Original article

Increasing antibiotic resistance in uropathogenic *Escherichia coli*: Is Fosfomycin a promising alternative?

Pooja Singla[1,*], Jyoti Sangwan[1], Prashant Singh[1], Pratibha Mane[1], Kumkum Yadav[1], N K Singh[2]

[1] Department of Microbiology, SHKMGMC Nalhar, Haryana, India

[2] Department of Community medicine, SHKMGMC Nalhar, Haryana, India

ARTICLE INFO:
Received: 06 Oct 2023
Accepted: 24 Oct 2023
Published: 31 Oct 2023

Corresponding author *
Dr Pooja Singla
Associate Professor,
Department of Microbiology,
SHKMGMC Nalhar, India.
E mail: pjsingla3@gmail.com

ABSTRACT:

Background: Antimicrobial resistance in uropathogenic Escherichia coli is encountered increasingly now a days. Fosfomycin has emerged as a promising solution for clinicians to treat UTI in current era of antibiotic resistance.

Objectives: This study was conducted to know the antibiotic susceptibility profile of uropathogenic Escherichia coli and to know the resistance rate of fosfomycin in UPEC at our hospital. Material and methods: A total of 167 consecutive, nonduplicate, uropathogenic Escherichia coli isolates were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method according to CLSI guidelines. Following antibiotics were used: ceftazidime (30µg), amoxycillin/clavulanic acid (20µg /10 µg), imipenem (10µg), gentamicin (10µg), ciprofloxacin (5µg), cotrimoxazole (25µg), aztreonam (30µg), nitrofurantoin (300µg) and fosfomycin (200µg). An isolate was considered as multidrug resistant (MDR) if it was resistant to at least three classes of antimicrobial agents.

Statistical Analysis: The statistical analysis was performed using standard tests. The data was represented as percentages and proportions. Chi-square test was applied when two or more set of variables were compared. If the p-value was <.05, it was considered significant.

Results: A total of 33% E. coli isolates were MDR. Maximum susceptibility was observed for fosfomycin (97%) followed by nitrofurantoin (95.8%), imipenem (95.2%), aztreonam (64%), gentamicin (57.4%), cotrimoxazole (39.5%), ciprofloxacin (38.3%), ceftazidime (32.9%) and lowest was for amoxicillin clavulanic acid (25.1%). A total of 5 isolates of E. coli were resistant to fosfomycin.

Conclusion: In view of low level fosfomycin resistance in UPEC, this drug may be considered as a better alternative drug for treatment of UTI.

Keywords: Uropathogenic, *Escherichia coli*, Urinary tract infections, Fosfomycin, Multidrug resistance, antibiotics.

1. INTRODUCTION

Urinary tract infections (UTIs) are one of the frequently occurring infections in community as well as in hospital settings. Worldwide approximately 150 million cases of UTI occur each year imposing a great financial burden on healthcare system. Uropathogenic *Escherichia coli* (UPEC) is the most significant causative agent of UTI accounting for 80-85% of all UTI cases. Inappropriate use of antibiotics for empirical treatment of UTI has led to emergence of multi drug resistant (MDR) strains in UPEC. MDR UPEC has been reported from almost all countries worldwide and had limited the therapeutic options available for UTIs [1].

Fosfomycin is a novel, broad spectrum, bactericidal antibiotic used primarily to treat acute uncomplicated lower urinary tract infections in adults. It is an old antibiotic known since 1969, gaining importance in recent times owing to its activity against MDR uropathogens especially *Enterobacteriaceae*. Fosfomycin acts by disrupting cell wall synthesis by inhibiting enzyme UDP-N-acetylglucosamineenolpyruvyltransferase (Mur A) which catalyse formation of N-acetylmuramic acid responsible for peptidoglycan synthesis. Easy to administer is another advantage of fosfomycin as single dose given orally can maintain high concentration in urine for upto 24 hours. In current times, fosfomycin has become a drug of choice among clinicians for treating lower UTI in pregnancy, in children, in patients with transplant as well as in immunocompetent adults. Emerging resistance to fosfomycin in UPEC, although at a low rate, has also been reported from various countries [2]. Therefore, this study was planned with the objective to evaluate the antibiotic susceptibility profile of uropathogenic *Escherichia coli* and to find out rate of fosfomycin resistance in UPEC at our hospital.

2. MATERIAL AND METHODS

Study design and settings:

This is a prospective, observational, cross sectional study conducted in department of Microbiology over a period of one year (June 2020- May 2021). Approval from Institutional ethical committee was taken prior to commencement of the study (Protocol No.EC/OA-25/2019).

Isolation and identification of UPEC:

A total of 167 consecutive, nonduplicate, uropathogenic *Escherichia coli* isolates were taken for the study. Midstream clean catch urine samples were collected from both indoor and outdoor patients irrespective of age and sex with clinical indications suggestive of UTI. Sample by suprapubic aspiration and from catheter was also collected wherever it was indicated by standard procedures. All samples were received in laboratory and were subjected to direct microscopy and culture. Direct microscopy was done to visualise pus cells, bacteria, crystals and Red Blood Cells. Culture was put on blood agar and MacConkey agar by semiquantitative method and plates were incubated at 370C overnight. A colony count of >105 colony forming units/ml was considered as significant bacteriuria and was further processed for proper identification of uropathogen by standard biochemical tests [3].

Antimicrobial susceptibility testing:

All the freshly isolated 167 UPEC strains were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method on Mueller Hinton agar media. The following antibiotics of HiMedia® brand were used: ceftazidime (30µg), amoxycillin/clavulanic acid (20µg/10µg), imipenem (10µg), gentamicin (10µg), ciprofloxacin (5µg), cotrimoxazole (25µg), aztreonam (30µg), nitrofurantoin (300µg) and fosfomycin (200µg). After overnight incubation at 370C, zone sizes were measured and interpreted as sensitive (S), intermediate sensitive (IS) and resistant (R) as per Clinical Laboratory Standard Institute (CLSI) guidelines.[4] An isolate was considered as multidrug resistant (MDR) if it was resistant to at least three classes of antimicrobial agents among penicillins and cephalosporins group including inhibitor combinations, monobactams, carbapenems, fluroquinolones, aminoglycosides, nitrofurantoin, cotrimoxazole and fosfomycin.

Statistical Analysis

The statistical analysis was performed using standard tests. The data was represented as percentages and proportions. Chi-square test was applied when two or more set of variables were compared. If the p-value was <.05, it was considered significant.

3. RESULTS

A total of 167 uropathogenic *Escherichia coli* isolates were obtained during the study period. Of these 167 isolates, 56.8% (95) were from adult patients and 43.1% (72) were from paediatric patients (p value 0.0159) mean age 33.4. From male patients 30.5% (51) UPEC isolates were cultured and 69.4% (116) were cultured from female patients (p value <0.00001) as shown in table 1. Out of 167 isolates, 36.5% (61) were grown from outdoor patients and 63.4% (106) *E. coli* isolates were grown from admitted patients (IPD) (p value <0.00001). Highest rate of isolation was from gynaecology ward followed by paediatric ward. Department wise distribution of *E. coli* isolates are shown in table 2. A total of 33% (84) *E. coli* isolates were MDR as depicted in figure 1. Maximum susceptibility was observed for fosfomycin (97%) followed by nitrofurantoin (95.8%) and imipenem (95.2%). Antibiotic susceptibility pattern is depicted in figure 2. A total of 5 isolates of *E. coli* were resistant to fosfomycin.

Table 1: Age and sex distribution of uropathogenic *E. coli* isolates

Age group	Male	Female	Total
	n(%)	n(%)	n(%)
Children			
<1 month	1 (1.9)	1(0.8)	2 (1.19)
1M-1year	7 (13.7)	7(6.0)	14 (8.3)
1year-18 years	11(21.5)	45(38.7)	56 (33.5)
Adults			
18years-40years	15(29.4)	55(47.4)	70 (41.9)
>40years	15(29.4)	10(8.6)	25 (14.9)
Total	51(30.5)	116(69.4)	167

Table 2: Department wise distribution of *E. coli* isolates

Department	Number (%)
Outdoor	61 (36.5)
Indoor	106 (63.4)
Surgery	27 (16.1)
Obstetrics& gynaecology	31 (18.5)
Paediatrics	28 (16.7)
Medicine	05 (2.9)
ICUs	15 (8.9)

Table 3: Comparison of antibiotic susceptibility profile of uropathogenic *Escherichia coli*

Antibiotic	Current study	Gopichand et al;2019	Zahrani et al;2019	Patel et al;2019	Neeraj et al;2018	Deepak et al;2017	Banerjee et al:2017	Neamati et al;2015	Sood et al;2012
		Pondicherry [11]	Saudi Arabia [13]	Gujarat, India [9]	Maharashtra [10]	Uttar Pradesh [8]	Kolkata [12]	Iran [14]	Jaipur, India [15]
Nitrofurantoin	4.20%	13.40%	30.50%	27.70%	6.30%	7.30%	21.30%	23.40%	5.77%
Fluoroquinolones	61.70%	0%	25.40%	81.10%	75.70%	88.70%	58.80%	63.30%	74.75%
Aminoglycosides	42.60%	95.40%	20.60%	46.90%	52.20%	28%	15.70%	46.70%	28.02%
Carbapenems	4.80%	20.30%	0%	8.31%	10.70%	-	11.20%	3.30%	-
Monobactams	36%	-	62.50%	51.40%	56.10%	-	-	52.70%	-
Cotrimoxazole	60.50%	-	72.20%	67.90%	62.10%	77.30%	51.90%	67.30%	67.80%
Colistin	-	-	0%	-	4.30%	-	100%	-	-
Beta lactams	67.10%	93.10%	23%	74.10%	-	92.70%	63.50%	51.30%	69.08%
Beta lactams beta lactamase inhibitors	74.90%	-	14.30%	55.90%	-	-	67.60%	28.70%	80.69%

Table 4: Comparison of Fosfomycin susceptibility in UPEC among different studies

Sr no	Study	Sample size and sample type	susceptibility	Method used
1.	Current study 2021	167 uropathogenic *E. coli*	97%	Kirby -Bauer Disc diffusion method
2.	Serretiello E et al;2021 Italy [22]	4121 urinary *E. coli* isolates collected over five years	2015-95.36% 2016-93.9% 2017-95.38% 2018-93.35% 2019-94.32%	Automated Vitek2
3.	Seok et al;2020 South korea [16	283 clinical isolates of *E. coli*	93.3%	Agar dilution method
4.	Bhattar et al; 2020 North India [17]	112 *E. coli* urinary isolates	91.07%	E test
5.	Amladi et al;2019 Vellore, India [18]	81 Carbapenem resistant isolates of *E. coli*	98.7%	E test
6.	Mueller L et al;2019 Switzerland [21]	1225 ESBL producing *E. coli* isolates	98.6%	Rapid NP test and Agar dilution method
7.	Gopichand et al;2019 Pondicherry [11]	217 clinical isolates of *E. coli*	100%	Agar dilution method
8.	Neeraj et al;2018 Maharashtra [10]	384 ESBL *E. coli*	99.6%	Broth microdilution method
9.	Dalai S et al;2018 [19]	289 *E. coli* urinary isolates	96.8%	Automated microbroth dilution test
10.	Banerjee et al:2017 Kolkata [12]	216 *E. coli*	98.1%	Kirby –Bauer Disc diffusion method and E test
11.	Deepak et al;2017 Uttar Pradesh [8]	150 MDR *E. coli*	100%	Kirby -Bauer Disc diffusion method
12.	Li Y et al;2015 China [20]	1109 *E. coli* isolates	92.2%	Agar dilution method

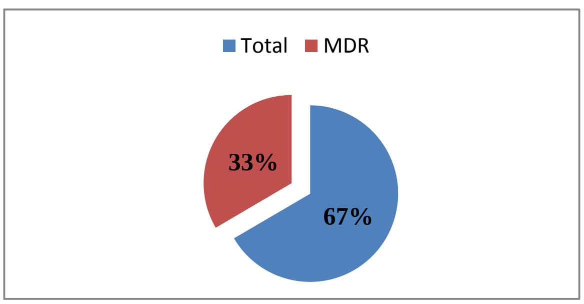

Fig 1: Distribution of MDR *E. coli* isolates

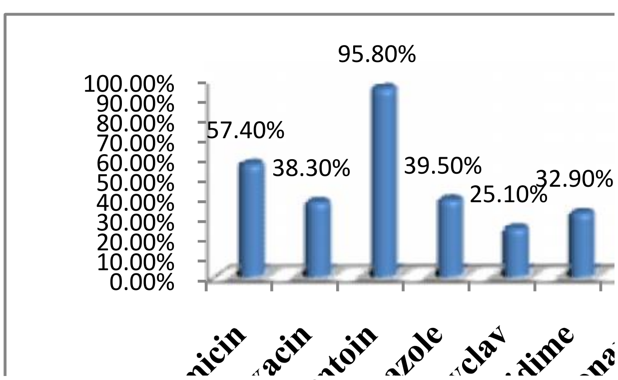

Fig 2: Antibiotic sensitivity pattern of *E. coli*

4. DISCUSSION

Worldwide, the antimicrobial resistance in uropathogens is increasing at an alarming rate. A five year study from Turkey showed that from 2014 to 2018, resistance to ciprofloxacin in UPEC, one of the commonly used drug for UTI, increased from 17% to 43%. Similarly, *E. coli* resistance to ampicillin (61.5%), nitrofurantoin, cefepime, ciprofloxacin, fosfomycin, and amoxicillin-clavulanic acid (34.6%) has also increased significantly with time (all $p = 0.001$) [5]. A SENTRY antimicrobial surviellance programme in United States in 2017 revealed that coresistance in extended spectrum beta lactamase (ESBL) producing UPEC was 93.6% for cefuroxime, 71.8% for fluoroquinolones, 59.2% for cotrimoxazole and 27.1% for amoxicillin clavulanic acid[6]. A surveillance report from South India noticed significant increase in resistance proportion for imipenem (29.8%), meropenem (18.3%), ertapenem (24.9%), ciprofloxacin (26.5%), nitrofurantoin (11.2%), amikacin (8.7%) and cefotaxime (7.4%) in 2017 as compared to 2011[7].

The results of current study showed high antimicrobial resistance in UPEC for almost all groups of antibiotics except carbapenems, nitrofurantoin and fosfomycin. These results are in agreement with previous studies as depicted in table 1. Deepak et al [8] and Patel et al [9] reported resistance against ciprofloxacin in 88.7% and 81.1% of the urinary *E. coli* isolates which is higher than the present study. Prevalence of resistance against cotrimixazole, another commonly used oral antibiotic for UTI, is reported between 51-77% in previous studies. In the present study, high resistance was noticed for amoxicillin clavulanic acid (74.9%) which is higher than the previous studies. In the current study, resistance against nitrofurantoin was noticed to be 4.2% which is lower than the past studies (6.3%-30.5%). Resistance to carbapenems was also lower in our study as compared to previous studies [10-15].

Various factors contribute to burden of antimicrobial resistance in a region. Inappropriate and irrational use of antimicrobials, prescribing of broad spectrum antibiotics without susceptibility checking, over the counter easy availability of drugs, self medication, poor compliance, limited healthcare access, non adherence to hospital antibiotic policy and poor infection control practices are some of the leading causes of high antimicrobial resistance in a region.

In the current study, 98.1% of the UPEC isolates were susceptible to fosfomycin. Most of the previous studies worldwide have reported a low prevalence of fosfomycin resistance, less than 2% in UPEC isolates. Authors from Pondicherry (2019) and Uttar Pradesh (2017) have reported 100% susceptibility to fosfomycin in MDR *E. coli* isolates.[11, 8] Other authors from Maharashtra (2018) and Kolkata (2017) have reported 99.6% and 98.1% fosfomycin susceptibility in UPEC isolates respectively [10,12]. However, on the other hand some authors have estimated higher resistance rate of fosfomycin in UPEC. Study from South Korea and North India in 2020 have estimated fosfomycin resistance rate to be 6.7% and 9.0% respectively [16, 17]. Other studies from various parts of the world conducted between 2015-2021 have reported varying prevalence of fosfomycin resistance ranging from 1.3% to 7.8% in urinary isolates of *E. coli* [18-22]. A comparative analysis of fosfomycin resistance among various studies is shown in table 4. Certain other differences are also present among various studies depicted in table 4 in terms of number of UPEC isolates taken for the study, types of UPEC isolates used like ESBL producing isolates or carbapenem resistant isolates and method used for detecting resistance.

5. CONCLUSION

This study shows high antimicrobial resistance against commonly used antibiotics for the empirical treatment of UTI. Fosfomycin seems to be a good promising agent in current scenario of high resistance to existing drugs and nonavailability of newer drugs.

6. REFERENCES

1. Kot B. Antibiotic resistance among uropathogenic Escherichia coli. Pol J Microbiol 2019;68(4):403-415.
2. Falagas ME, Vouloumanou EK, Samonis G, Vardakas KZ. Fosfomycin. Clin Microbiol Rev 2016;29(2):321–347.
3. Collee JG, Mles RB, Watt B. Tests for identification of bacteria. In: Collee JG, Fraser AG, Marmon BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology, 14th ed. New York: Churchill Livingstone; 1996. p. 131-49.
4. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests: approved standard. 9th ed. CLSI document M2-A9, Vol 26(1). Wayne, PA;2006.
5. Caskurlu H, Culpan M, Erol B, Turan T, Vahaboglu H, Caskurlu T: Changes in antimicrobial resistance of urinary tract infections in adult patients over a 5 Year period. Urol Int 2020;104:287-292.
6. Critchley IA, Cotroneo N, Pucci MJ, Mendes R. The burden of antimicrobial resistance among urinary tract isolates of Escherichia coli in the United States in 2017. Plos One 2019;14(12):e0220265.
7. Ravishankar U, P. S, Thayanidhi P. Antimicrobial resistance among uropathogens: surveillance report from South India. Cureus 2021;13(1): e12913.
8. Kumar D, Das A, Purbey MK, Gupta N, Nath G. Susceptibility of uropathogenic multidrug resistant Escherichia coli to fosfomycin. J Acad Clin Microbial 2017;19:101-4.
9. Patel HB, Soni ST, Bhagyalaxmi A, Patel NM. Causative agents of urinary tract infections and their antimicrobial susceptibility patterns at a referral center in Western India: An audit to help clinicians prevent antibiotic misuse. J Family Med Prim Care 2019;8:154-9.
10. Tulara NK. Nitrofurantoin and Fosfomycin for Extended Spectrum Beta-lactamases producing Escherichia coli and Klebsiella pneumoniae. J Global Infect Dis 2018;10:19-21.
11. Gopichand P, Agarwal G, Natarajan M, Mandal J, Deepanjali s, Parameswaran S, et al. In vitro effect of fosfomycin on multi-drug resistant gram-negative bacteria causing urinary tract infections. Infection and Drug Resistance 2019:12:2005–2013.
12. Banerjee S, Sengupta M, Sarker TK. Fosfomycin susceptibility among multidrug-resistant, extended-spectrum beta-lactamase-producing, carbapenem-resistant uropathogens. Indian J Urol 2017;33(2):149-54.
13. Zahrani JA, Dossari KA, Gabr AH, Ahmed AF, Shahrani SAA, Ghamdi SA. Antimicrobial resistance patterns of uropathogens isolated from adult women with acute uncomplicated cystitis. BMC Microbiology 2019;19:237:1-5.
14. Neamati F, Firoozeh F, Saffari M, Zibaei M. Virulence Genes and Antimicrobial Resistance Pattern in Uropathogenic Escherichia coli Isolated From Hospitalized Patients in Kashan, Iran. Jundishapur J Microbiol. 2015;8(2):e17514.
15. Sood S, Gupta R. Antibiotic resistance pattern of community acquired uropathogens at a tertiary care hospital in Jaipur, Rajasthan. Indian J Community Med 2012;37(1):39-44.
16. Seok H, Choi JY, Wi YM, Park DW, Peck KR, Ko KS. Fosfomycin resistance in Escherichia coli isolates from South Korea and in vitro activity of fosfomycin alone and in combination with other antibiotics. Antibiotics 2020;9(112):1-13.
17. Bhattar S, Shingare P, Tigga RA, Qureshi S, Sharma V. Emerging resistance among uropathogens: is fosfomycin revival the best hope? J Clin Diag Res 2020;14(7):DC06-10.
18. Amladi AU, Abirami B, Devi S M, Sudarsanam TD, Kandasamy S, Kekre N, Veeraraghavan B, Sahni RD. Susceptibility profile, resistance mechanisms & efficacy ratios of fosfomycin, nitrofurantoin&colistin for carbapenem-resistant Enterobacteriaceae causing urinary tract infections. Indian J Med Res 2019;149:185-91.
19. Dalai S, Modak M, Lahiri K, Fosfomycin susceptibility among uropathogenic E.coli, and K. Pneumoniae Int J Sci Res 2018 8(4):282-84.
20. Li Y, Zheng B, Li Y, Zhu S, Xue F, Liu J. Antimicrobial susceptibility and molecular mechanisms of fosfomycin resistance in clinical Escherichia coli isolates in Mainland China. Plos One 2015;10(8):e0135269.
21. Mueller L, Cimen C, Poirel L, Descombes MC, Nordmann P. Prevalence of fosfomycin resistance among ESBL-producing Escherichia coli isolates in the community, Switzerland. Eur J Clin Microbiol Infect Dis 2019;38:945-949.

22. Serretiello E, Folliero V, Santella B, Giordano G, Santoro E, Caro FD, et al. Trend of bacterial uropathogens and their susceptibility pattern: study of single academic high volume center in Italy (2015-2019). Int J Microbiol 2021;2021:1-10.

ACKNOWLEDGEMENT: Work is credited to Shaheed Hasan Khan Government Medical College, Nalhar, Nuh

CONFLICT OF INTEREST: The authors declare no conflict of interest, financial or otherwise.

SOURCE OF FUNDING: None.

AVAILABILITY OF DATA AND MATERIALS: The raw data used in this study can be obtained from the corresponding author upon reasonable request.

CONSENT FOR PUBLICATION: Not applicable.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE: Protocol No.EC/OA-25/2019