



Original Research Article

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Prevalence and Antibiotic Susceptibility Pattern of *Escherichia coli* Positive Urinary Tract Infections in a Rural Tertiary Care Hospital in Rohtas, Bihar, India

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A B S T R A C T

Urinary tract infection (UTI) is a common condition affecting all age groups. *E.coli* and several other uropathogens account for most UTIC cases. Although definitive diagnosis is based on culture reports, most of the UTI are treated empirically. Multidrug Resistance (MDR) cases of UTI is also on the rise. A cross sectional study was conducted to find out the prevalence of *E.coli* positive UTI cases and to know its susceptibility pattern against commonly used antibiotics. Urine samples were collected from patients attending OPD's who were suspected to be having UTI. Identification of organism by urine culture and susceptibility pattern was based on standard conventional bacteriological techniques using Kirby-Bauer disc diffusion method. Clinical and Laboratory Standard Institute (CLSI) guidelines were followed. 64.57% isolates were positive for *E.coli*. There were 56.6% females and 43.4% males. 44.2% of patients with *E.coli* positive UTI were found within the age range of 20-39 years. *E.coli* exhibited highest resistance to Nalidixic acid. Amoxycillin, Cefixime, Cotrimoxazole, Ceftriaxone and Ofloxacin also showed high resistance. 87.6% *E.coli* were MDR cases which may be attributed to their prevailing usage and abuse in the area under study. Empirical treatment should be carefully selected based upon the sensitivity data of uropathogens of that particular area. Frequent monitoring of antimicrobial susceptibility for uropathogens in the community and hospital setting is recommended.

Keywords

Antibiotic Susceptibility Pattern, *Escherichia coli* Urinary Tract Infections.

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Introduction

Urinary tract infection (UTI) is a very common condition and a major cause of seeking medical consultation (Omoregie *et al.*, 2008). UTI are also prevalent in community in all age groups and requires urgent treatment (Orrett *et al.*, 2006). Most of the UTI cases are treated on an empirical basis of geographical distribution of

antibiotic susceptibility in absence of any laboratory investigations (Bercion *et al.*, 2009). UTI can be caused by several uropathogens. In developing countries like India, *E.coli* accounts for most of the UTI cases across all age groups, in both sexes with variable clinical presentation ranging from mild asymptotic cases to severe complicated UTI.

Diagnosis and definitive treatment of UTI is mainly based on bacteriological culture and antibiotic sensitivity. In past decade, indiscriminate use of antibiotics resulted in worldwide rise of Multidrug Resistance (MDR) cases (Davies *et al.*, 2010).

Antibiotic resistant uropathogens makes empirical treatment of UTI even more difficult. Hence analysing antibiotic susceptibility pattern of uropathogens will help to overcome the therapeutic dilemmas and guide in selection of appropriate antibiotics for empirical treatment. The aim of this study was find out the prevalence, age and sex distribution of *E.coli* positive UTI in this area and to know its susceptibility pattern against commonly used antibiotics.

Materials and Methods

This hospital based cross-sectional study was conducted in Department of Microbiology at Narayan Medical College & Hospital, situated in a rural area of Sasaram, Bihar in collaboration with the Department of Community Medicine and Pharmacology. The study period was from June 2016 to august 2016. The study was approved by Institutional Ethical Committee. Urine samples were collected from patients attending OPD's who were suspected to be having UTI after getting their consent.

Clean-catch midstream urine was collected into a wide mouth sterile universal container. A proper instruction was given to the patients regarding the method of collection. Males were asked to retract prepuce, cleanse the glans with soap and water and then collect the sample. Females were instructed to thoroughly clean the anogenital area from front to back, pass urine with labia separated and collect sample. Later labelled specimens with patient's age

and sex on container were transported to the laboratory.

Inadequate urine samples (<10 mL), urine bag collected samples, specimens collected more than 2 hours before submission, specimens in leaking, or dirty unsterile containers and specimens revealing growth of more than two types of bacteria on culture were excluded from the study. Patients on long term antibiotic therapy prior to or during the investigation were also excluded.

Urine culture was done by standard loop method, a semi-quantitative method. Before processing urine is mixed well and a loop-full (0.001 ml) urine was streaked onto the surface of blood agar and Mac Conkey's agar. These plates were incubated aerobically at 37°C FOR 18-24 hours. Bacterial counts were expressed in colony forming units (CFU) per millilitre (mL). A count of $\geq 10^5$ CFU/mL was considered significant bacteriuria which conferred to "Kass concept of significant bacteriuria". Identification of organism was based on standard conventional bacteriological techniques (Forbes *et al.*, 2002).

Antimicrobial susceptibility testing was performed as per Kirby-Bauer disc diffusion method as per guidelines. The entire disc was obtained from Hi Media Laboratories, India. Quality controls were regularly checked by standard strains of *E.coli* ATCC 25922. Clinical and Laboratory Standard Institute (CLSI) guidelines were followed to interpret sensitivity and resistance on the basis of the diameters of zones of inhibition of bacterial growth as recommended by the disc manufacturer (Clinical and Laboratory Standard Institute, 2013).

Antibiotics against which sensitivity was tested in the present study included Amoxycillin, Cefixime, Ceftriaxone, Amikacin, Tobramycin, Nalidixic acid,

Ciprofloxacin, Ofloxacin, Levofloxacin, Azithromycin and Cotrimoxazole. The concentrations at which they were used is given in Table 2. An isolate was considered as MDR if found resistant to three or more antimicrobials to different classes/ groups of antimicrobials

(http://ecdc.europa.eu/en/activities/diseaseprogrammes/ARHAI/Documents/table-3Clinical_Microbiology_and_Infection.pdf).

Data was entered in MS Excel and analyzed by the statistical package SPSS software, version 16.0. Descriptive and inferential statistics were applied (percentages and Z test). The values of *p* were kept significant at the level of 0.05.

Results and Discussion

Bacteriological aetiology of UTI in patients of this hospital was determined in 538 urine samples. Bacteria were successfully isolated from 175 (32.5%; 175/538) samples. Out of 175 bacterial isolates, 113 (64.57%) isolates were positive for *E.coli*. Thus *E.coli* was most common isolates, followed by *Klebsiella pneumonia* (13.71%), *Pseudomonas aeruginosa* (10.86%), *Proteus spp.* (5.14%), *Acinetobacter baumanii* (2.85%), *Staphylococcal aureus* (1.71%) and *Candida spp.* (1.16%).

Among *E.coli* positive UTI cases, there were more females (56.6%, 64/113) than males. (43.4%, 49/113) and this difference was statistically significant ($Z = -1.996$; $p=0.0459$). The incidence of UTI was ranged in patients between 4-80 years old. The highest number (50; 44.2%) of patients with *E.coli* positive UTI were found within the age range of 20-39 years. In this age group, proportion of females (72.0%; 36/50) was significantly more as compared to males (28.0%; 14/50).

In comparison, *E.coli* isolates were found less in age group ≤ 19 years and ≥ 60 years.

Considering the overall incidence among all age groups, females of age group 20-39 years (56.25%; 36/64) and males of 40-59 years (42.86%; 21/49) were more affected (Table 1).

Antibiotic resistant pattern of *E.coli* against a number of antibiotics was also tested out. *E.coli* exhibited highest resistance to Nalidixic acid (93.6%) followed by Amoxycillin (91.1%), Cefixime (77.9%), Cotrimoxazole (76.1%), Ceftriaxone and Ofloxacin (69.9%). Out of 113 *E.coli*, 99 (87.6%) were multidrug resistance (MDR) as the isolates were non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories. (Table 2)

The culture positive rate in this study was 32.5%. A similar rate was observed in other studies. In this study we have excluded sterile samples, samples showing insignificant growth ($<10^5$ CFU/mL) and samples contaminated with polymicrobial growth of more than three types of organism (Arjunan *et al.*, 2010; Taneja *et al.*, 2010; Singhal *et al.*, 2014).

The most common uropathogen in our study was *E.coli*, found in 65% isolates. Similar pattern was seen in study done by other studies (Singhal *et al.*, 2014; Kamat *et al.*, 2009). The second most common uropathogen in this study was *Klebsiella pneumonia*, followed by *Pseudomonas aeruginosa* and *Proteus spp.* (Manikandan *et al.*, 2014).

Infection frequency of *Acinetobacter baumanii*, *Staphylococcal aureus* and *Candida spp.* was found less in this study in comparison to *E.coli*. While most other studies have reported the involvement of *Enterobacter spp.* and *enterococcus faecalis* as one of the common pathogen in UTI cases, our study in this area has not shown any such finding (Niranjan *et al.*, 2014).

The etio-pathological variation in UTI cases may be due to differences in host factors, environmental factors, health care and hygiene practices and socio-economic conditions in different part of the world. In this study, we have described the socio-demographic factors, prevalence of antibiotic susceptibility and resistance in patients of UTI predominantly caused by *E.coli*. The sex distribution of the UTI patients in this study is similar to other reported studies showing a predominance of females (56.6%).

The increased incidence of UTI among females is related to the anatomical differences between male and female genitourinary symptoms and alteration in normal vaginal flora. Among females, more cases were seen in reproductive age group because of their increase sexual activities, for example "honeymoon cystitis" and "Pyelitis of Pregnancy".

In this study, males of 40-59 years were more affected which may be related to the increased incidence of bladder outlet obstruction such as prostatitis and bladder stone. *E.coli* isolates were found less at the extremes of ages in this study probably because of their dependence for consultation in this tertiary care centre.

The most effective antimicrobial agents in this study were Amino glycosides (Amikacin, Tobramycin), followed by Fluoroquinolone (Levofloxacin) and Macrolide (Azithromycin). In this study Amikacin is the most effective antimicrobial against *E.coli*. Our result is further supported by different authors (Shalini *et al.*, 2011).

E.coli showed considerable resistance to Amoxycillin and Nalidixic acid (>90% in both) followed by Cefixime and Cotrimoxazole. It is important to note that

the *E.coli* isolates showed considerable resistance to beta-lactam antibiotics probably because they belonged to Extended spectrum beta lactamase (ESBL) group. Another important finding is the resistance to most of the older generation fluoroquinolones and sensitive to only levofloxacin.

The rising resistance for fluoroquinolones is based on several factors (Hooton, 2003). Recent guidelines from the Infectious Diseases Society of America recommended that empiric antibiotic therapy for UTIs should be based on local resistance data, drug availability, and antibiotic intolerance/allergy history of treated patients (Gupta *et al.*, 2011).

Fluoroquinolones (Ciprofloxacin, Ofloxacin) and Cotrimoxazole are most recommended antimicrobials for empirical treatment of UTI in developing countries like India. But in this study we found the resistance against both these groups which reflects the geographical variation of the sensitivity data. Hence based upon the data of this study for our setting in this zone, the recommended empirical therapy before culture and sensitivity will be amino glycosides like Amikacin, Levofloxacin and/or Azithromycin.

However, in older patients amikacin should be used carefully due to risk of ototoxicity and nephrotoxicity.²¹ 87.6% isolates were MDR *E.coli*.

It could be due to overall increase in prevalence of MDR pathogens in the community, which is associated with the injudicious irrational use of available antibiotics as well as newer molecules and improper personal hygiene by health staff and patients.

Table.1 Distribution of UTI patients according to age groups and gender.

Age Groups	Males (%)	Females (%)	Total	%of Total	Z	p
≤ 19 yrs	7 (58.4%)	5 (41.6%)	12	10.6%	0.8	0.410
20- 39 yrs	14 (28.0%)	36 (72.0%)	50	44.2%	4.4	<0.0001
40- 59 yrs	21 (61.7%)	13 (38.3%)	34	30.1%	1.9	0.0537
≥ 60 yrs	7 (41.2%)	10 (58.8%)	17	15.1%	1	0.304
Total	49 (43.4%)	64 (56.6%)	113	100%	2	0.0472
$\chi^2 = 10.6$; df= 3; p= 0.014						

Table.2 Antibiotic sensitivity pattern of *E.coli* isolates

Antibiotics	Codes	Disc content (μ g)	Diffusion zone breakpoint (mm)	Sensitive		Intermedi ate		Resistant	
				n	%	N	%	n	%
Amoxycillin	AMX	30	≤ 14	7	6.2	3	2.7	103	91.1
Cefixime	CFM	5	≤ 15	20	17.7	5	4.4	88	77.9
Ceftriaxone	CTR	30	≤ 13	23	20.4	11	9.7	79	69.9
Amikacin	AK	30	≤ 14	87	77.0	2	1.7	24	21.3
Tobramycin	TOB	10	≤ 12	80	70.8	9	8.0	24	21.2
Nalidixic Acid	NA	30	≤ 13	4	3.5	3	2.9	106	93.6
Ciprofloxacin	CIP	5	≤ 15	21	18.6	20	17.7	72	63.7
Ofloxacin	OF	5	≤ 28	18	16.0	16	14.1	79	69.9
Levofloxacin	LE	5	≤ 29	77	68.1	23	20.3	13	11.6
Azithromycin	AZM	15	≤ 18	72	63.7	8	7.1	33	29.2
Cotrimoxazole	COT	1.25/ 23.75	≤ 26	23	20.3	4	3.6	86	76.1

In conclusion, there is variation in antibiotic susceptibility pattern of *E.coli* positive UTI. Hence empirical treatment should be carefully selected based upon the susceptibility data of isolates of that

particular area. Frequent monitoring of antimicrobial susceptibility for uropathogens in the community and hospital setting is recommended. To limit the increasing incidence of drug resistance, strict national

and hospital antibiotic policies especially to the newer antibiotics, is the need of the hour.

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