



# Virulence factors and multidrug resistance of uropathogenic *Escherichia Coli* isolated from men with urinary tract infection in and around Coimbatore, South India

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## ABSTRACT

Urinary tract infection (UTI) has become prominent disease caused predominantly by uropathogenic *Escherichia coli* (UPEC) and other members of Enterobacteriaceae family. The bacteria will enter the urethra and infect bladder (cystitis), kidney (pyelonephritis) and blood stream (septicemia). The virulence factors and serotypes influence the pathogenicity. Unwanted usage of low standard antibiotics leads to multidrug resistance of the bacteria. The UPEC produces extended spectrum beta lactamase (ESBL) in order to degrade certain drugs. The prevalence of UTI is more in women when compared to men due to urogenital anatomy. Therefore, treatment till date given is based on the data from women patients. Since the UPEC isolated from men behaved differently in their sensitivity pattern, they must be treated separately. In this way we have isolated 100 UPEC from men attending hospitals in and around Coimbatore, South India and analyzed. No cases were identified in adult between 21 and 30 years, above 60 years more cases were reported. The highest expressed virulence factor was hemolysis (64%) followed by serum resistance (40%) and MSHA (36%). Most of them (72%) were ESBL producers and the antibiotic sensitivity pattern revealed that the isolates were highly resistant to ampicillin (84%), cephazolin and cefepime (72%). The least resistant was against imipenem and meropenem (4%). Commonly encountered serotypes were rough, O8, O9 and O2. To conclude, men of age above 60 years were vulnerable to UTI by UPEC which are hemolytic. Above 70% were ESBL producers and highly resistant to ampicillin, cephazolin and cefepime. They were sensitive to imipenem and meropenem towards which resistance started developing. Common serotypes of UTI were absent and random serotypes were detected. This work may help treating UTI in men and research in this field is essential to understand and treat UTI in men.

**Keywords:** Uropathogenic, *Escherichia coli*, Virulence factor, Multidrug Resistant, Serotype.

## 1. INTRODUCTION

Studies unveiled that urinary tract infections (UTI) affected nearly 50% of the people worldwide [1]. The

occurrence of UTI varies with gender, age and susceptibility conditions. Women are more prone to the disease when compared to men of all ages [2].

The large distance between the urethral meatus and the anus, the length and dryness of male urethra and the antibacterial activity of prostatic fluids play important role in preventing males from infection. [3]. Guidelines on UTI always depicts infections of women because of high prevalence than men regardless the difference in the genito-urinary system [4]. Most common pathogen of UTI is uropathogenic *Escherichia coli* (UPEC) [5,6] the pili or fimbriae possessed by UPEC initiates the infection by adhering to the cells in the urinary tract [7]. The infection starts from urethra and spontaneously advances to the bladder, kidney and to the bloodstream [8-10]. The pathogenicity is linked with certain O serotype [11] and virulence factors like mannose resistant and sensitive hemagglutination (MRHA and MSHA), hemolysin, biofilm formation and serum bactericidal activity [12]. The UPEC shows increased resistant to commonly used antibiotics [13] by producing enzymes like extended spectrum beta lactamase (ESBL) that hydrolyses ceftazidime, cefotaxime (extended spectrum cephalosporins) and/or the monobactam aztreonam [14]. The treatment of UTI between genders differs only in the duration of drug administration [15]. Since the male patients were not given importance and not treated separately, the pathogens were never studied properly. The purpose of this study is to analyze the virulence factors and antimicrobial resistant pattern of UPEC isolated from male patients suffering from UTI in and around Coimbatore.

## 2. MATERIALS AND METHODS

### 2.1 Collection of sample

The urine samples received at Bioline Laboratories, Coimbatore, are from patients visiting various hospitals in and around Coimbatore for a period of three years from 2012 to 2015. A total of 270 urine samples of males were processed to get 100 UPEC isolates. For identification Vitek 2 compact, ID/AST instrument, Biomerieux Diagnostics was used. For further analyses the samples were sub cultured and transferred to Microbiology lab, CMS college of Science and Commerce.

### 2.2 Hemolysis Analysis

The isolates were inoculated in alkaline meat extract broth and incubated at 37<sup>0</sup> C for 2 hours and 30 mins. Then spinned at 2415x g for 30 mins and to the supernatant equal volume of 2% RBC was added and kept at 37<sup>0</sup> C for another 2 hours. Hemolysis was observed under microscope [16].

### 2.3 Hemagglutination Assay

A drop of erythrocyte in phosphate buffered saline is mixed with a drop of bacterial culture on a VDRL slide. Hemagglutination was noted in the presence of 3% of mannose is called as mannose resistant hemagglutination (MRHA) and clumping found only in the absence of mannose and not in its presence is called as mannose sensitive hemagglutination (MSHA) [17].

### 2.4 Serum Bactericidal assay (SBA)

Hank's Balanced Salt Solution (HBSS) was prepared and inoculated with 18 hour UPEC cultures grown on blood agar. The culture is mixed with serum of equal volume (0.05 ml) and incubated at 37<sup>0</sup> C for 3 hours. The mixture (10 µl) was inoculated on blood agar for viable count. Serum resistant is 90% survival and serum sensitive is 1% survival rate [17].

### 2.5 Biofilm formation

Ten ml of Trypticase Soy Broth was inoculated with overnight UPEC culture and incubated for 24 hours at 37<sup>0</sup> C with glucose. The broth discarded, washed with Phosphate Buffer Saline (pH 7.3) and stained with 0.1% crystal violet. The tubes were observed after drying upside down. Biofilm formation could be observed by a thin film on wall and bottom [18].

### 2.6 ESBL screening

All the isolates were subjected to double disc approximation test for ESBL production. Muller Hinton Agar (MHA) was uniformly inoculated with UPEC. Antibiotic discs of ceftazidime / clavulanic acid were placed 20 mm apart and incubated for 24 hours at 37<sup>0</sup>C. The ESBL positive cultures will produce a zone of 5 mm or more. [19].

### 2.7 Antibiogram

Sensitivity pattern of UPEC against antibiotics were done using disc diffusion technique by CLSI guidelines [20]. Antibiotic discs used were Ampicillin (10µg), Ampicillin -sulbactam (20µg), Amikacin (30 µg), Amoxicillin (10µg), Cephazolin (30µg), Ciprofloxacin (5µg), Cephalothin (30 µg), Cefepime (30µg), Ertapenem (10µg), Imipenem (10µg), Meropenem (10µg), Norfloxacin (10µg), Tozabactam (10µg), Tigecycline (4µg), Co-trimaxazole (25µg) and Gentamycin (10µg).

### 2.8 Serotyping

25 representative cultures were selected randomly for serotyping and were sent to National Salmonella and

Escherichia Center, Central Research Institute (CRI), Kasauli, Himachal Pradesh, India.

### 3. RESULTS AND DISCUSSION

A total of 100 isolates were checked for virulence factors, ESBL production, antibiotic sensitivity and few isolates were serotyped. For serotyping, 25 isolates were randomly selected and sent to NSEC, CRI, Kasauli, India. During transport 3 isolates were contaminated and reported as non *E. coli*, 2 were untypeable and remaining 20 were serotyped and reported.

To obtain 100 UPEC isolates, we have screened urine samples from 270 males which represented a 37% prevalence of *E. coli* among UTI infected males. Previous studies reported ranges of prevalence like 17% [21], 56% [22] and 60% [23]. Age plays a role in pathophysiology of UTI in males [24]. Increase in incidence of UTI was noticed with increase in age, particularly in men as they are prone to prostatic hypertrophy [24]. In our study, the age group 1 to 20 years was less infected when compared to patients above 60 years. There were no cases in the age group 21 to 30 years. Ulleryd et al., [2] mentioned it is very rare to find infection in young, healthy and middle aged men. Considerable number of individuals was infected in the age group 41 to 60 years which increased in the age above 60 years (Table 1).

Pyelonephritis was diagnosed in 40% of the patients and cystitis in 60% individuals. Desai et al., [25] reported the occurrence of cystitis nearly two times of pyelonephritis. They also demonstrated the virulence factors as 30% MSHA, 36% MRHA, 54% hemolytic and 52% serum resistant which could be related to our study where the highest percentage of virulence factor was found in hemolysis followed by serum resistance. Hemolysis was reported in 60% of *E. coli* from urine [26]. Hemolytic *E. coli* are often occurred in UPEC [27] and symbolize severity of the infection [28]. Mannose sensitive hemagglutination was observed for more isolates than MRHA. The hemagglutination property of the UPEC denotes the presence of fimbriae [29], the MRHA possibly have P fimbriae [30] and MSHA indicates presence of type 1 fimbriae which is important in biofilm formation [31]. Biofilm formation was also detected in significant number of isolates (Table 2.).

A study documented that more than 50% biofilm producer in all bacterial infections. Normal human serum can kill bacteria by complement pathway [32] and serum resistant was documented in 33% of UPEC [33]. The ESBL production was observed in

72% of the UPEC which is similar to 79% in previous study by Yadav et al., [34].

The treatment for UTI in men requires urine culture analysis rather than empirical antimicrobial treatment [35-37]. Livermore and Pearson [38] said that sensitivity varies over time and region. The UPEC isolated from male UTI patients in Coimbatore showed different sensitivity pattern. Highest resistance was noted towards the antibiotics ampicillin, cephazolin, amoxicillin, ciprofloxacin and cephalothin. Akram et al. [39] demonstrated high resistant to ampicillin and amoxicillin in India. Approximately 50% of our isolates show resistance against ampicillin – sulbactam, co-trimoxazole, tozabactam and gentamycin.

In Mexico, 74% of UPEC showed resistant to ampicillin, 60% to co-trimoxazole and 32% against ciprofloxacin [40,41]. Significant resistance was seen towards amikacin, ertapenem and norfloxacin. Enwuru et al., [42] stated that low resistance was found against ceftazidime, ofloxacin, gentamycin since their usage is limited when compared to others. In our work lower resistance was found against the antibiotics imipenem, meropenem and tigecycline. Kader and Angamuthu, [43] reported sensitivity towards imipenem and meropenem. Intermediate sensitivity was observed for some of the antibiotics like norfloxacin, tozabactam, ampicillin -sulbactam, meropenem and tigecycline (Table 3.). Sharma et al., [44] say, as a consequence of uncontrolled usage resistance have developed against commonly used antibiotics. Donnenberg and Welch [45] stated that O1, O2, O4, O6, O7, O16, O18, O25 and O75 were consistent in 81% of UPEC. In our 20 isolates we were able to identify the serotypes as rough (4 isolates), O8 (4 isolates), O9 (3 isolates), O2 (3 isolates), O141, O35, O39 and O55 (each one isolate). The most frequent serotypes are O4 and O6 [46, 47] were not encountered by us.

### 4. CONCLUSION

The treatment of UPEC is complicated unless we understand the characteristics of the pathogen like virulence factors, serotype and antibiotic sensitivity. There was less information available on treatment of UTI infected men since the prevalence is more in women. We have analyzed the UPEC from 100 men which showed old age men were more prone to UTI. The prevalence of virulence factors in descending order was hemolysis, serum resistance, MSHA, biofilm production and MRHA. Most of them were

**Table 1.** Prevalence of Uropathogenic *Escherichia coli* (UPEC) in men.

Age	01 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 60	61 - 70	71 - 80
No of Patients	4	4	Nil	12	16	16	28	20

Nil – no UPEC was found in the age group

**Table 2.** Distribution of virulence factors of UPEC isolates of men.

S. No	Virulence factors	Total N= 100 (%)
1	Hemolysis	64 (64)
2	MRHA	28 (28)
3	MSHA	36 (36)
4	Biofilm formation	32 (32)
5	Serum Resistance	40 (40)

MRHA – Mannose Resistant Hemagglutination, MSHA – Mannose Sensitive Hemagglutination, N – Number of isolates. (%) – Percentage.

**Table 3.** Antibiotic susceptibility report of UPEC isolated from UTI infected men.

S. No	Antibiotic	No. of Isolates (Percentage)		
		Sensitive (%)	Resistant (%)	Intermediate (%)
1	Ampicillin	16	84	0
2	Ampicillin -sulbactam	40	56	4
3	Amikacin	88	12	0
4	Amoxicillin	32	68	0
5	Cephazolin	28	72	0
6	Ciprofloxacin	32	68	0
7	Cephalothin	32	68	0
8	Cefepime	28	72	0
9	Ertapenem	84	16	0
10	Imipenem	96	4	0
11	Meropenem	92	4	4
12	Norfloxacin	44	24	32
13	Tozabactam	28	48	24
14	Tigecycline	88	8	4
15	Co-trimaxazole	44	56	0
16	Gentamycin	52	48	0

-multidrug resistant and the highest resistant was against ampicillin, cephalosporin and cefepime. Drug resistance has even started against imipenem, ertapenem and meropenem which were previously effective drugs. Some random serotyping showed repeated serotypes of rough, O8, O9 and O2. This information will help in treating the male patients of UTI of Coimbatore. Similar work is essential to treat patients elsewhere.

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### Conflicts of Interest

There are no conflicts of interest.

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