

FOSFOMYCIN SUSCEPTIBILITY AMONG EXTENDED-SPECTRUM BETA LACTAMASE PRODUCING AND CARBAPENEM RESISTANT UROPATHOGENS.

Shakthi R¹, Venkatesha D²

Received : 27/01/2023
Received in revised form : 14/03/2023
Accepted : 29/03/2023

Keywords:
Fosfomycin, ESBL, Carbapenemase, Uropathogens.

Corresponding Author:
Dr. Shakthi R.
Email: drshakthir@bgsaims.edu.in

DOI: 10.47009/jamp.2023.5.2.251

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (2); 1185-1188



¹Assistant Professor, Department of Microbiology Adichunchanagiri Institute of Medical Sciences, Adichunchanagiri University, B.G. Nagara, Mandya, Karnataka, India

²Professor and Head, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, Adichunchanagiri University, B.G. Nagara, Mandya, Karnataka, India

Abstract

Background: Urinary tract infections (UTI) are one of the most commonly encountered infections in clinical practice. Increasing multidrug-resistant (MDR) pathogens contribute considerably to increasing proportion of urinary tract infections (UTIs) as they limit treatment options. This study is to determine the Fosfomycin susceptibility pattern against MDR uropathogens by disk diffusion and Agar Dilution Method. **Materials and Methods:** Prospective study was conducted for a duration of 6 months in a tertiary care hospital of South India. A total of 1928 urine samples were tested during the study period, the samples with significant bacteriuria were further processed, identification and antibiotic susceptibility pattern was determined as per standard procedure. The Multidrug resistant (MDR) Gram negative bacilli (GNB) isolates were tested for susceptibility to Fosfomycin by disk diffusion. Minimum inhibitory concentration against Fosfomycin was detected using Agar Dilution Method. Statistical Analysis: Data analysis was done using Microsoft Excel and Percentages were used to analyze variables. **Result:** Out of 1928 urine samples, 385 yielded significant growth of GNB. Among these isolates 152(39.48%) were extended-spectrum beta lactamase producers (ESBL), 98 (25.45%) were carbapenem resistant. However, 368(95.58%) of total isolates, 142(93.42%) of ESBL producers and 90 (91.83%) of carbapenem resistant isolates were susceptible to Fosfomycin. **Conclusion:** Fosfomycin is the most active antimicrobial agent against all the uropathogens isolated in this study and in this era of antimicrobial resistance, it can be considered for the treatment of MDR UTI.

INTRODUCTION

Urinary tract infections are one of the most common infections responsible for antibiotic resistance.^[1] Increasing Multi Drug Resistant (MDR) bacteria has limited the treatment options. The most common mechanism of resistance among Enterobacteriaceae is by enzymatic inactivation of the beta lactams by beta-lactamases.^[2]

Organisms producing ESBLs hydrolyze penicillin, cephalosporins and monobactams.^[3] They are plasmid coded and are easily transmissible from one organism to the other.^[4] Carbapenems are often used as drug of choice to treat infections due to ESBL and plasmid mediated Amp C producing organisms, increase in the use of these drug contributes to the emergence and spread of carbapenem resistant organisms.^[5]

Increased incidence of emergence and spread of MDR organisms prompted for the use of non-traditional antibiotics like Fosfomycin. It is a bactericidal drug which acts by inactivating the enzyme phosphoenolpyruvate synthetase, required in assembly of glycan and peptide portion of peptidoglycan, thus disrupting bacterial cell-wall synthesis.^[6] It is best absorbed if given before food intake and is excreted in urine.^[7] It maintains high level concentration (2000 µg/ml) in urine for over 24 hours and can be recommended for single time oral therapy.^[8]

This study was conducted to determine the Fosfomycin susceptibility among extended-spectrum beta lactamase producing and carbapenem resistant uropathogens.

MATERIALS AND METHODS

Source of Data

This prospective study was conducted at department of Microbiology in a tertiary care centre of South India from July to December 2022. The institutional ethics committee clearance was obtained to conduct the study.

Collection of bacterial isolates

A total of 1928 non duplicate, clean catch mid-stream urine samples with suspected Urinary Tract Infection (UTI) were included in the study. Gram negative Urinary pathogens with significant growth were isolated as per standard Laboratory techniques.^[9]

Antimicrobial susceptibility test-

The antibiotic susceptibility testing was done as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.^[10] The test was done on the Mueller Hinton agar with the following discs obtained from HiMedia, Mumbai: Cefepime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Nitrofurantoin (300µg), Fosfomycin (200µg) with 50 µg of glucose-6-phosphate.

Methods for detection of ESBL and Carbapenamase resistance:^[9]

Screening test:

A bacterial suspension of 0.5 McFarland's unit is lawn cultured on Mueller Hinton agar (MHA). Amoxicillin-clavulanic acid disc (20 µg+10 µg) was placed in the centre of the plate and Cefpodoxime (10 µg), Ceftazidime (30µg) disc was placed on either side of Amoxicillin-clavulanic acid disc at a distance of 20 mm and Meropenem (10 µg) disc was placed at a distance of >25mm from other discs. Plates were incubated at 35⁰ C for 16-18 hrs.

Interpretation:

1. Extension of zone of inhibition of Cefpodoxime or Ceftazidime towards amoxyclav disc was taken as ESBL screening positive.
2. Zone of inhibition around Meropenem disc <21mm was taken as Carbapenamase screening positive.

Confirmatory tests:

All Screening test positive isolates were subjected to respective confirmatory tests.

1. Confirmatory test for ESBL: combination disc method:

Lawn culture of bacterial suspension was done on MHA plate. Ceftazidime (30µg), Ceftazidime +clavulanic acid (30µg+10µg) were placed >30mm apart. Plates were incubated at 37⁰ C for 16-18 hrs. Interpretation: ≥ 5mm increase in the zone of inhibition of Ceftazidime+clavulanic acid disc as compared to Ceftazidime alone was taken as ESBL positive. [Figure 1]

2. Confirmatory tests for Carbapenamase: Modified and remodified Hodge test:

Lawn culture of *Escherichia coli* ATCC 25922 was done on MHA. Imipenem (10 µg) disc and

Imipenem (10 µg) +zinc (140 µg) disc were placed on the inoculated plate. Test strains were streaked at right angles to each other from. Imipenem and Imipenem +zinc disc, without touching the edge of the disc. The plates were incubated at 37⁰ C for 16-18 hrs.

Interpretation: Enhancement of growth of indicator stains around Imipenem+ Zinc disc as compared to only Imipenem disc was considered as positive.

Determination of Fosfomycin MIC by agar dilution method.^[9]

Agar dilution was performed with Mueller-Hinton agar medium supplemented with 25 µg/ml of glucose-6-phosphate (Hi-media) to reduce the rates of false resistance. Fosfomycin trometamol was used as fosirol powder (Cipla Ltd.). Muller- Hinton agar with different concentrations of Fosfomycin (2,4,8,16,32,64,128 and 256µg/ml) was used. After adjusting the turbidity with 0.5 McFarland standards, 10µl of bacterial culture of test organism was spot inoculated on Mueller- Hinton agar plate with different concentrations of Fosfomycin. Plates were incubated overnight at 37°C and examined for growth. *E. coli* ATCC 25922 was used as control strains. The MIC values for testing and reporting of urinary tract isolates. [as per CLSI guidelines 2021]^[10]. [Figure 2]

Zone diameter (mm)	Interpretation
≥64	Susceptible (S)
128	Intermediate (I)
≤256	Resistant(R)

RESULTS

Out of 1928 urine samples included in this study, 385 yielded significant growth of GNB.



Figure 1: ESBL confirmatory combination disc test



Figure 2: Agar dilution method for detection of Fosfomycin MIC

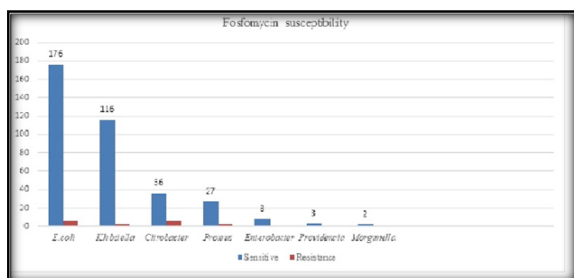


Figure 3: Fosfomycin susceptibility among uropathogens

Of these 385 patients, majority were female (289, 75.05%) followed by male (82, 21.29%) and children (14, 3.63%). *E. coli* (182, 47.27%) was the major pathogen isolated followed by *Klebsiella* (118, 30.64%), *Citrobacter* (42, 10.9%) and *Proteus* (29, 7.5%).

Of 182 *E. coli* isolates, high rate of resistance was observed for Cephalosporins, Fluoroquinolones and Co-trimoxazole. Similarly, high rate of resistance to Cephalosporins, Fluoroquinolones was noted in *Klebsiella* isolated in this study. [Table 1]

Among 385 isolates, 152 (39.48%) were extended-spectrum beta lactamase producers (ESBL), 98 (25.45%) were carbapenem resistant. Out of 152, ESBL producers, 115 (75.6%) were *E. coli*, 32 (31.05%) were *Klebsiella* and out of 98 carbapenem resistant isolates, 56 (57.1%) were *E. coli*, 41 (41.8%) were *Klebsiella* and 1 (1.02%) *Proteus*.

Among the total isolates tested, 368 (95.58%) were susceptible to Fosfomycin (figure 3), and 142 (93.42%) of ESBL producers and 90 (91.83%) of carbapenem resistant isolates were susceptible to Fosfomycin. [Table 2 & 3]

Table 1: Organism wise antibiotic susceptibility among Gram negative uropathogens

Organisms	<i>E. coli</i> (n=182) (%)		<i>Klebsiella</i> (n=118) (%)		<i>Citrobacter</i> (n=42) (%)		<i>Proteus</i> (n=29) (%)		<i>Enterobacter</i> (n=09) (%)		<i>Providencia</i> (n=03) (%)		<i>Morganella</i> (n=02) (%)	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
CPM	31.5	68.4	35.2	64.7	45.3	54.7	41.5	58.5	35.6	64.4	33.3	66.6	00	100
CAZ	22.6	77.4	33.3	66.6	42.8	57.2	33.33	66.7	33.3	66.7	33.3	66.6	00	100
CTR	26.3	73.6	32.5	67.5	36.8	63.2	45.8	54.2	44.4	55.6	33.3	66.6	00	100
CTX	21	78.9	33.3	66.7	47.3	52.7	48.7	51.3	44.4	55.6	33.3	66.6	00	100
CIP	21	78.9	33.3	66.7	23.5	76.5	24.7	75.3	33.3	66.7	00	100	50	50
NIT	78.9	21.1	75.3	24.7	71.5	28.5	66.2	33.8	56.2	43.8	100	00	100	00
COT	57.8	42.1	66.7	33.3	43.1	56.9	34.3	65.6	61.3	38.7	100	00	50	50

(S-Sensitive, R-Resistant, CPM-Cefepime, CAZ-Ceftazidime, CTR-Ceftriaxone CTX-Cefotaxime, CIP-Ciprofloxacin, NIT-Nitrofurantoin, COT-Co-trimoxazole)

Table 2: Fosfomycin sensitivity among ESBL producers

Organisms	ESBL producers(%)
<i>E. coli</i>	109/115 (94.78)
<i>Klebsiella</i>	30/32 (93.75)
<i>Proteus</i>	03/05 (60)
Total	142/152 (93.42)

Table 3: Fosfomycin sensitivity among Carbapenem resistant isolates

Organisms	ESBL producers(%)
<i>E. coli</i>	50/56 (89.28)
<i>Klebsiella</i>	39/ 41 (95.12)
<i>Proteus</i>	01 /01 (100)
Total	90/98 (91.83)

Table 4: Comparison of Fosfomycin susceptibility among uropathogens in various studies from India

Author(y)	Fosfomycin susceptibility (%)
Present study	95.58
Banerjee et al 2017, ^[14]	95.18
Mittal et al 2015, ^[15]	100
Sabharwal ER et al 2015, ^[16]	94.4
Rajenderan et al 2014, ^[17]	90
Sahni et al 2013, ^[18]	83

DISCUSSION

UTIs are the most prevailing ailment affecting all age groups and both genders. The most common uropathogens are showing multiple drug resistance (MDR) mechanisms against the commonly used oral antimicrobial agents. UTIs are emerging as treatment challenges for the clinicians. Hence, there is an urgent need to re-evaluate old antibiotics which were not much in clinical use.

In the present study females (75.05%) were predominant over males (21.29%) among UTI patients, which is similar to the study of Shraddha *et al.*^[11]

Of 385 isolates, majority were *E. coli* (47.27%) followed by *Klebsiella* (30.64%), which is in accordance with other studies.^[12,13]

In this study 39.48% were ESBL producers and 25.45% were Carbapenemase resistant isolates, majority of these isolates showed susceptibility to Fosfomycin 95.58% and 91.83% respectively. These findings are similar to other studies [Table 4].^[14-18]

Fosfomycin was found to have a powerful activity against *E. coli*, *Klebsiella* and *proteus*.^[19] Marie *et al* has reported that Fosfomycin and Colistin were the two most effective antimicrobial agent against Multidrug resistant uropathogens (MDR).^[20]

In spite of this high rate of susceptibility, Fosfomycin is an undervalued agent for complicated Urinary tract infections (UTI) with MDR pathogens.

CONCLUSION

Fosfomycin is the most active antimicrobial agent against all the uropathogens isolated in this study and in this era of antimicrobial resistance, it can be considered for the treatment of MDR UTI. It maintains high level concentration in urine and can be recommended for single time oral therapy of UTIs for outpatient and inpatients.

Acknowledgments

We are thankful to all the technical staff for their support.

REFERENCES

1. Faraz MAA, Mendum S, Swamy MV and Patil S. Prevalence of urinary tract infections and related antimicrobial resistance in India: a systemic review and meta-analysis. *Int J Pharm Sci& Res* 2021;12(8): 4314-21.doi:10.13040/IJPSR.0975-8232.12(8).4314-4321
2. Laghawe A, Jaitly N, Thombare V. The simultaneous detection of ESBL and Amp C β -lactamases in gram negative bacilli. *J Clin Diagn Res*.2012;6(4):660-663
3. Thomas KS. Controversies about extended spectrum betalactamases. *Emerg Infect Dis*.2001;7(2):333-336
4. Rupp ME, Fey PD. Extended spectrum β lactamase (ESBL)-producing Enterobacteriaceae-Consideration for diagnosis, prevention and drug treatment.2003;63(4):353-365
5. Hara GL, Gould I, Endimiani A, et al. Detection, treatment and prevention of Carbapenemase producing Enterobacteriaceae: Recommendation from an international working group. *J Chemother*.2013 Jun;25(3):129-140
6. Sabharwal ER, Sharma R. Fosfomycin: An alternative therapy for the treatment of UTI amidst escalating antimicrobial resistance. *J Clin Diagn Res* 2015 Dec;9(12):6-9
7. Falagas ME, Vouloumanou EK, Samonis G, et al. Fosfomycin. *Clin Microbiol Rev* 2016;29:321-347
8. Garg R, Gupta V, Chander J, et al. Report of carbapenem resistant *P. aeruginosa* isolates carrying ESBLs, AmpC, MBL enzymes based on phenotypic methodology and susceptibility to Fosfomycin. *Indian J Med Microbiol* 2015;33:160-161
9. Collee JG, Marr W. Culture of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, eds. Mackie and McCartney Practical Medical Microbiology, 14th ed. New York: Churchill Livingstone; 2006:113-129
10. CLSI. Performance Standards for Antimicrobial susceptibility testing. 31st ed. CLSI supplement M100. Clinical and Laboratory Standard Institute; USA 2021.
11. Shraddha S, Pankaj KV, Vinita R, et al. Fosfomycin Sensitivity Pattern among Uropathogens Isolated from Patients Visiting Day Care Facility of Sushila Tiwari Hospital in Kumaun Region, Uttarakhand, India. *Journal of Clinical and Diagnostic Research*. 2021 Jun;5(6):30-3
12. Kucheria R, Dasgupta P, Sacks SH, Khan MS, Sheerin NS. Urinary tract infections: New insights into a common problem. *Postgrad Med J*. 2005;81(952):83-6
13. Gupta V, Rani H, Singla N, Kaistha N, Chander J. Determination of extended-spectrum β -lactamases and Amp C production in uropathogenic isolates of *Escherichia coli* and susceptibility to Fosfomycin. *J Lab Physicians* 2013; 5:90-93
14. Banerjee S, Sengupta M, Sarker TK. Fosfomycin susceptibility among multidrug-resistant, extended β -spectrum beta lactamase-producing, carbapenem-resistant uropathogens. *Indian J Urol* 2017;33:149-154
15. Mittal S, Sharma M, Chaudhary U. Fosfomycin use in multidrug resistant uropathogenic *Escherichia coli*. *Infect Disord Drug Targets* 2015;15:196-201
16. Sabharwal ER, Sharma R. Fosfomycin: An alternative therapy for the treatment of UTI amidst escalating resistance. *J Clin Diagn Res* 2015;9:DC6-9
17. Rajenderan S, Balaji V, Anandan S, et al. Determination of MIC distribution of arbekacin, cefminox, Fosfomycin, biapenem and other antibiotics against Gram-negative clinical isolates in south India: A prospective study. *PLoS One* 2014;9:e103253
18. Sahni RD, Balaji V, Barghese R, et al. Evaluation of Fosfomycin activity against uropathogens in a Fosfomycin-naïve population in South India: A prospective study. *Future Microbiol* 2013;8:675-680
19. Dastidar SG, Mazumdar A, Mookerjee M, Chakrabarty AN. Antimicrobial potentiality of new beta-lactam antibiotic Fosfomycin. *Indian J Exp Biol* 1997;33:300-301
20. Marie MA, Krishnappa LG, Lory S. In vitro activity and the efficacy of arbekacin, cefminox, Fosfomycin, biapenem against Gram-negative organisms. New treatment options? *Proc Natl Acad Sci India Sect B Biol Sci* 2015;1-7.