

Demographic characterization, risk factors and antimicrobial susceptibility pattern of UPEC associated UTI

Shivani Sinha¹, Atul Kumar², Preeti Kori^{3*}

¹*Demonstrator, Department of Microbiology, Government Medical College, Ratlam, Madhya Pradesh, India*

²*Associate Professor, Department of Surgery, Government Medical College, Ratlam, Madhya Pradesh, India*

³*Assistant Professor, Department of Medicine, Government Medical College, Ratlam, Madhya Pradesh, India*

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Abstract

Objective: To determine the demographic characterization of patient with urinary tract infection and antimicrobial susceptibility pattern of UPEC in our region. **Methods:** This study was a cross sectional analytical study done for period of one year. All the patients suspected or diagnosed with UTI, their urine specimen were sent for culture and sensitivity. **Results:** Catheterization (27.27%) is the most common risk factor followed by bladder outlet obstruction due to BPH. The highest antibiotic resistance was observed for all isolates with Ampicillin (96 %) followed by Amoxycyclav (80%) and highest sensitivity was found with Cotrimoxazole (85%) and Nitrofurantoin (85%) among oral antibiotics. Ceftriaxone (80 %) and Cefotaxim (75%) were found to be highly resistant among parenteral antibiotics whereas highest sensitivity of the isolates was observed with Imipenem (98 %) followed by Piperacillin + Tazobactam (95 %) and Amikacin (85 %). Imipenem was found to be 100 % sensitive in UPEC isolated from uncomplicated UTI whereas only 1 isolate was resistant to Piperacillin + Tazobactam. **Conclusion:** In the present study, highest percentage of resistance was observed with Ampicillin (96%) followed by Amoxicillin + clavulanate (80%) Ceftriaxone (80%) and Gentamycin (75%). Antibiotic sensitivity pattern varies according to the pathogens associated with the disease and also depend on the antibiotic policies in that region. In most of the places especially in rural areas where culture facilities are not available ampicillin, amoxicillin + clavulanic acid and fluoroquinolones are frequently prescribed as oral agents and Ceftriaxone and Aminoglycosides as a parenteral treatment leads to development of resistant strains.

Keywords: Uropathogenic *E. Coli* (UPEC), Urinary tract infections (UTI), Benign Prostatic Hypertrophy (BPH)

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Introduction

Urinary tract infections (UTIs) are the most common bacterial infections affecting approximately 150 million per annum across the world.[1] They are also the most common hospital acquired infections accounting for about 30% - 40% of the total burden. UPEC is the most important group of pathogens causing UTIs.[1] More than two third of the urinary tract infections were complicated by them. With the widespread availability of various groups of antibiotics it has been managed effectively for past few decades. As the antibiotics are used empirically, the increasing bacterial resistance of UPEC to a large number of antibiotics is causing major health concerns worldwide.[2] Now a days, infectious pathogens are mostly resistant to several antibiotics leads to reinfections and urosepsis. The unique ability to adhere to urothelium and capacity to develop resistant to various groups of antibiotics makes them difficult to treat. Virulence factors of UPEC are capsular antigen, production of toxins like hemolysin and biofilm formation over indwelling urinary catheter.[3]

The antibiotic resistance genes can also be transferred to other non resistant colonies via plasmid or integrins.[4,5] Many molecular studies had also proved that urovirulent *E.coli* expresses many cell surface factors and secretes toxins and enzymes, among them few are peculiar in causing UTIs. As the incidence of re infections and complicated UTI is increasing and changing the sensitivity pattern due to evolution of various resistance mechanism by UPEC, the pattern and resistant pattern should be studied and determined frequently to decrease the morbidity and mortality associated with urosepsis. Hence, the present study was undertaken to determine the demographic characterization of patient with urinary tract infection and antimicrobial susceptibility pattern of UPEC in our region.

Materials and methods

Design of study

This study was a cross sectional analytical study done for period of one year from December 2012 to December 2013 in Rohilkhand Medical College and Hospital, Bareilly, U.P. All the patients suspected or diagnosed with UTI in OPD or admitted in the hospital and urine specimen were sent for culture and sensitivity was included in this study. Urine samples were collected via clean catch mid stream void, suprapubic aspiration and catheters as per standard protocols.

*Correspondence

Dr. Preeti Kori

Assistant Professor, Department of Medicine, Government Medical College, Ratlam, Madhya Pradesh, India.

E-mail: drshivani1705@gmail.com

Specimen Processing

The collected samples were properly labeled and processed without delay to get maximum number of possible colonies. Only *E. coli* isolates were included in this study. Colonies of multiple different species of bacteria in association with *E. coli* were excluded from the study. The significant isolates were further processed for identification of *E. coli*. Antimicrobial susceptibility testing was done by Kirby-Bauer disk diffusion method. The *E. coli* isolates were tested against the commonly used antibiotics against UPEC in our region. Growth inhibition zone diameters were measured in millilitres and results interpreted as recommended by the Clinical laboratory standards institute (CLSI) guidelines 2011. Minimal inhibitory

concentration (MIC) determination was done by Broth micro-dilution technique and was interpreted as per CLSI guidelines 2011. The wet mount findings and colony count in the culture plates were correlated with the patient's demographic details and like age and gender, associated risk factors, co-morbidities, previous antibiotic history and associated diseases if any.

Consent

Written consent was obtained from the relatives of patients after explaining them the nature and purpose of the study. They were assured that confidentiality would be strictly maintained. The option to withdraw from the study was always open.

Observation chart**Table1: Pathogen isolated from various clinical specimens**

| Sample | Total No. of samples | <i>E. coli</i> | <i>Klebsiella</i> | <i>Pseudomonas</i> | <i>S. aureus</i> | <i>Proteus</i> | <i>Enterococcus</i> | Other org.* | Sterile |
|--------------|----------------------|----------------|-------------------|--------------------|------------------|----------------|---------------------|-------------|---------|
| Urine | 2160 | 317 | 150 | 120 | 17 | 60 | 56 | 30 | 1410 |
| Blood | 1280 | 97 | 98 | 122 | 130 | 40 | 5 | 132 | 656 |
| Pus | 1400 | 143 | 139 | 179 | 281 | 31 | 33 | 27 | 567 |
| Vaginal swab | 91 | 10 | 1 | 3 | 1 | 2 | 4 | 7 | 63 |
| Throat swab | 41 | - | 1 | 1 | 3 | - | - | 4 | 32 |
| Sputum | 1400 | 170 | 242 | 240 | 195 | - | - | 45 | 508 |
| Body fluids | 200 | 7 | 6 | 9 | 10 | 3 | 2 | 10 | 153 |
| Total | 6572 | 1127 | 672 | 723 | 637 | 148 | 100 | 295 | 2870 |

Other organism are *Citrobacter*, *Acinetobacter*, *Staphylococcus Saprophyticus*, *Providencia*, *Morganella*.

Table 2: Bacteria isolated from urinary specimens on culture.

| Species identified | No. of Isolates (n=750) | Percentage (%) |
|---------------------|-------------------------|----------------|
| <i>E. coli</i> | 317 | 42.26 % |
| <i>klebsiella</i> | 150 | 20 % |
| <i>Pseudomonas</i> | 120 | 16 % |
| <i>Proteus</i> | 60 | 08 % |
| <i>Enterococcus</i> | 56 | 7.4 % |
| <i>S. aureus</i> | 17 | 2.2 % |
| other organisms | 30 | 04 % |

Table 3: Distribution of patients with various associated factors

| Associated Factors | Frequency N= 220 | Percentage (%) |
|-----------------------------------|------------------|----------------|
| Catheterization | 60 | 27.27% |
| Urosepsis | 10 | 4.5% |
| Urolithiasis | 20 | 9 % |
| Septicemia | 15 | 6.8% |
| Recurrent urinary tract infection | 15 | 6.8 % |
| Bladder outlet obstruction | 40 | 18.1% |
| Vesico ureteric reflex | 05 | 2.2% |
| Acute epididymo-orchitis | 15 | 6.8% |
| Fournier's gangrene | 10 | 4.5% |
| Pregnancy | 20 | 9 % |
| Diabetes Mellitus | 40 | 18.1% |
| Chronic Kidney Disease(CKD) | 10 | 4.5 % |
| Neurogenic bladder | 30 | 13.6% |

Table 4. Antimicrobial susceptibility pattern of UPEC

| Drugs | Sensitive | Resistance |
|-------------------------------|-----------|------------|
| Ampicillin | 12 | 305 (96 %) |
| Amoxicillin + Clavulanic acid | 63 | 254 (80%) |
| Ciprofloxacin | 95 | 222 (70%) |
| Nitrofurantoin | 269 | 48 (15%) |
| Cotrimoxazole | 269 | 48 (15%) |
| Ceftriaxone | 63 | 254 (80%) |
| Cefotaxime | 79 | 238 (75%) |
| Ceftazidime | 158 | 159 (50%) |
| Gentamycin | 79 | 238 (75%) |
| Amikacin | 269 | 48 (15%) |
| Piperacillin-Tazobactam | 301 | 16 (5%) |

| | | | |
|--|----------|-----|---------|
| | Imipenem | 310 | 07 (2%) |
|--|----------|-----|---------|

Table 5: Resistance pattern of UPEC among complicated and uncomplicated UTI

| Drugs | Complicated (n=220) | Uncomplicated (n=97) |
|--------------------------|---------------------|----------------------|
| Ampicillin | 215 (97.7%) | 90 (92 %) |
| Amoxclav | 184 (83.6 %) | 70 (72.1%) |
| Amikacin | 38 (17.2%) | 10 (10.3 %) |
| Nitrofurantoin | 36 (16.36%) | 12 (12 .3%) |
| Ciprofloxacin | 157(71.3%) | 65 (67%) |
| Cotrimoxazole | 70 (31.8%) | 10 (10.3%) |
| Ceftriaxone | 174(79 %) | 70 (72.16 %) |
| Cefotaxime | 168(76.3%) | 70 (72.1%) |
| Piperacillin+ Tazobactam | 15(6.8%) | 1(1%) |
| Imipenam | 7(3.1%) | 0 (0%) |

Results

6572 clinical specimens were referred for bacteriological cultures. Among which include 2160 urine, 1280 blood samples, 1400 pus, 91 vaginal swabs, 41 throat swabs, 200 Body fluids, 1400 sputum. Total 2160 urine samples were received in Microbiology department during the study period were processed for culture and sensitivity. Out of these, 1410 samples (65.27 %) were sterile and 750 (34.72 %) showed growth of various species of bacteria. Gram negative bacilli were found to be predominant isolates followed by Gram positive cocci. Fungal growth was not included in this study. Among the Gram negative bacilli, *E. coli* was isolated in 317 samples (42.26%) followed by *Klebsiella* 150 (20 %), *Pseudomonas aeruginosa* 120 (16%) and *Proteus* 60 (8%). In Gram positive cocci, *Staphylococcus aureus* were isolated in 17 (2.2%) and *Enterococcus* in 56 (7.4%).

Demographic details of UPEC isolates

Out of 317 *E. coli* isolated, 202 (63.72%) were isolated from the female patients and 115 (36.27%) from males. The mean age was 44 years with a standard deviation of 22.41 years. The incidence of UTI in females was highest in age group of 21 to 40 years (44.55%) followed by the age group of 41 to 60 years (34.65 %). Among the male patients, organisms were isolated mostly in age group above 61 years (45 %) and the lowest was less than 20 years (2 %).

Distribution of cases according to clinical diagnosis

In the present study, out of 317 *E. coli* isolated, 220 were associated with various risk factors leading to diagnosis of complicated UTI whereas in 97 samples no risk factor association was observed. Diabetes mellitus was found to be present in 40 of the cases while 30 cases were associated with 2 or more risk factors other than DM.

Catheterisation (27.27%) was found to be the most common risk factors followed by bladder outlet obstruction due to BPH, stricture urethra leading to cystitis (18.1%) and neurogenic bladder (13.6%). Urolithiasis was found to be present in 9 % of the cases while history of recurrent UTI was recorded in 6.8% of the cases.

In this study, the highest antibiotic resistance was observed for all isolates with Ampicillin (96 %) followed by Amoxy-clavulanic acid (80%) and highest sensitivity was found with Cotrimoxazole (85%) and Nitrofurantoin (85%) among oral antibiotics commonly used against UPEC. Ceftriaxone (80 %) and Cefotaxim (75%) were found to be highly resistant among parenteral antibiotics whereas highest sensitivity of the isolates was observed with Imipenem (98 %) followed by Piperacillin + Tazobactam (95 %) and Amikacin (85 %). Antibiotic resistance was found to be more commonly associated with UPEC isolated from cases of complicated UTI as compared to uncomplicated UTI. No major difference was found in the resistance pattern of UPEC from both the complicated and uncomplicated UTI. Imipenem was found to be 100 % sensitive in UPEC isolated from uncomplicated UTI whereas only 1 isolate was resistant to Piperacillin + Tazobactam.

Statistical analysis

Statistical analysis was done by using SPSS software version 13.0. Variables were analyzed for mean, standard deviation, frequencies and percentages. Chi-square test was used as test of association, for which p value was calculated. Significance level was set as p value of

0.05. Data was compiled using MS excel 2007 and analysis was done with the help of Epi-Info 7 software. Frequency and percentage were calculated & statistical test (Chi Square) was applied wherever applicable; $p < 0.05$ was taken as statistically significant.

Discussion

Demographic characterization of UPEC isolates

In our study out of 2160 samples collected from out patients and in patients, 750 (34.7%) samples had showed growth of bacteria. It has been shown by many studies in various regions of India that the prevalence rate of community acquired UTIs was 37-61%.[2,6] However few studies have reported in their study on community acquired UTIs that the prevalence rate was about 10-17 % in OPD patients.[2,7] In our study 15 % of the samples had shown significant growth among out patients. *E. coli* was the predominantly isolated from urinary samples in our study followed by *Klebsiella* and *Enterococcus* which is similar to other studies.[6,7] Enterobacteriaceae were found to be predominant isolate in most of the studies due to their ability to adhere to urothelium and colonize there with the help of pili, fimbriae, and P1-blood group phenotype receptor. Few studies has shown *Enterococcus* as the second common pathogen followed by *Klebsiella*.[8]. Some studies has also shown *Citrobacter* as a second common pathogen isolated from urinary samples in about 14 -20 %.[9,10]

Due to short urethra, proximity of urethra to vagina and rectum and exposure to pathogens during sexual activity, UTI is common in females.[8] As the age advances especially in older age group UTI is commoner in males as compared to females that could be due to bladder outlet obstruction, incontinence, neurogenic bladder and associated co-morbidities.[8] The prevalence of UTI is multifactorial and depends on age, gender, personal hygiene, social culture and other environmental factors. About 20% of women experiences at least one episode of UTI during their lifetime, and about 3% of women have more than one episode of UTI per year.[11] Out of 317 *E. coli* isolated, 202 (63.72%) were isolated from the female patients and 115 (36.2%) from males. The mean age was 44 years. The incidence of UTI in females was highest in age group of 21 to 40 years (44.5%) followed by the age group of 41 to 60 years (34.6 %). Among the male patients, organisms were isolated mostly in age group above 61 years (45 %) and the lowest was less than 20 years (2 %). Devanand Prakash et al had also reported high prevalence rate (30.30%) among older males above 48 years of age in their study.[12] In the study done by Dash et al in Odisha over community acquired UTIs the prevalence was highest in age group 18-27 years (29.2%) followed by 28-37 years (26.2%) among the female patients. In males, majority of the isolates (41.8%) were obtained from male patients aged ≥ 68 years.[8]

Antimicrobial susceptibility of UPEC

In the present study, highest percentage of resistance was observed with Ampicillin (96%) followed by Amoxicillin + clavulanate (80%) Ceftriaxone (80%) and Gentamycin (75%). Antibiotic sensitivity pattern varies according to the pathogens associated with the disease and also depend on the antibiotic policies in that region. In most of the places especially in rural areas where culture facilities are not

available ampicillin, amoxicillin + clavulanic acid and fluoroquinolones are frequently prescribed as a oral agents and ceftriaxone and aminoglycosides as a parenteral treatment. The culture of not to send urinary samples for culture and sensitivity is highly prevalent in sub-urban and rural areas in India. Resistance to ampicillin, amoxicillin + clavulanic acid, fluoroquinolones, ceftriaxone and aminoglycosides had been documented in most of the studies in India done in different demographic region.[8,13] Among the third generation cephalosporins, Ceftriaxone resistance was found to be 80 % our study. Resistance to third generation Cephalosporines has been reported to be more than two third of the isolates in various studies.[8,12] Nitrofurantoin and Cotrimoxazole were found to have less resistance among oral agents commonly used for the treatment of UTI.[8,12] In our study also sensitivity to nitrofurantoin and Cotrimoxazole was found to be 85 % and 85 % respectively.

The other disadvantage of injudicious use of antibiotics is emergence of multi drug resistant among UPEC. Apart from that delay in seeking treatment, poor catheterization technique, increase incidence of Diabetes and other immunosuppressive state with advancement of age and prolonged stay in hospital are few possible reasons for increase incidence of re infection and intake of multiple drugs which promote the pathogen to develop resistance mechanism. In our study, 48 isolates were found to be resistant to two or more group of antibiotics. MDR E.coli isolates among urinary pathogens are also increasing from past two decades. It has been observed in various studies that MDR were isolated from 8 -52 % of the UPEC isolated in recent years.[4,14] The resistant was transferred rapidly through plasmids as well as through gene clustering in the integrons.[4,5] MDR isolates with intrgron commonly show resistance with ampicillin, triazole, tetracycline and gentamycin.[4]

Resistance pattern of UPEC isolates among Complicated and uncomplicated UTI

In our study it was found that 69.4 % and 30.5% of patients had complicated and uncomplicated UTI respectively whereas in the study carried out by Desai et al 22% had complicated and 78% were uncomplicated UTI. In our study, antibiotic resistance was found to be more commonly associated with UPEC isolated from cases of complicated UTI as compared to uncomplicated UTI. No major difference was found in the resistance pattern of UPEC from both the complicated and uncomplicated UTI. Imepenam was found to be 100 % sensitive in UPEC isolated from uncomplicated UTI whereas only 1 isolate was resistant to Piperacillin + Tazobactam. It has been shown in many studies that most of the antibiotics were multidrug resistant strains were more commonly isolated in patients with complicated UTI than those suffering from uncomplicated UTI.[15]

Conclusion

In the present study, highest percentage of resistance was observed with Ampicillin (96%) followed by Amoxicillin + clavulanate (80%) Ceftriaxone (80%) and Gentamycin (75%). Antibiotic sensitivity pattern varies according to the pathogens associated with the disease and also depend on the antibiotic policies in that region. In most of the places especially in rural areas where culture facilities are not available ampicillin, amoxicillin + clavulanic acid and fluoroquinolones are frequently prescribed as a oral agents and Ceftriaxone and Aminoglycosides as a parenteral treatment leads to development of resistant strains.

What this study add to existing knowledge

As the incidence of re infections and complicated UTI is increasing and changing the sensitivity pattern due to evolution of various resistance mechanism by UPEC, the pattern and resistant pattern should be studied and determined frequently to decrease the morbidity and mortality associated with urosepsis.

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