expresses, the more severe an infection it is able to cause[13]. The occurrence of multiple virulence factors in UPEC strains further strengthens the concept of association of UPEC with urinary pathogenicity[3]. These virulence factors enable some members of the normal flora to elicit an infection by overcoming the host defence mechanisms. Virulence factors enable E. coli to colonize selectively the mucosal uro-epithelium, evoke an inflammatory reaction and eventually proceed from lower urinary tract to renal cavities and tissue invasion. The capacity of E. coli to produce many virulence factors contributes to its pathogenicity[5]. Incidence of UTI was more common in females (66.72%) than in males (33.27%) in our study. Piatti et al also reported a higher prevalence of UTI in females (77%) [14]. 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, close proximity to anal canal, presence of normal flora in vagina and pregnancy.

Hemolysin production is associated with human pathogenic strains of E.coli, especially those causing more clinically severe forms of UTI[11]. It is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury and impaired host defenses[13]. In the present study, 53.63% E.coli isolates produced hemolysin. In other studies conducted by Raksha et al [3],Siegfried et al[9], Hughes et al[15], Shruthi et al [16] hemolysin production was detected in 41.36%, 59.6%, 59.7% and 41.9% isolates respectively.

The role of bacterial adherence in the pathogenesis of UTI is that colonization of the urogenital epithelium of susceptible individuals by specific bacteria is associated with successful microbial invasion of the urinary tract[17] 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. This concept has been supported in many researches, e.g., Seigfried et al.[9], Vagarali et al. [7] , Raksha et al. [3] , Kauser et al.[18] 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 26.17% and of these isolates 30.9% showed MDR.

Urinary antibodies resist UTI by preventing the adherence of bacteria to uroepithelial cells. [17] Serum resistance is the property by which the bacteria resist killing by normal human serum due to lytic action of complement system. It is likely that complete resistance to serum results from the accumulation of several distinct components at or near the cell surface. [15] In the present study, serum resistant was found in 53.27% isolates. In other studies, Kauser et al. [18] and Sharma et al. [6] have demonstrated the serum resistance in 49.5% and 86.8% of the urinary E. coli isolates. Hughes et al.[15] stated that the increased degree of serum resistance is associated with increased virulence of the organisms. In the

present study, gelatinase producing UPEC isolates 42.72% had correlation with MDR which is not statistically significant.

Siderophore production, promotes bacterial growth in the limiting iron concentrations encountered during infection and act as a virulence factor in the pathogenesis of UTI. In our study the siderophore production was seen in 252 (45.81%) isolates and 46% of these isolates were MDR statistically insignificant. In other studies, the incidence of siderophore production has been reported to be 76% [19] and 98%, [7,20] which is in concordance with our study.

Antibiotic susceptibility pattern was studied for all E.coli isolates. These isolates were most commonly resistant to ampicillin, amoxyclavulanic acid and cotrimoxazole. The increasing prevalence of MDR has been reported by other workers as well, which is due to dissemination of MDR strains in hospital settings. In this study, the maximum sensitivity was shown to nitrofurantoin (89%) followed by imipenem (88%) and ceftazidime (47%). The present study has shown the production of various virulent factors and developing drug resistance in UPEC. 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.

Conclusion

UTI's are considered acute, self-limiting infections despite the prevalence of recurrent symptoms, two or more times within months of a primary infection. It is more common in middle- aged females. Uropathogenic E. coli the major causative agent is more common in community as compared to hospital because of poor sanitation and unhygienic practices. The knowledge of virulence factors of E. coli will help in better understanding of organisms and its empirical treatment.

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