

Escherichia coli* as uropathogen: antibiotic susceptibility profiling*Anuja Jha¹, Manju Agrawal^{1*}, Arvind Neral², Rajesh Hishikar¹, Basant Maheshwari¹**¹Department of Pharmacology,²Department of Microbiology,
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Raipur, Chhattisgarh, India**Received:** 25 November 2017**Accepted:** 28 December 2017***Correspondence to:**Dr. Manju Agrawal,
Email: manjuaagrawal@yahoo.com**Copyright:** © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.**ABSTRACT****Background:** Empirically chosen antibiotics based on the local resistance pattern of uropathogens remain the principle treatment of urinary tract infections (UTI).**Methods:** Antibiogram of most frequent uropathogen was determined. Based on the antibiogram result, authors compared effectiveness of drugs recommended for UTI by National centre for disease control (NCDC), India, and assessed age and gender based variability in the effectiveness of these drugs.**Results:** 1278 urine samples were accounted, of which 405 samples showed significant growth. *E. coli* was the most common uropathogen (n=146, 36%) followed by enterococcus species (31%) and *Klebsiella pneumoniae* (10%). Using McNemar's test authors found that nitrofurantoin (90% sensitivity) was statistically the most effective drug among drugs recommended by NCDC for uncomplicated cystitis. Furthermore, authors used Fisher's exact test on adults and paediatrics and found that significant difference in effectiveness was observed for nitrofurantoin (p-value <0.001) and cotrimoxazole (p-value 0.034). Using logistic regression, authors found that with age, effectiveness of ciprofloxacin and cotrimoxazole deteriorate significantly (p-value 0.021 and 0.002 respectively). Additionally, authors observed that cotrimoxazole has significantly better efficacy in males compared to females (p-value 0.022).**Conclusions:** In accordance with present study, nitrofurantoin can be used as first line treatment for uncomplicated cystitis. Age and gender should be considered while prescribing empirical treatment for UTI. Periodic surveillance should be carried out to identify the on-going pattern of antibiogram to update the guideline for empirical therapy.**Keywords:** Antibiogram, NCDC guidelines, UTI**INTRODUCTION**

Urinary tract infections (UTIs) are a major public health problem globally affecting around 150 million patients yearly.^{1,2} These are the second most common type of infection in the body, accounting for about 8.1 million visits to health care providers each year.³

UTIs are caused by various types of pathogens, known as uropathogens, with special virulence factor which facilitate their invasion into urinary tract.⁴ *Escherichia coli* are the most common uropathogen isolated worldwide.⁵ UTI can be diagnosed by dipstick method, urine analysis, and urine culture. Urine culture is the gold standard of the treatment.⁶

Oral therapy with empirically chosen antibiotic is the mainstay of the treatment. Empirical therapy is based on a clinical diagnosis combined with evidence from the literature and from the educated experience of the probable pathogens causing the infection. It is prescribed while waiting for the report of the culture. These antibiotics are chosen based on the local resistance pattern of the organism. Indiscriminate antimicrobial use leads to antimicrobial resistance and this is becoming a global public health concern. Antimicrobial resistance makes the treatment difficult, prolonged and costlier.⁷

As per National treatment guidelines for antimicrobial use in infectious diseases by NCDC, Ministry of health and

welfare, Government of India, treatment of UTI is as follows:⁷

NCDC recommend nitrofurantoin, cotrimoxazole, ciprofloxacin, and cephalosporins for uncomplicated cystitis and for pyelonephritis it is gentamicin, piperacillin-tazobactam, cephalosporins, and imipenem.

Several recent studies in India have shown nitrofurantoin as one of the most effective drugs for UTI.^{8,9} No significant resistance has been reported with nitrofurantoin.¹⁰ Nitrofurantoin is a urinary antiseptic with no other systemic use. It can be used as the most preferred presumptive drug for uncomplicated cystitis. The other drugs for uncomplicated cystitis by NCDC may be prescribed in place of nitrofurantoin, if they have higher effectiveness. To compare the effectiveness of cotrimoxazole, ciprofloxacin, cephalosporin vis-à-vis nitrofurantoin we formulate the following hypotheses.

Hypothesis 1

Effectiveness of nitrofurantoin is equal to effectiveness of cotrimoxazole, ciprofloxacin and cephalosporins. Multidrug resistant *E. coli* are becoming more prevalent and common cause for pyelonephritis. Pyelonephritis can lead to several long-term complications. Hence it is essential to treat pyelonephritis accurately. For this it is desirable to know which of the four drugs (gentamicin, piperacillin-tazobactam, cephalosporins and imipenem) recommended by NCDC for pyelonephritis work better than others. To compare the effectiveness of these four drugs among each other, authors formulate the following hypotheses:

Hypothesis 2

Gentamicin, piperacillin-tazobactam, cephalosporin, and imipenem which are recommended for pyelonephritis by NCDC are equally efficacious (using pair-wise comparisons). It has been reported in the literature that multidrug resistant *E. coli* are becoming more prevalent in the pathogenesis of UTI in the paediatric age group (≤ 18 years) also. They are prone for recurrent UTI too. More selective targeting will decrease the incidence of recurrence. Authors compare the effectiveness of all the seven mentioned drugs on paediatric age group (≤ 18 years) vis-à-vis adults (≥ 19 years). Hence, authors formulate hypothesis 3.

Hypothesis 3

Effectiveness of all the seven UTI drugs on paediatric age group is same as their effectiveness on adults. Further, pathogenesis and treatment of UTI varies with the gender and with age. As for e.g. prostatitis, treatment of which requires a drug that can reach the prostatic secretions in sufficient concentration. However, in the NCDC guidelines, age has not been included as a factor while suggesting a presumptive antibiotic for the treatment of

UTI in adults. Hence, authors formulate our next hypothesis.

Hypothesis 4

Gender and age do not influence the effectiveness of any of the seven drugs that are recommended for uncomplicated cystitis and pyelonephritis. In this study, authors evaluated the uropathogens and their antibiogram in our hospital Dr. BRAM hospital, Raipur, Chhattisgarh, India. An antibiogram is an overall profile of antimicrobial susceptibility testing results of a specific microorganism to a battery of antimicrobial drugs.¹¹ Author report experimental design and results in the next sections.

METHODS

Study area

The present study was conducted at Department of Pharmacology and Microbiology, Pt. JNM Medical College and Dr. BRAM Hospital, Raipur, Chhattisgarh. It is a tertiary care hospital with 1129 beds. The study was conducted after approval from the institutional ethics committee.

Objective of this study was to find out the antibiogram of the most frequent uropathogen isolated. Authors also compared effectiveness of drugs included in the guidelines of National centre for disease control (NCDC) and assessed gender and age based variability in the effectiveness of antimicrobials.

Study design

It was a retrospective study. All the urine samples which were subjected for culture test during October 2016 to December 2016 were included in present study. Mid-stream freshly voided urine samples collected in a sterile container were received in the microbiology department. One sample per patient was included. These samples were inoculated in blood agar and MacConkey agar media plates. Then they were incubated at 37° C for 16-18 hours. Samples showing growth with colony count more than 10⁵ CFU/ml were subjected to antibiogram.

Antibiogram

Antibiotic susceptibility profiling was carried out by Kirby-Bauer method using Mueller Hinton agar. Antibiotic discs against which uropathogens were tested were from HIMEDIA. It included nitrofurantoin (200mcg), cotrimoxazole (25mcg), norfloxacin (10mcg), amikacin (10mcg), ampicillin (25mcg), piperacillin/tazobactam (100/10mcg), vancomycin (30mcg), imipenem (10mcg), cefixime (5mcg), colistin (10mcg), polymyxin B (50 U), ceftazidime (30mcg), ceftazidime (30mcg), cefotaxime (30mcg) and tigecycline (15mcg). Discs were applied using aseptic technique and

were placed 24mm apart. These preparations were incubated immediately at 37°C and examined after 16 hours. Zones showing complete inhibition were measured. Zone size used for the interpretation was in accordance to performance standards for antimicrobial disk susceptibility tests, CLSI (The Clinical and Laboratory Standards Institute) and EUCAST (The European Committee on Antimicrobial Susceptibility Testing).

Table 1: Organism distribution.

Organism	Sample proportion (%)
<i>Escherichia coli</i>	36.04
<i>Enterococcus</i>	30.86
<i>Klebsiella pneumonia</i>	09.87
<i>Pseudomonas aeruginosa</i>	06.91
CONS	05.18
<i>Candida albicans</i>	03.70
<i>Acinetobacter baumannii</i>	03.20
<i>Staphylococcus aureus</i>	01.71
<i>Citrobacter</i>	01.48
<i>Proteus</i>	00.74
Non-fermenter	00.31

CONS- Coagulase Negative *Staphylococcus aureus*

All precautions required for accuracy of the test were followed. Control tests using known culture was included with the sensitivity test. Controls used were *E. coli* ATCC 25922. Ampicillin disc was used for susceptibility to amoxicillin as well.

Statistical design

The outcome of antibiogram for each sample-drug combination is dichotomous (success/failure). Hence, for our 1st and 2nd hypotheses, authors use McNemar's test for comparing drugs with matched pairs of subjects. This test is analogous to paired-t test except for the fact that variables that are compared are categorical.¹² It uses chi square (or binomial distribution for lower sample sizes) to determine if the 2x2 contingency table has marginal homogeneity.

Further, authors test hypothesis 3 using Fisher's exact test. This test is used when the groups that are compared (here paediatrics vs. adults for each of the drugs) are independent and have categorical data (success/failure).

To test hypothesis 4, authors use logistic regression analysis in which the outcome is categorical.¹³ The dependent variable in the model is log odds of success and the predictor variables are the categorical variables for drugs and interaction variables drug-gender and drug-age. Author use IBM SPSS version21 for the statistical analysis. Author report our findings in the results section.

RESULTS

1278 urine samples were accounted of which 405 (31.69%) samples showed significant growth. 193 (48%) samples were from males and 212 (52%) were from females.

Table 2: Antibiotic susceptibility profiling of *E. coli* by age and gender.

Age	Gender	Count ¹	Drug sensitivity							
			Nitrofurantoin	Cotrimo-xazole	Ciproflo-xacin	Cephalo-sporin	Piperacillin-tazobactam	Gentamicin	Ampicillin	Imipenem
0-18	Female	10	70.0%	0.0%	50.0%	20.0%	66.7%	50.0%	0.0%	100.0%
	Male	28	74.1%	23.1%	14.3%	11.5%	83.3%	72.2%	0.0%	100.0%
	Overall	38	73.0%	16.7%	25.0%	13.9%	78.8%	66.7%	0.0%	100.0%
19-30	Female	27	95.8%	61.1%	62.5%	44.0%	80.8%	86.4%	25.0%	100.0%
	Male	10	100.0%	85.7%	40.0%	30.0%	88.9%	80.0%	66.7%	100.0%
	Overall	37	96.9%	68.0%	57.1%	40.0%	82.9%	85.2%	36.4%	100.0%
31-50	Female	22	85.7%	16.7%	30.8%	10.5%	75.0%	85.0%	0.0%	87.5%
	Male	11	100.0%	75.0%	50.0%	27.3%	62.5%	70.0%	33.3%	60.0%
	Overall	33	90.6%	31.3%	35.3%	16.7%	71.4%	80.0%	14.3%	76.9%
51-76	Female	16	100.0%	14.3%	22.2%	26.7%	80.0%	100.0%	50.0%	100.0%
	Male	22	100.0%	25.0%	18.2%	22.7%	80.0%	73.7%	0.0%	85.7%
	Overall	38	100.0%	21.1%	20.0%	24.3%	80.0%	83.9%	25.0%	91.7%
Overall		146	89.9%	37.2%	34.6%	23.9%	78.6%	79.5%	18.0%	91.7%

Count reflects the number of urine samples subjected to a drug and it is not necessary that a sample was subjected to all the drugs.

Author report the results of uropathogens isolated *E. coli* were the most common uropathogens (n=146, 36%) followed by *Enterococcus species* (n=125, 31%) and *Klebsiella pneumoniae* (n= 40, 10%). From their preliminary analysis, authors found that cotrimoxazole has better efficacy in case of male. Efficacy of cotrimoxazole and ciprofloxacin decreases with age in the adult group (Table 2). Four samples were subjected to test against

colistin, tigecycline, and Polymyxin B and showed 100% sensitivity.

To test hypothesis 1 and 2, McNemar's test is used. The low p-value with McNemar's test, for comparison of nitrofurantoin with ciprofloxacin (p-value <0.001), cotrimoxazole (p-value <0.001) and cephalosporin (p-value <0.001) indicate that these three drugs have

statistically significant lower effectiveness compared to nitrofurantoin. Author also compare gentamicin, piperacillin-tazobactam, imipenem and cephalosporin using McNemar's test. Based on significant difference of these tests, the drugs in decreasing orders of effectiveness are: imipenem^a, gentamicin^{a,b}, piperacillin-tazobactam^b, cephalosporins^c. The superscripts represent the classification of the drugs based on effectiveness (Table 1). To test hypothesis 3, Author compare effectiveness of drugs on paediatrics and adults with Fisher's exact test. It summarizes the effectiveness of nitrofurantoin, ciprofloxacin, cotrimoxazole cephalosporins, imipenem, gentamicin, and piperacillin-tazobactam on these groups. On these two groups (paediatrics and adults) statistically significant difference in effectiveness was observed for nitrofurantoin (p-value <0.001) and cotrimoxazole (p-value 0.034). The same test with cephalosporin gives a p-value of 0.075. Consequently, given our alpha of 0.05, author conclude that nitrofurantoin and cotrimoxazole are less effective on paediatrics compared to adults. Results can be referred from table 2 in appendix.

Logistic regression analysis is used to test hypothesis 4. Among the categorical variables, piperacillin-tazobactam (in drugs) and female (in gender) are chosen as reference categories. Since, authors already used McNemar's test to do pair-wise comparison between drugs, with logistic regression analysis authors focus on analysing the impact of age and gender for each of the drug. Note that instead of using separate logistic regression for each drug, author has one logistic regression with each term having interaction effect with drug. The outcome does not change with this method.

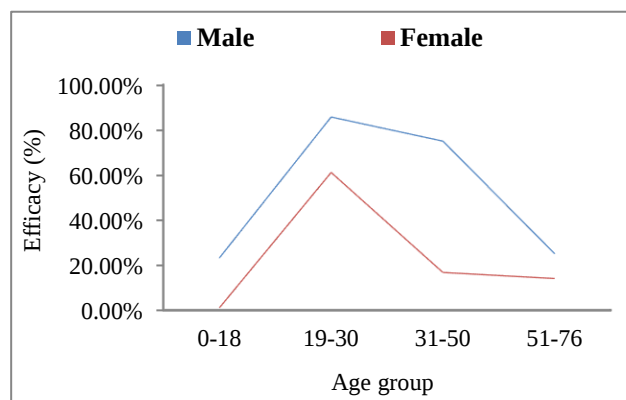


Figure 1: Pattern of efficacy of cotrimoxazole with age and gender.

Author observed that cotrimoxazole has significantly better efficacy in males compared to females (p-value 0.022). Hence, null hypothesis of exponential (B)=1 (meaning equal effectiveness w.r.t. female), is rejected for cotrimoxazole. Further, controlling for age, the odds of success with cotrimoxazole is 5.6 times for males compared to females (Figure 1). Author also observe that with age, effectiveness of ciprofloxacin and cotrimoxazole deteriorates significantly (p-value 0.021 and 0.002

respectively). This reduction in efficiency is extremely significant for cotrimoxazole. For every increase in age by 1 year, odds of success decrease by a factor of 0.958 and 0.935 (i.e. 4.2% and 6.5% decrease) for ciprofloxacin and cotrimoxazole respectively.

DISCUSSION

Etiology and sensitivity pattern of uropathogens keep changing over the years.⁹ Therefore, there is a need for periodic surveillance program that will help in the formulation of regional guideline based on the local antibiogram pattern of uropathogens.

In present study 31.69% samples showed significant growth. *E. coli* were the most common uropathogens isolated and accounts for 36.04% and Enterococci were isolated in 30.86% samples. Various studies show a different pattern where *E. coli* is present in 64.33%, 61.84%, 42.3% and 17.7% samples respectively.^{9,14-16} This difference may be due to the fact that our samples included both community acquired and nosocomial infections.

E. coli is the most common uropathogen as its peritrichate flagella facilitate their entry into the urinary tract.¹⁷ Furthermore, the proximity of urethra to the rectal opening favours migration of *E. coli*, which is a normal inhabitant in the large intestine. According to recent national nosocomial infections surveillance system (NNIS) survey, prevalence of enterococci has been increased in case of UTI, blood stream infection, and wound infection and it is due to the rise in the use of third generation cephalosporins.¹⁸

Present antibiogram shows *E. coli* sensitivity towards nitrofurantoin in 90% cases, while sensitivity to cotrimoxazole is 37.97%, cephalosporins is 25.18%, and, ciprofloxacin is 33.33%. According to the infectious diseases society of America (IDSA) if drug resistance is more than 20% and 10% for cotrimoxazole and fluoroquinolone respectively, they should not be used as empirical treatment alone.¹⁹ In present study resistance against both these drugs are much higher than the cut off value. Sensitivity against cephalosporin is also very low compared to other studies where it is 98% and 50% respectively.^{20,21} This difference can be due to indiscriminate use of cephalosporins in our area. present antibiogram results favour the use of nitrofurantoin as the preferred agent for uncomplicated cystitis.

Sensitivity to piperacillin-tazobactam, gentamicin and imipenem were 78.79%, 79.46% and 91.67% respectively. Specimens which were subjected to colistin, tigecycline, and polymyxin B (after the failure of first line drugs) showed 100% sensitivity towards them. Sentry antimicrobial surveillance program, 1997 in Europe shows that susceptibility of *E. coli* to piperacillin-tazobactam, amikacin and imipenem were 98%, 99.8% and 100% respectively which is different from present study.²⁰ In another study in Bangladesh by Akhtar et al also showed

that *E. coli* were sensitive to gentamicin (52.2%), amikacin (87.3%) and imipenem (90.35%).²¹ Imipenem demonstrate superior sensitivity compared to other agents in studies compared while gentamicin and piperacillin-tazobactam shows variable result. These differences may be due to variability in the rate and choice of antimicrobials over the period of time and place. Hence a single guideline cannot be applied globally.

Further, in comparison of antibiotics use in paediatrics and adults, nitrofurantoin and cotrimoxazole were having significantly inferior efficacy in paediatric age group. Cephalosporin is also less effective in paediatric population compared to adults, but p-value is 0.075, which is statistically not significant (due to small sample size). Cotrimoxazole, amoxicillin-clavulanic acid and cephalosporins are drugs recommended by NCDC for paediatric UTI but nitrofurantoin is not recommended.⁷ In present study good response is seen with imipenem (100%), piperacillin-tazobactam (79%) and gentamicin (66%). Better response to these drugs in paediatric patients may be due to the fact that they usually present with complicated UTIs, and multidrug resistance *E. coli* are more common among them.^{22,23} And this is a reason for recurrence and poor response to cotrimoxazole and cephalosporins too.

In comparison of various antibiotics among the different age groups, efficacy of cotrimoxazole and ciprofloxacin decreases at extremes of age in both genders (Figure 1). This may be due to increased prevalence of complicated UTI among them.²² In postmenopausal women, major risk factors for complicated UTI include anatomical factors affecting bladder emptying, such as cystoceles, urinary incontinence, and residual urine. In men most commonly, urinary obstruction secondary to prostatic hypertrophy is responsible for complicated UTI. Among male neonates and infants, majority of UTIs are due to the congenital urinary tract anomalies.²⁴ It is important to treat these populations with appropriate drug as they are more prone for UTI complications like chronic pyelonephritis, renal insufficiency and hypertension. Optimizing treatment as per our findings will help in the prevention of treatment failure, complications, and drug resistance.

Cotrimoxazole is significantly more efficacious in case of males as it is found in therapeutic concentrations in prostatic secretions.²⁵ Present finding supports the recommendation for use of cotrimoxazole in case of bacterial prostatitis by NCDC.⁷

For treating UTI, an ideal agent should be efficacious, used for short duration and must be economic. From present study, authors conclude that nitrofurantoin (90% sensitivity) should be preferred as the first line treatment of uncomplicated cystitis as it fulfils the ideal agent criteria. Tigecyclin, colistin, and polymyxin B also show good sensitivity and should be reserved for resistant cases only. Extremes of ages should be treated with more caution as the multidrug resistant UTI are common among them

and show decreased sensitivity to common antimicrobials like cotrimoxazole and ciprofloxacin. Appropriate usage of drugs on patients by physicians increases its effectiveness, prevents antimicrobial resistance which is a global concern and decreases pharmacoeconomic burden too.

Present study will help in the development of a database for our country. As present study duration was for three months only, there should be a long-term study to identify the on-going pattern of antibiogram to update the guideline for empirical therapy.

ACKNOWLEDGEMENTS

Authors would like to thank Department of Microbiology, Pt. JNM Medical College, Raipur, Chhattisgarh, India. Authors convey our sincere thanks to Mr. Amit Vatsa who helped us in the statistical analysis in this study.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Jha A, Agrawal M, Neral A, Hishikar R, Mahesh B. *Escherichia coli* as uropathogen: antibiotic susceptibility profiling. *Int J Basic Clin Pharmacol* 2018;7:433-8.