

Original research article

Study on phenotypic assays to determine virulence factors of uropathogenic escherichia coli (UPEC) isolates and their correlation with antibiotic resistance pattern in tertiary care hospital of western Uttar Pradesh

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Abstract

Introduction: Urinary tract infections (UTI) forms the largest single group of hospital acquired infections and this infection can account for about 40-50% of nosocomial infections. Uropathogenic *Escherichia coli* (UPEC) are the the important organisms causing 80-90% of community-acquired UTIs and more than 30% of nosocomially acquired UTIs. Multidrug-resistant *E. coli* (MDR-ECO) strains have increased with the application of broad-spectrum antibiotics. Therefore, this demands the need for common bacterial pathogens periodic screening in different communities for their antibiotic susceptibility profiles.

Aim & Objectives: To determine the presence of different phenotypic virulence factors in UPEC isolates and determine their correlation with antibiotic resistance pattern.

Methods: Total 60 uropathogenic *Escherichia coli* was isolated from the urine samples of the patients suffering from UTIs with the significant bacterial counts ($\geq 10^5$ CFU/mL) obtained in the culture. The isolated organisms were identified and characterized on the basis of standard microbiological tests such as Gram staining and various biochemical reactions. Features tested from all the uropathogenic *Escherichia coli* isolated from patients sample were haemolysin production, antimicrobial resistance and ESBL production.

Results: Out of 60 UPEC isolates, hemolysin production was observed in 34% of the UPEC isolates. The remaining 66% isolates showed no hemolysis. Out of 60 urine samples 17 samples (28 %) were ESBL producers. Our results also showed a relationship between hemolysin production and ESBL production. The UPEC showed the highest resistance to amoxicillin/clavulanate, norfloxacin, piperacillin, aztreonam.

Conclusion : There is a correlation between the phenotypic virulence traits of the UPEC isolates and antibiotic resistance. Studies of phenotypic characteristics of UPEC isolates may help us to get novel insights into pathogenesis of UPEC isolates. Methods of detection of the virulence markers used in this study are reasonably easy and screening them in a clinical microbiology laboratory on routine basis will be helpful in early detection and treatment of the patients. This study will help the clinicians for prescribing timely and cost effective treatment of patients in healthcare settings.

INTRODUCTION

Urinary tract infections (UTI) forms the largest single group of hospital acquired infections and this infection can account for about 40-50% of nosocomial infections. There are about 150 million UTIs per annum world wide.^[1] UTI includes cystitis and pyelonephritis that are considered to be the second most common infections in human. Approximately ~150 – 250 million people causes this infection per year.^[2,3] *Escherichia coli* is a major

human pathogen, that can cause 80% of uncomplicated UTI and near about 30% of bacteraemia cases.^[4] Uropathogenic *Escherichia coli* (UPEC) are the general microbes that can cause UTIs.^[5] It is the important organisms causing 80-90% of community-acquired UTIs and more than 30% of nosocomially acquired UTIs.^[6] UTI affects women more than men at all ages, except in the first year of life, it is very uncommon in young, healthy and middle aged men. ^[7] . UTI patients are classified in asymptomatic or symptomatic cases. Asymptomatic bacteriuria (ABU) present itself in small number of healthy individuals and sometimes they don't need treatment, that makes it different from symptomatic. It can affect pregnant women, it depends on various factors like age, sex, sexual activity and the presence of genital abnormalities.^[8,9,10]

The Virulence factors of *E.coli* are multiple in numbers and they show unusually complex affecting pathogenicity in combination with one another. There are some common Virulence factors that include surface hydrophobicity, capsule, serum resistance, colonization factor, resistance to phagocytosis, enterotoxins and siderophore, hemagglutination and fimbriae.^[11] These markers of uropathogenic *E.coli* are expressed in different diseases state ranging from asymptomatic to chronic pyelonephritis. These factors support their ability to attach to uroepithelial cells that help to resist the bactericidal effect of serum and augment cell surface hydrophobicity thereby they leading directly to tissue damage. ^[12]

The antibiotic susceptibility of bacterial isolates is not constant and varies with time and environment. Multidrug-resistant *E. coli* (MDR-ECO) strains have increased with the application of broad-spectrum antibiotics. Therefore, this demands the need for common bacterial pathogens periodic screening in different communities for their antibiotic susceptibility profiles.

Lack of data regarding the prevalence and antibiotic resistance pattern among the UPEC isolated from patients of UTIs in our hospital prompted us to carry out this study in the department of Microbiology at KD Medical college hospital & Research Centre located in Mathura city of Uttar Pradesh.

MATERIAL AND METHODS

The present study was carried out in the Department of Microbiology at KD Medical college hospital & Research Centre, Mathura for period of 6 months between January 2018 to June 2018. Total 60 uropathogenic *Escherichia coli* was isolated from the urine samples of the patients suffering from UTIs with the significant bacterial counts ($\geq 10^5$ CFU/mL) obtained in the culture. The isolated organisms were identified and characterized on the basis of standard microbiological tests such as Gram staining and various biochemical reactions.

Clinical symptoms, infection history, treatment summary and all the necessary relevant information from patients were taken from hospital records, laboratory reports and interviewing patients. Following features were tested from all the uropathogenic *Escherichia coli* isolated from patients sample:

Hemolysin production

This is the ability of the UPEC isolates to induce hemolysis on blood agar was evaluate to detect the hemolysin producer isolates. The bacteria was inoculated into blood agar plate and incubated overnight at 37°C. Hemolysin production was detected by the partial or complete zone of haemolysis around the colonies.

Antimicrobial susceptibility pattern

Antibiotic susceptibility patterns against UPEC isolates was interpreted as recommended by the Clinical and Laboratory Standard Institute (CLSI). The following antibiotics were included in this study: Aztreonam (30ug), Imipenem (10ug), Ceftriaxone (30ug), Amikacin (30ug), Meropenem (10ug), Ciprofloxacin (5ug), Gentamicin

(30ug), Piperacillin (10 units), Ceftazidime (30ug), Eretapenem (10ug), Tetracyclin (30ug), Ampicillin / Sulbatum (10/10), Piperacilin / Tazobactam (100/10), Chloramphenicol (30ug), Cotrimoxazole (1.25/23.75ug), Tobramycin (10ug), Norfloxacin (10ug), Nitrofurantion (300ug), Ampicillin (10 ug), Amoxicillin / Clavulanate (20/10ug), Fosfomycin were placed on agar plate with lawn culture. Inhibition zone diameter (mm) of each antimicrobial disc was measured, and the isolates were classified as susceptible and resistant. The E.coli ATCC 25922 was used in this study as a control.

Detection of ESBL producers

As described by recent 2018 CLSI guidelines all the UPEC isolates were resistant to third generation cephalosporins (3GCs) by disc diffusion test, ceftazidime, ceftriaxone or cefotaxime were selected for confirmation of ESBL production by double disk synergy test. E.coli ATCC 25922 were used as control.

RESULTS

1. Prevalence of UTI in relationship with profile of patients (Tables 1, 2, 3 & 4)

Of the 60 UTI patients, the rate of UTI in females (63.3%) was higher than males (36.6%). The incidence of UTI in female patients with age group of 1 to 10 years (2.6%), 11 to 20 years (10.52%), 21 to 30 years (55.26%), 31 to 40 years (13.1%), 41 to 50 years (10.52%), 51 to 60 years (13.1%), 61 to 70 years (18.42%) and 71 to 80 years (2.6%) was highest while the age group 21 to 30 years had the incidence of UTI (55.26%). Among male patients, the highest prevalence of UTI was observed in the age group of 61 to 70 years (33.3%) and the lowest was seen in the age group of 31 to 40 years (5.5%).

Table-1 Distribution percentage of urinary tract infection in the hospital:

Patient status	% Urinary tract infection
Outpatients (community acquired UTI)	26%
Inpatients (hospital acquired UTI)	74%

Table-2 Gender Distribution percentage of urinary tract infection :

Sex	Urinary tract infection
Male	36 %
Female	64 %

Table-3 Distribution of age and sex among samples of urine isolates

Age (years)	Female (no.of isolates) N=38	Male (no.of isolates) N=22
1 to 10	1	1
11 to 20	4	1
21 to 30	11	1
31 to 40	5	1
41 to 50	4	2
51 to 60	5	5
61 to 70	7	8
71 to 80	1	3

Among the 60 samples of E.coli obtained, most of them are from the department of General Medicine followed by General Surgery, Paediatrics, Psychiatry and Obst & Gynaecology. (Table-4)

Table- 4 Distribution of E.coli among various departments

Department	No. of samples
General medicine	23
General surgery	18
Obst & Gynaecology	16
Paediatrics	2
Psychiatry	1

Virulence characteristics of UPEC isolates

Hemolysin production

According to our results, hemolysin production was observed in 34% of the UPEC isolates. The remaining 66% isolates showed no hemolysis. In this the 8.3% isolates are ESBL producers. (fig.1)



Figure-1 Beta hemolysis on blood agar plate.

ESBL production

Double disc synergy test (DDST) showed that 28.3% of the UPEC isolates were positive for ESBL production. Out of 60 urine samples 17 samples are ESBL producer. (figure 2)

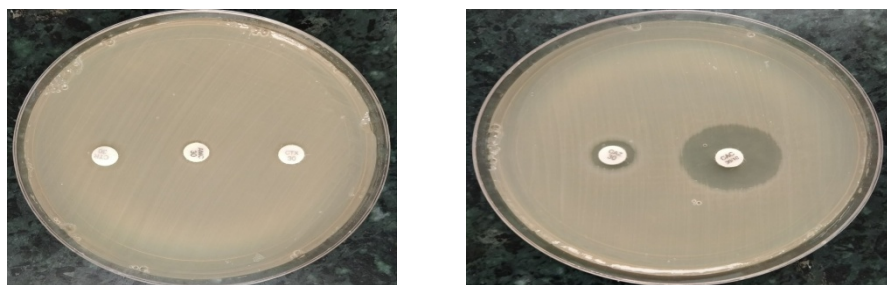


Figure 2. ESBL production shown by *Escherichia coli* isolates

Antimicrobial resistance profile of UPEC

The resistance pattern of UPEC isolates to the different antimicrobial agents is shown in **Fig. 3**. Among the antibiotics tested Amoxicillin / Clavulanate resistance prevalence was the highest (96.7%), followed by, Eretapenem (61.9%), Chloramphenicol (63.3%) and Tobramycin (56.6%). The isolates showed the highest sensitivity to antibiotics such as imipenem (98%) and meropenem (78.3%), amikacin (85%), piperacillin/tazobactam (91.6%), and nitrofurantion (80%). Totally, 60 isolates tested (79%) were multidrug-resistant (isolates with resistance to three or more different classes of antibiotics).

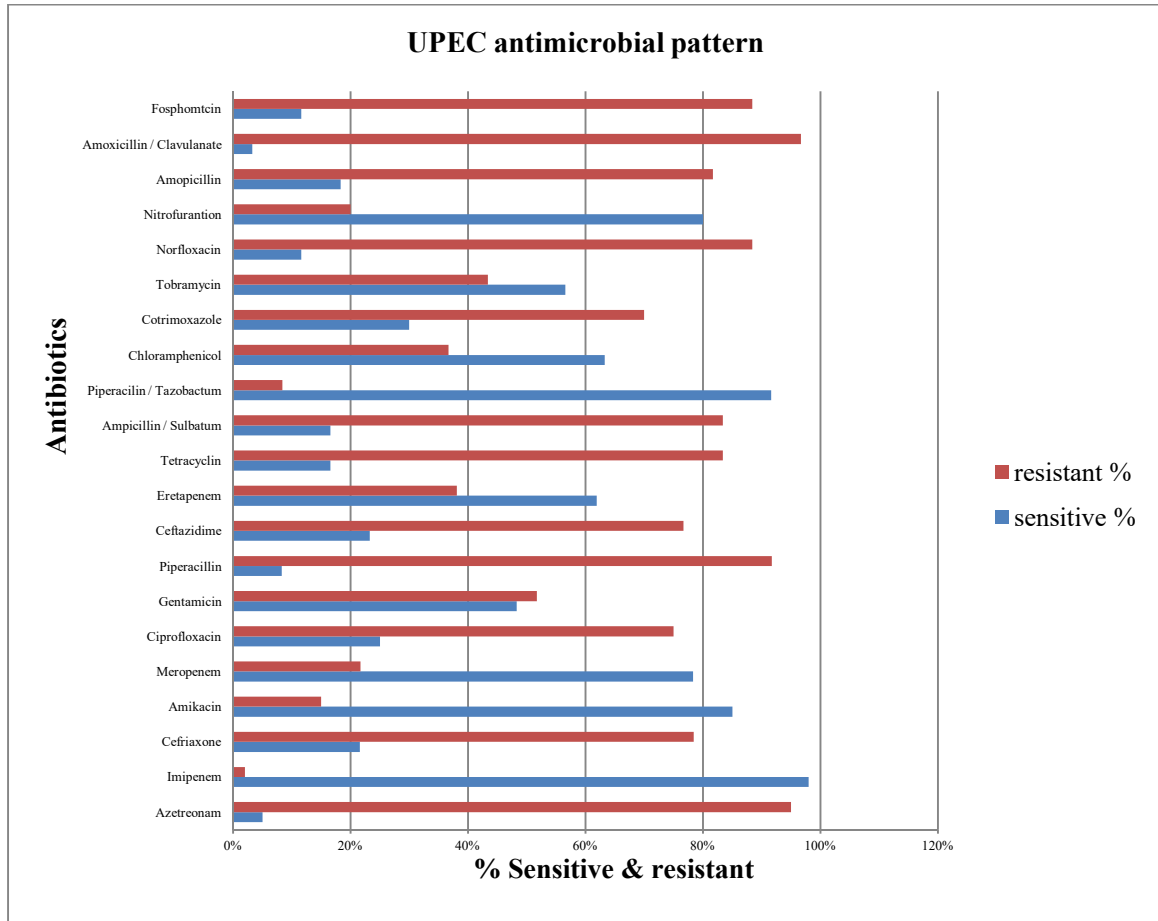


Figure-3 Antimicrobial sensitive-resistant pattern of UPEC isolates from patients with urinary tract infection.

Relation of antimicrobial resistance with virulence determinants of UPEC (Table 5)

According to our results resistance rates of the antibiotics tested among the UPEC isolated from male patients were higher than females.

Table-5 Relationship between antimicrobial resistance and hemolysin production.

Virulence Markers		Antimicrobial agents							
		CAZ %R	CPM %R	COT %R	AMP %R	NX %R	TE %R	PI %R	CTR %R
Hemolysin production	Positive	60%	56%	52%	76%	64%	56%	68%	64%
	Negative	40%	44%	48%	24%	36%	44%	32%	36%

DISCUSSION

Phenotypic characteristics of UPEC isolates and their correlation with antibiotic resistance patterns in patients with UTI are not well known and few data have been reported in this study Identification of virulence markers of UPEC will help to understand their contribution with the antimicrobial resistance. [2,3] Our findings in accordance with other studies indicated that females have a notable frequency of UTI versus males. [13, 14] This difference in frequency could be due to several clinical factors, including anatomic differences, hormonal effects, and behavioral patterns. Moreover, our investigations reveal that overall resistance rates of antimicrobial drugs were higher in men than women. These findings were similar to Yilmaz et al. [15] The goal of the present study was to determine the phenotypic characteristics of uropathogenic E.coli (UPEC) isolates virulence factors and their correlation with antibiotic resistance pattern.

These observations were similar to the findings of Yilmaz et al and Mohsen Tabasi et al. In this study we had taken 60 E.coli isolates from urine samples. The age of patients ranged from 5 to 80 years.

Our study samples obtained were more in the age group 21 to 30 years in women and our study obtained that women had a higher incidence of UTI than men. Similarly, by the study of Annapurna et al, 2014 women are especially prone to UTIs for anatomical reasons with the life time risk of having UTIs are greater than 50%.

In our study out of 60 isolates we found 73.3% from IPD and 26.6% from OPD. In the study of Sayan Bhattacharyya et al, only 3 were from in patients, and 97 were from out patients isolates.

Out of 60 isolates 28.3% and 44.6% isolates showed ESBL and Beta hemolysin production respectively. In a study from Himachal Pradesh, 10.24% of E. coli isolates were haemolytic,[4] whereas we isolated 25% of haemolytic *E. coli*. In the study of Sneha Kukanur et al, reported 26.9% ESBL producers from urine samples. [17] Similarly, a study by Mathur et al, showed 41% of E.coli to be ESBL producers.

Our study revealed that 98%, 91.6% and 80% of urine isolates were sensitive to Imipenem, Piperacillin/Tazobactam and Nitrofurantoin. A study from KVG Medical college & hospital, Sullia DK, Karnataka, India, have showed the sensitivity in Nitrofurantoin (92.5%), Amikacin (75%) and Gentamicin (57.1%). [16] A study from CMC Vellore, (India) had showed that older drugs like Nitrofurantoin appeared to be useful and could be considered as a choice for treating uncomplicated lower urinary tract infections. [18] Our study aimed to find out the virulence factors of uropathogenic E.coli and their correlation with antibiotic resistance pattern.

CONCLUSION

Urinary tract infections caused by *Escherichia coli* are common among elderly patients in residential care facilities, as well as in the hospital settings. There is a correlation between the phenotypic virulence traits of the UPEC isolates and antibiotic resistance. Studies of phenotypic characteristics of UPEC isolates may help us to get novel insights into pathogenesis of UPEC isolates. The antibiotic susceptibility of bacterial isolates is not constant and varies with time and environment. Emergence of Multidrug-resistant *Escherichia coli* strains have increased with the application of broad-spectrum antibiotics. Methods of detection of the virulence markers used in this study are reasonably easy and screening them in a clinical microbiology laboratory on routine basis will be helpful in early detection and treatment of the patients. This study will help the clinicians for prescribing timely and cost effective treatment of patients in healthcare settings.

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