



# ANTIMICROBIAL SUSCEPTIBILITY OF UROPATHOGENS IN COMMUNITY ACQUIRED ACUTE UNCOMPLICATED CYSTITIS IN ADULT WOMEN IN WESTERN UTTAR PRADESH, INDIA

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## ABSTRACT

**Introduction:** Aim of the present study is to identify bacteria causing community acquired acute uncomplicated cystitis in adult women and detection of local antibiotic resistance pattern of isolates .

**Materials and Methods:** The present prospective study was conducted at a tertiary care hospital and post graduate teaching institute. Total 149 patients of acute uncomplicated cystitis who had significantly positive bacterial culture for single bacterium species were selected for the study. Eight antibiotics were tested by modified Kirby Bauer disc diffusion method.

**Results:** The uropathogens identified were Escherichia coli (78.53%), Klebsiella pneumoniae (4.70%), Proteus mirabilis (2.01%), Staphylococcus saprophyticus (12.75%) and Enterococcus faecalis (2.01%). Most isolates showed resistance against cotrimoxazole (trimethoprim plus sulfamethoxazole) and coamoxiclav (amoxicillin plus clavunalate). They were susceptible to fluoroquinolones, third generation cephalosporin (cefepodoxime) and nitrofurantoin.

**Conclusions:** The most effective antibiotics in the present study for acute uncomplicated cystitis were levofloxacin and cefepodoxime

**Key Words:** Antibiotic sensitivity, Antibiogram, Bacterial resistance

## INTRODUCTION

Acute uncomplicated cystitis is the most common bacterial urinary tract infection in a premenopausal, non pregnant women with no known urologic abnormalities or comorbidities.<sup>[1]</sup>

Bacterial spectrum of acute uncomplicated cystitis consists of Escherichia coli, Staphylococcus saprophyticus (coagulase negative staphylococcus aureus; CoNS), Klebsiella pneumoniae, Enterococcus species, Group B Streptococcus (GBS; Streptococcus agalactiae) Salmonella species, Gordenerella vaginalis, Mycoplasma hominis and Ureaplasma urealyticum. Other gram negative and gram positive species are rarely isolated.<sup>[2]</sup>

The clinical manifestations of acute uncomplicated cystitis

consists of the acute onset of one or more symptoms such as dysuria, urgency, frequency, stanguria, or hesitancy. Gross hematuria is uncommon.. Physical examination of patients with acute uncomplicated cystitis is typically normal except in 10-20 % women with suprapubic tenderness.<sup>[2]</sup>

Antimicrobial susceptibility pattern of uropathogens vary considerably between different regions of a country. Therefore regional antimicrobial susceptibility pattern of uropathogens should be taken in to consideration for selection of empirical therapy of community acquired acute uncomplicated cystitis. Studies have reported a trend towards increasing resistance for most antibiotics, necessitating continued monitoring of this data.<sup>[3]</sup>

Hence the present study was planned to evaluate the in-vitro antimicrobial susceptibility of bacterial uropatho-

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gens in community acquired acute uncomplicated cystitis in adult women in Western Uttar Pradesh, India.

## MATERIALS AND METHODS

The present prospective study was conducted in a Medical College and Research Centre in Western Uttar Pradesh from February 2013 to March 2014. The study was approved by Institutional Ethical committee. Adult women patients in good general health who attended Out Patient Department for treatment of acute onset of one or more of the classical symptoms of cystitis for less than seven days duration and were sent to microbiology department for urine culture were screened via check list of inclusion and exclusion criteria. Written informed consent from each eligible patient was taken prior to their inclusion in the study group.

### Inclusion criteria were as follows:

- (a) Immunocompetant.
- (b) Non pregnant.
- (c) Premenopausal.
- (d) No known urologic abnormalities.
- (e) No comorbidities.

### Exclusion criteria were as follows:

- (a) Immunocompromised.
- (b) Pregnant.
- (c) Lactating.
- (d) Postmenopausal.
- (e) Known functional or anatomical urologic abnormalities eg. stone, stents, indwelling catheter, neurogenic bladder, polycystic kidney disease etc.
- (f) Comorbidities (Diabetes, cancer, renal insufficiency, vaginal discharge etc).
- (g) History of recurrent cystitis (more than three episodes in previous year).
- (h) No signs or symptoms of upper urinary tract infection (Fever, chills or flank pain and tenderness).
- (i) On immunosuppressant drugs.
- (j) Had antibiotics in past two weeks.
- (k) Had undergone urologic procedure in past two weeks.

### Sample collection:

All study group patients were advised to collect freshly voided clean catch midstream urine (mid portion of urine) sample in a wide mouth leak proof sterile container to hold 50 ml of urine.

### Direct microscopy and Dipstick nitrite test:

The unspun urine samples were screened by direct microscopy for pyuria (presence of 6 to 10 or more neutro-

phils per high power field) and urine dipstick nitrite test for bacteriuria (positive for nitrate reducing bacteria).

### Urine culture:

The urine sample was inoculated within 15 minutes of collection to Cysteine Lactose Electrolyte Deficient medium and Nutrient Agar medium and then incubated in an oxygen incubator at 37 °C for 16 (maximum 24) hours. Sheep Blood Agar medium was also used. It was incubated at 37 °C in an atmosphere of 5% carbon dioxide for 18 to 48 hours.

Standard thresholds used for bacterial count after urine culture were greater than or equal to 10<sup>3</sup> colony forming units per ml for Enterobacteriaceae and Staphylococcus saprophyticus; and greater than or equal to 10<sup>5</sup> colony forming units per ml for other pathogens. Patients whose urine culture report did not meet standard thresholds were excluded from study group. Urine culture growing 2 or more bacteria were also excluded from study group.

### Identification of pathogens:

Pathogens were identified on the basis of colony character, motility, Gram staining and biochemical tests (Catalase test, Oxidase test, Indole fermentation test, Methyl red test, Voges Proskauer test, Citrate utilization test, Triple sugar iron agar test, Urea hydrolysis, Coagulase test, Bile esculin hydrolysis test). Quality control: For gram negative bacteria, E. coli ATCC 25922, E. coli ATCC 35218 and Klebsiella pneumoniae ATCC 700603 were used as quality control strains. For gram positive bacteria, Staphylococcus aureus ATCC 25923 and Enterococcus faecalis ATCC 29212 strains were used as controls.

### Antimicrobial Susceptibility Testing: Modified Kirby Bauer Disc Diffusion method.

**Inoculum preparation:** Five well isolated colonies of same morphological types were selected from agar plate culture. Tip of each colony was touched with a loop and growth was transferred in to a tube containing 5 ml Mueller Hinton Broth medium. The broth culture was then incubated at 37 °C for two to six hours until it achieved the turbidity of 0.5 McFarland standard.

### Inoculation of Test plate:

Within fifteen minutes after adjusting turbidity of the inoculum suspension, a sterile cotton swab was dipped into adjusted suspension. It was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. Then, the dried surface of Mueller Hinton Agar plate was inoculated by streaking the swab over the entire agar surface. This process was repeated three times, rotating the plate approximately 60 degree each time to ensure even distribution of inoculum.

### **Application of discs to inoculated agar plate:**

Four predetermined battery of antibacterial discs were dispensed on to the surface of the each inoculated agar plate (100 mm). Each disc was pressed down to ensure complete contact with the agar surface. They were evenly distributed (no closure than 24 mm from centre to centre). Within 15 minutes, the plates were inverted and placed in incubator set at 37 ° C for 16 to 18 hours. MHA supplemented with 5 % sheep blood was also used. It was incubated at 37 ° C in an atmosphere of 5 % Carbon dioxide for 72 hours.

### **Reading of plates and interpreting the result:**

After incubation period was over, the plates were examined for zone of inhibition (uniformly circular clear area). It was measured in millimeter (mm) using a measuring scale (sliding calliper or ruler) from the edge of the clear area, across the disc to the other edge of clear area. Zone diameter =/> 20mm indicated susceptible, 15-19 mm indicated intermediate and =/< 14 mm indicated resistant strains. (01-CLSL-M02-2012 guideline)

Eight orally active drugs used as antibacterial discs during the study were : nitrofurantoin (100 mcg), cotrimoxazole (trimethoprim plus sulphamethoxazole) (23.75 /1.25 mcg), norfloxacin (10 mcg), ciprofloxacin (5 mcg), levofloxacin (5 mcg), coamoxiclav (amoxicillin plus clavunate) (20/10 mcg), cefuroxime (30 mcg) cefpodoxime (10 mcg). (Hi Media, Mumbai, India)

### **Data analyses:**

The information was recorded and analyzed using Microsoft Excel (2007 version).

## **RESULTS**

One hundred ninety one (191) patients who met the inclusion criteria were enrolled in the study group. Out of which 149 (78.01 %) patients who had standard threshold for bacterial count in urine culture (significantly positive urine culture) were finally selected for study. Rest 42 (21.99 %) patients were excluded from study group because 39 (20.42 %) had negative urine culture (did not reveal standard threshold for bacterial count in urine culture) and 3 (1.57 %) had contaminated growth.

### **Distribution of cases with respect to age:**

Total 149 cases were studied, out of them majority 82 (55.04 %) had 21-30 years age, while 43 (28.86 %) had 31-40 years, 15 (10.06 %) had 41-50 years and remaining 9 (6.04 %) had 18-20 years age.

### **Isolates obtained from urine samples:**

Gram negative bacilli accounted for 127 (85.24 %), while gram positive cocci accounted for remaining 22 (14.76 %) cases. Gram negative bacilli isolated were Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis. Escherichia coli alone accounted for 117 (78.53 %) urinary isolates followed by Klebsiella pneumoniae 7 (4.70 %) and Proteus mirabilis 3 (2.01 %). Gram positive cocci isolated were Staphylococcus saprophyticus and Enterococcus faecalis which accounted for 19 (12.75 %) and 3 (2.01%) urinary isolates respectively. [Table 1] [Fig 1]

### **Susceptibility pattern of gram negative bacteria:**

Escherichia coli showed least resistance to levofloxacin (14.53 %), ciprofloxacin (15.38%) and cefpodoxime (15.38 %). Klebsiella pneumoniae showed least resistance to levofloxacin (14.29 %), cefpodoxime (14.29 %) and nitrofurantoin (14.29 %). Proteus mirabilis showed least resistance to levofloxacin (00.00 %) and cefpodoxime (00.00 %). Escherichia coli and Klebsiella pneumoniae isolates showed the highest resistance to cotrimoxazole (50.43 % and 57.14 % respectively) while Proteus mirabilis showed 66.67 % resistance to both nitrofurantoin and coamoxiclav. [ Table 2 3, 4 ] [ Fig 2 ]

### **Susceptibility pattern of gram positive bacteria :**

Staphylococcus saprophyticus showed least resistance to norfloxacin, ciprofloxacin, levofloxacin (5.26 %) and nitrofurantoin (15.79 %) while Enterococcus faecalis showed least resistance to nitrofurantoin, ciprofloxacin and levofloxacin (00.00 %). Staphylococcus saprophyticus showed the highest resistance to coamoxiclav (31.58 %) and Enterococcus faecalis showed highest resistance (100.00 %) towards cotrimoxazole, cefuroxime and cefpodoxime. [ Table 5, 6].

## **DISCUSSION**

Local antibiotic susceptibility pattern of Escherichia.coli has been recommended by various guidelines to be considered in empirical antibiotic selection for acute uncomplicated cystitis in adult women. In the present study bacterial spectrum of acute uncomplicated cystitis consisted 78.53 % of Escherichia.coli. Its local resistance pattern not exceeding 20 % has been observed with nitrofurantoin, levofloxacin and cefpodoxime. As regards the susceptibility pattern, 73.51 % has been observed with nitrofurantoin, 83.76 % with levofloxacin and 82.05 % with cefpodoxime.

Several studies conducted in west by Gupta et al,<sup>[4]</sup> Naber KG et al,<sup>[5]</sup> Etienne M et al,<sup>[6]</sup> Dalhoff A,<sup>[7]</sup> Knottneurs RS

et al,<sup>[8]</sup> Jancel T and Duadas V,<sup>[9]</sup> Wagenlehner FM et al,<sup>[10]</sup> Ekelund O, Kahlmeter G et al <sup>[11]</sup> have reported nitrofurantoin, cotrimoxazole, fluoroquinolones, fosfomycin trometamol and pivmecillinam as choice of therapy for acute uncomplicated cystitis in adult women.

The present study conducted at a tertiary care centre in Uttar Pradesh, India revealed *Escherichia coli* resistance to cotrimoxazole to be as high as 50.43 % and fosfomycin as well as pivmecillinam are not available at the place of study and hence their resistance pattern could not be determined.

Thomas and Hooton <sup>[12]</sup> reported cefpodoxime to be not inferior to ciprofloxacin in the treatment of acute uncomplicated cystitis in adult women. The present study observation is also similar and noted cefpodoxime to be almost equal to levofloxacin.

## CONCLUSION

The present study concludes that nitrofurantoin, levofloxacin and cefpodoxime could be helpful in making decisions about empirical therapy of community acquired acute uncomplicated cystitis in adult women in this region of country.

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**Table 1 : Pattern of etiological agents isolated from patients of acute uncomplicated cystitis**

Bacteria	Patients	
	No.	%
Escherichia coli	117	78.53
Klebsiella pneumonia	7	4.70
Proteus mirabilis	3	2.01
Staphylococcus saprophyticus	19	12.75
Enterococcus fecalis	3	2.01
Total	149	100

No. = Number, % = Percent

**Table 2: Antibiotic Resistance in Escherichia coli in adult women with acute uncomplicated cystitis. (n = 117)**

Antibiotics	Susceptible		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Nitrofurantoin	86	73.51	11	9.40	20	17.09
Cotrimoxazole	54	46.15	4	3.42	59	50.43
Norfloxacin	82	70.08	8	6.84	27	23.08
Ciprofloxacin	93	79.49	6	5.13	18	15.38
Levofloxacin	98	83.76	2	1.71	17	14.53
Coamoxiclax	48	41.03	17	14.53	52	44.44
Cefuroxime	80	68.38	11	9.40	26	22.22
Cefpodoxime	96	82.05	3	2.57	18	15.38

**Table 3: Antibiotic Resistance in Klebsiella pneumoniae in adult women with acute uncomplicated cystitis (n = 7)**

Antibiotics	Susceptible		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Nitrofurantoin	4	57.14	2	28.57	1	14.29
Cotrimoxazole	3	42.86	0	0.00	4	57.14
Norfloxacin	3	42.86	0	0.00	4	57.14
Ciprofloxacin	4	57.14	2	28.57	1	14.29
Levofloxacin	6	85.71	0	0.00	1	14.29
Coamoxiclax	2	28.57	1	14.29	4	57.14
Cefuroxime	4	57.14	1	14.29	2	28.57
Cefpodoxime	5	71.42	1	14.29	1	14.29S

**Table 4 : Antibiotic Resistance in Proteus mirabilis in adult women with acute uncomplicated cystitis. (n = 3)**

Antibiotics	Susceptible		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Nitrofurantoin	1	33.33	0	0.00	2	66.67
Cotrimoxazole	2	66.67	0	0.00	1	33.33
Norfloxacin	1	33.33	1	33.33	1	33.33
Ciprofloxacin	2	66.67	1	33.33	0	0.00
Levofloxacin	3	100	0	0.00	0	0.00
Coamoxiclax	1	33.33	0	0.00	2	66.67
Cefuroxime	2	66.67	0	0.00	1	33.33
Cefpodoxime	3	100	0	0.00	0	0.00

**Table 5 : Antibiotic Resistance in Staphylococcus saprophyticus in adult women with acute uncomplicated cystitis. (n = 19)**

Antibiotics	Susceptible		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Nitrofurantoin	13	68.42	3	15.79	3	15.79
Cotrimoxazole	14	73.68	2	10.53	3	15.79
Norfloxacin	16	84.21	2	10.53	1	5.26
Ciprofloxacin	17	89.48	1	5.26	1	5.26
Levofloxacin	18	94.74	0	0.00	1	5.26
Coamoxiclav	7	36.84	6	31.58	6	31.58
Cefuroxime	10	52.63	5	26.52	4	21.05
Cefpodoxime	15	78.94	2	10.53	2	10.53

**Table 6 : Antibiotic Resistance in Enterococcus faecalis in adult women with acute uncomplicated cystitis. (n = 3)**

Antibiotics	Susceptible		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Nitrofurantoin	2	66.67	1	33.33	0	0.00
Cotrimoxazole	0	0.00	0	0.00	3	100
Norfloxacin	1	33.33	1	33.33	1	33.33
Ciprofloxacin	2	66.67	1	33.33	0	0.00
Levofloxacin	2	66.67	1	33.33	0	0.00
Coamoxiclav	1	33.33	0	0.00	2	66.67
Cefuroxime	0	0.00	0	0.00	3	100
Cefpodoxime	0	0.00	0	0.00	3	100

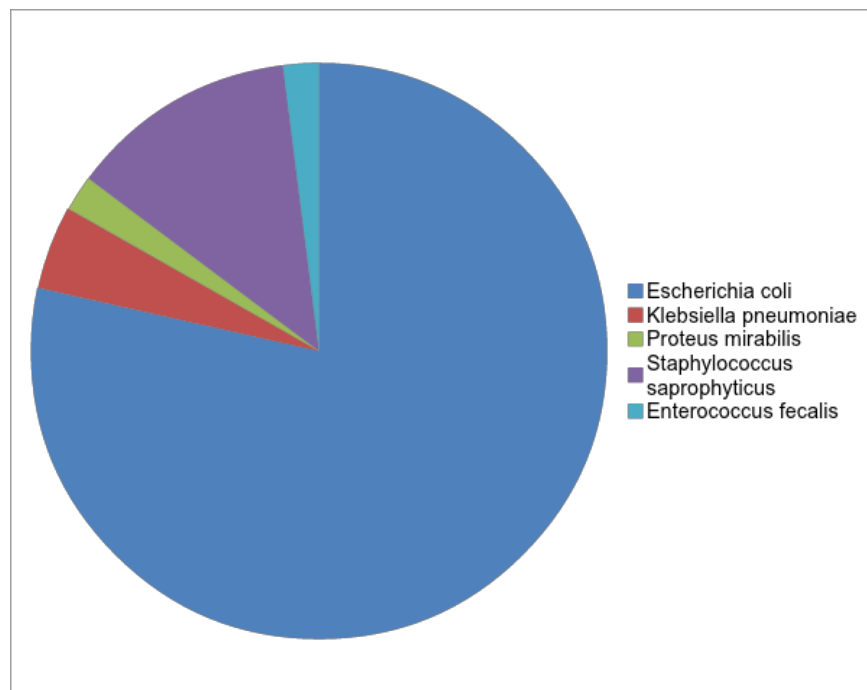


Figure 1: “Pattern of etiological agents isolated from patients of acute uncomplicated cystitis”

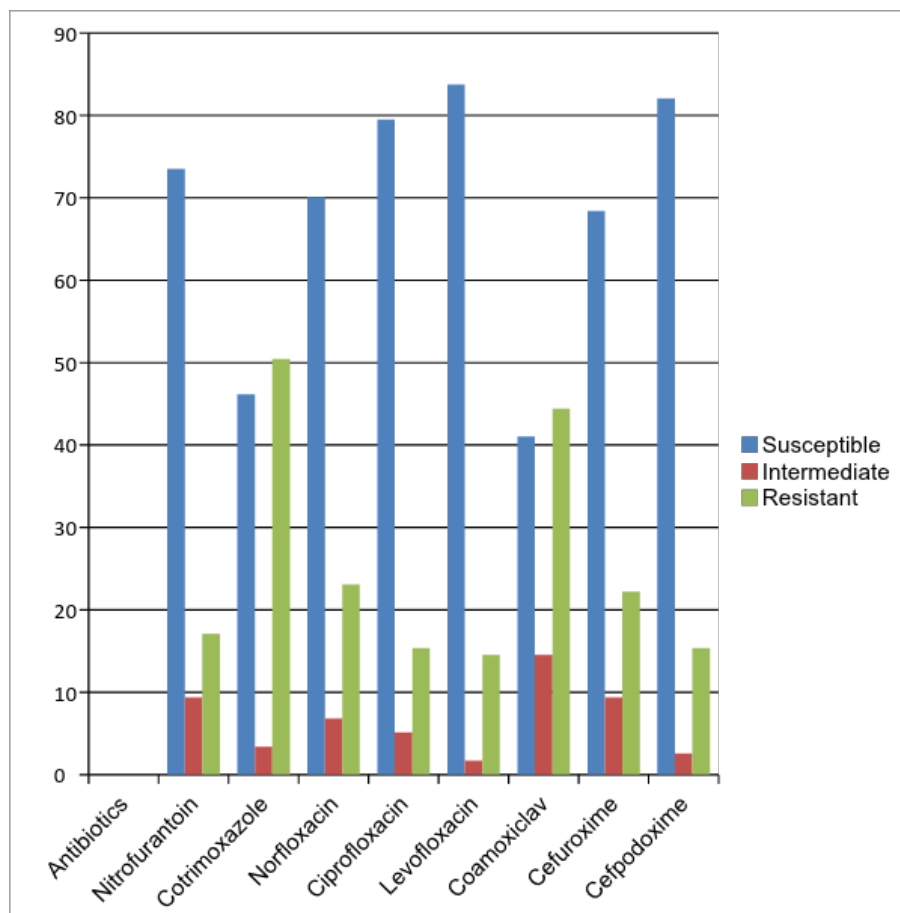


Figure 2: “Antibiotic Resistance in Escherichia coli in adult women with acute uncomplicated cystitis. (n = 117)”