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Uropathogenic *Escherichia coli:* Correlation between virulence factors and serotypes

Pooja Tomar[1], K. P. Ranjan[2]*, Neelima Ranjan[2], Savita B. Jain[2], Himanshi Bansal[2]

[1]Department of Microbiology, Sri Venkateshwara University, Amroha, Uttar Pradesh, India, [2]Department of Microbiology, Gajra Raja Medical College, Gwalior, Madhya Pradesh, India

ABSTRACT

Background: Urinary tract infections (UTIs) are the most commonly encountered bacterial infections both in community and health-care settings. **Objective:** We determine the prevalence of virulence factors and serotypes associated with uropathogenic Escherichia coli (UPEC). In addition, we also compare the possible relationship between virulence factors with serotypes. **Materials and Methods:** A total of 200 E. coli isolates were collected from clinically suspected UTI and detection of virulence factors such as hemolysin production, hemagglutination, biofilm formation, and also serotyping was done. **Results:** The incidence of UTI was more in the age group of 21–30 years and more in females. Hemolysin production was seen in 23% strains. Hemagglutination was shown by total 127 (63.5%) isolates. One hundred nine (54.5%) isolates showed production of moderate and high-value biofilms. Among the serotypes, O1, O2, O8, O90, O101, and O125 were the most commonly isolated uropathogens. Among all the serotypes of UPEC isolates, maximum hemolysin production and hemagglutination were seen in O8 serotype. **Conclusion:** Certain serotypes of UPEC are more commonly associated with UTI. These serotypes have a wide array of virulence factors and their identification may facilitate the application of more precise approaches in diagnosis of UTIs

Key words: Biofilm, serotype, urinary tract infections, uropathogenic *Escherichia coli,* virulence factors

INTRODUCTION

Urinary tract infections (UTIs) are the most commonly encountered bacterial infections both in community and health-care settings.[1] Common UTI causing bacterial pathogens belong to family *Enterobacteriaceae*, *Escherichia coli* being the most common member isolated. Certain serotypes of *E. coli* which are consistently associated with uropathogenecity are designated as uropathogenic *E. coli* (UPEC). These UPEC possess wide array of virulence factors that help them colonize and infect the urinary tract. Virulence factors of UPEC are multiple and unusually complex affecting pathogenicity in combination with one another. The common virulence factors include surface hydrophobicity, colonization factor, capsule, serum resistance, resistance to phagocytosis, hemolysin, enterotoxin and siderophore, fimbriae, and hemagglutination.[2] The different virulence factors act in concert, their expression may be turned on or off during the course of the infection and can be regulated by environmental signals. Many different factors have been implicated in UPEC pathogenesis; however, the specific factors that differentiate UPEC strains responsible for the different clinical manifestations of UTIs remain unclear.[3] *E. coli* strains are identified by serological typing of their flagellar, lipopolysaccharide, and in some cases, capsular surface antigens. The prevalence of different serotypes varies in different geographical regions. We determine the prevalence of virulence factors and serotypes associated with UPEC. In addition, we also compare the possible relationship between virulence factors with serotypes of UPEC.

MATERIALS AND METHODS

This prospective study was carried out in the department of microbiology of the institute for the duration of 2 years. A total of 200 *E. coli* isolates were recovered from urine samples of patients with clinically suspected UTIs of all age groups belonging to the departments of urology, nephrology, medicine, pediatrics, etc. Urine samples were inoculated on blood and MacConkey agar by standard loop method and a colony count of $>10^5$ CFU/ml was taken to be significant bacteriuria. Isolates were identified as *E. coli* by standard biochemical tests.[4,5] Standard UPEC serotypes O4 and O6 and *E. coli* ATCC 25922 were used as controls for detection of the virulence markers.

Detection of Virulence Factors

Hemolysin production

The test was done for the detection of α-hemolysin produced by the *E. coli*. The bacteria were inoculated onto 5% sheep blood agar and incubated overnight at 35°C. Hemolysin production was detected by the presence of a zone of complete lysis of the erythrocytes around the colony and clearing of the medium.[6]

Hemagglutination

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 nutrient broth culture, and the slide was rocked at room temperature for 5 min. The presence of

Address for correspondence:
Dr. K. P. Ranjan, Department of Microbiology, Gajra Raja Medical College, Gwalior, Madhya Pradesh, India. Phone: +91-9009021907.
E-mail: drkpranjan@gmail.com

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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.[6]

Biofilm formation

E. coli isolates were tested for biofilm production by tissue culture plate method.[2]

Serotyping

All biochemically confirmed *E. coli* isolates were serotyped at Central Research Institute, Kasauli (National Salmonella and Escherichia Centre), India.[7,8]

RESULT

A total of 200 *E. coli* were isolated from the urine samples of patients with clinically suspected UTI. The incidence of UTI was more in the age group of 21–30 years (33%) followed by 31–40 years (32.5%), 41–40 years (15.5%), 11–20 years (11%), 1–10 years (6.5%), and >50 years (1.5%), respectively. The incidence of UTI was slightly more in females (51%) than males (49%). The age group of 31–40 years was showing highest incidence of UTI in males, whereas the age group of 21–30 years was exhibiting highest incidence in females.

Hemolysin production was seen in 46 (23%) strains. Maximum hemolysin production was seen in 31–40 years of age group and least in >50 years age group. In which, 45.6% males and 54.34% females showed hemolysin production, respectively.

Hemagglutination was shown by total 127 (63.5%) *E. coli* isolates. Isolates from both male and female showed equal hemagglutination, 63 (49.6%) and 64 (50.4%), respectively. Maximum hemagglutination was seen in the age group of 31–40 years in males, 21–30 years in females, and 31–40 years overall. MRHA was seen in 30% isolates while 33.5% exhibited mannose-sensitive hemagglutination.

Of 200 isolates of *E. coli,* 109 (54.5%) isolates showed the production of moderate and high-value biofilms. Among the serotypes, O1, O2, O8, O90, O101, and O125 were the most commonly isolated uropathogens. 43 isolates could not be typed as they were either rough or non-typeable. Among all the serotypes of UPEC isolates, maximum hemolysin production and hemagglutination were seen in O8 serotype, whereas maximum biofilm production was seen among serotype O8, O101, and O125 [Table 1].

Of total 200 isolates of UPEC studied, 76 isolates (38%) showed the presence of anyone virulence marker, 73 (36.5%) presence of any two virulence markers, and only 20 (10%) showed all three virulence markers. 31 isolates (15.5%) showed the presence of no virulence marker [Table 2].

Table 1: Distribution of virulence factors among various serotypes of *E. coli*

Serotypes	*n* (%)	Hemolysis (%)	Hemagglutination (%)	Biofilm (%)
O1	10 (5)	3 (6.5)	6 (4.7)	5 (4.5)
O2	20 (10)	6 (13)	14 (11)	11 (10)
O4	1 (0.5)	0	1 (0.7)	1 (0.9)
O6	6 (3)	1 (2.1)	4 (3.1)	3 (2.7)
O8	20 (10)	0	12 (9.4)	10 (9.1)
O26	4 (2)	0	1 (0.7)	3 (2.7)
O40	2 (1)	1 (2.1)	1 (0.7)	2 (1.8)
O50	2 (1)	1 (2.1)	2 (1.5)	2 (1.8)
O60	2 (1)	0	1 (0.7)	0
O66	2 (1)	0	1 (0.7)	1 (0.9)
O68	2 (1)	1 (2.1)	2 (1.5)	0
O76	2 (1)	0	1 (0.7)	2 (1.8)
O84	2 (1)	2 (4.3)	1 (0.7)	1 (0.9)
O86	1 (0.5)	0	0	1 (0.9)
O87	3 (1.5)	0	3 (2.3)	2 (1.8)
O88	2 (1)	1 (2.1)	2 (1.5)	2 (1.8)
O90	10 (5)	3 (6.5)	7 (5.5)	7 (6.4)
O101	17 (8.5)	3 (6.5)	14 (11)	10 (9.1)
O102	1 (0.5)	1 (2.1)	0	0
O116	4 (2)	1 (2.1)	3 (2.3)	2 (1.8)
O118	1 (0.5)	0	1 (0.7)	0
O121	6 (3)	3 (6.5)	3 (2.3)	2 (1.8)
O125	9 (4.5)	1 (2.1)	6 (4.7)	7 (6.4)
O126	2 (1)	0	2 (1.5)	1 (0.9)
O141	8 (4)	1 (2.1)	4 (3.1)	3 (2.7)
O148	2 (1)	0	0	0
O149	2 (1)	0	1 (0.7)	2 (1.8)
O154	2 (1)	2 (4.3)	0	0
O157	3 (1.5)	1 (2.1)	3 (2.3)	1 (0.9)
O163	4 (2)	2 (4.3)	4 (3.1)	1 (0.9)
O172	5 (2.5)	2 (4.3)	3 (2.3)	2 (1.8)
Rough	19 (9.5)	8 (17.3)	13 (10.2)	13 (11.9)
UT	24 (12)	2 (4.3)	11 (8.6)	12 (11)
Total	200	46	127	109

E. coli: Escherichia coli, UT: Urinary tract

Table 2: Distribution of various virulence markers among UPEC isolates

Presence of virulence markers	Number of isolates (%)
No virulence markers	31 (15.5)
One virulence marker	76 (38)
Two virulence markers	73 (36.5)
All three virulence markers	20 (10)
Total	200 (100)

UPEC: Uropathogenic *Escherichia coli*

DISCUSSION

UPEC is the most important group of microorganisms responsible for UTI. They differ from non-uropathogenic *E. coli* by the production of specific virulence factors which enable the bacteria to adhere to uroepithelial cells and to establish UTI. Besides, adhesion factors, toxins, modulins, capsules, iron uptake system, and other bacterial products contribute to virulence of strains.[9]

Based on what was observed from the results obtained, women have higher percentage rate of UTI than men. This difference could be attributed to several factors such as the anatomical differences

between the male and female urethra, improper cleaning of the perineum, the use of napkins, sanitary towels, and tampons together with pregnancy and intercourse.[10] In addition, the urine of females was found to have a more suitable pH and osmolarity for the growth of *E. coli* and other enteric pathogens.[11]

In the present study, most of the UPEC were isolated from patients aged between 21 and 30 years (33%) and 31–40 years (32.5%), respectively, and least were isolated patients over 50 years of age group (1.5%). The incidence was similar as the study conducted by Polse *et al*. in Iraq.[12] They also isolated UPEC mostly from people aged 11 to 44 years. This result was also comparable to a study conducted by Kiffer *et al*. as they found higher percentage of *E. coli* isolates in people of age group of 13–60 years and lower percentage in people younger than 13 years or older than 60 years.[13] Another study found that the lowest percentage of *E. coli* was among age group <10 years and high within the age group of 26–36 years.[14]

Hemolysin production is associated with human pathogenic strains of *E. coli*, especially those causing more clinically severe forms of UTI.[15] It is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury, and impaired host defenses.[16] Of total 200 isolates studied, hemolysin production was shown by 45 isolates (24.5%). Hemolysin contributes to tissue injury and survival of pathogen in renal parenchyma, and it has been suggested that colonization with hemolytic strains of *E. coli* is more likely to develop into UTIs.[17]

Mannose-sensitive hemagglutination was exhibited by 33.5% isolates and 30% showed mannose-resistant hemagglutination in this study. In the study of Saikia *et al.*, hemagglutination was observed in 39% cases.[18] In a study conducted at Bijapur in 2009, 30% isolates were MRHA positive and 36% were MSHA positive.[9] Hemagglutination by a strain is an indirect evidence of fimbriae possession by that strain. *E. coli* strains exhibiting MRHA have been associated with severe form of UTIs.[19,20]

In our study, 109 (54.5%) isolates showed production of moderate and high-value biofilms. In a study conducted in Iran, among 130 *E. coli* isolates, 61.53 % were able to make biofilm, whereas Suman *et al*. have reported a higher rate (92%) of biofilm production.[18,21] Biofilm production promotes persistence in the urinary tract and endows bacteria with several advantages such as acquisition of antibiotic tolerance, expression of several virulence factors, and increased resistance against phagocytosis. As a result, there are limited treatment options, which can make the infections difficult to treat.[2]

Among the serotypes, O1, O2, O8, O90, O101, and O125 were the most commonly isolated uropathogens. UPEC belonging to certain serogroups possesses certain specific virulence factors which enhance their ability to cause infection. In most studies, UPEC belonged to serotypes 01, 02, 04, 06, 07, 08, 016, 018, 025, and 075.[22]

Among all the serotypes of UPEC isolates, maximum hemolysin production and hemagglutination were seen in O8 serotype, whereas maximum biofilm production was seen among serotype O8, O101, and O125. In the study of Saikia *et al.*, O25 (26.66%), O15 (20.0%), and O16 (13.33%) had the highest biofilm producing serogroups while O2, O4, O6, O8, O21, and O22 had the lowest biofilm producing serogroups which showed (6.66%) among UPEC isolates detected.[18]

Of total 200 isolates of UPEC studied, 31 isolates (15.5%) showed the presence of no virulence marker. 76 isolates (38%) showed the presence of anyone virulence marker, 73 (36.5%) presence of any two virulence markers, and only 20 (10%) showed all three virulence markers. In the study by Kausar *et al*., 21 (10.5%) isolates showed the presence of all three virulence factors, 71 (35.5%) showed two virulence factors and one virulence marker in 68 (39%). This study was similar to our study in distribution of virulence markers.[2,9]

CONCLUSION

In the end, we would like to conclude that certain serotypes of UPEC are more commonly associated with UTI. These serotypes have a wide array of virulence factors and their identification may facilitate the application of more precise approaches in diagnosis of UTIs.

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