ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 07 | July - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

"ANTIMICROBIAL SUSCEPTIBILITY TESTING OF 87 URINARY ISOLATES OF ESCHERICHIA COLI WITH EMPHASIS ON FOSFOMYCIN"

Microbiology

Dr. Banashankari G S Professor, Department of Microbiology, M S Ramaiah Medical College, Bangalore, Karnataka, India

Dr. Betu Rama Soujanya* Senior Resident, Department of Microbiology, M S Ramaiah Medical College, Bangalore, Karnataka, India. *Corresponding Author

ABSTRACT

Objectives: Urinary tract infections (UTIs) affect all age groups and genders and are one of the most common infectious diseases. In the present era, there is an increased rate of isolation of multidrug-resistant (MDR) Gram-negative organisms. Fosfomycin a broad-spectrum antimicrobial with high renal concentration is one of the therapeutic options for these UTIs. In this study, an antimicrobial susceptibility pattern with emphasis on Fosfomycin of isolates of Escherichia coli from urine was done. **Materials and methods:** Antimicrobial susceptibility testing was performed on 87 urinary isolates of Escherichia coli (E. coli) by the Kirby-Bauer disc diffusion method. Statistical software: Analysis of data was done by SPSS version 21. Graphs have been generated using Microsoft Word and Excel. **Results:** All the isolates were (100%) susceptible to fosfomycin, 90.8% were susceptible to nitrofurantoin, followed by meropenem which is 81.6%, and Piperacillin-Tazobactam was being 71.3%. **Conclusion:** With the non-availability of a new group of antimicrobials and an increase in multidrug-resistant pathogens, it is come to a point to reuse the older drug fosfomycin with a broad spectrum of activity with minimal side effects and cross-resistance which can be used as monotherapy or in combination.

KEYWORDS

Escherichia coli, Urinary tract infection, antimicrobial susceptibility testing, Fosfomycin

INTRODUCTION:

One of the most commonly encountered infections in a health care setting as well as in outpatient departments is Urinary tract infections (UTIs).[1] *The most commonly isolated organism is Escherichia coli, followed by other Gram-negative bacilli like Klebsiella* species, *Enterobacter* species, *Proteus* species, *and Citrobacter species Pseudomonas aeruginosa and Acinetobacter* species in UTIs followed by Gram-positive organisms like *Staphylococcus saprophyticus, Enterococcus* species, and *Staphylococcus aureus* cause UTIs.[2] In a community as well as healthcare-associated infections one of the most commonly isolated organisms is *Escherichia coli (E. coli).* But due to misuse or overuse of antimicrobials, underdosing and incomplete course of antimicrobials led to their resistance which is of great concern in the present era.[3] It has become difficult to treat multidrug-resistant (MDR) organisms as we are limited with very few antibiotics to choose from or to revert to an older class of drugs.

Fosfomycin has a broad-spectrum antimicrobial activity (Members of family Enterobacteriaceae, including those that produce ESBL, carbapenemase-producing *Klebsiella pneumoniae*, vancomycin-resistant enterococci) and is an oral antimicrobial with minimal adverse effects can be also given to pregnant women with UTI.[4,5]

In 1969 in Spain, from *Streptomyces* species cultures Fosfomycin was discovered to act by inhibiting phosphoenolpyruvate transferase and thereby interfering with the synthesis of the cell wall of gram-negative and gram-positive bacteria.[5,6] It is seen to have a very low minimum inhibitory concentration & is most active against *E. coli* & *Enterococcus faecalis* and is usually administered in the dose of 3g single oral dose making this drug more patient compliant. [7]

It is also observed that apart from being the first-line treatment for UTI it can also be used to treat other infections like Respiratory infections including pneumonia, skin, and soft tissue infections, central nervous system infections (encephalitis or meningitis), Blood and Cardiovascular system (septicemia or endocarditis). [8] It is also tried as a part of combination therapy along with aminoglycosides, beta-lactams, and polymyxins. [9] In the present study, the susceptibility of Fosfomycin was tested against drug-resistant *E. coli* from urine samples.

OBJECTIVES:

1. To isolate *E. coli* from urine samples
2. Test for antimicrobial susceptibility especially for Fosfomycin

MATERIALS AND METHODS:

The urine samples received in the Department of Microbiology, a tertiary care hospital for 3 months were inoculated on Cysteine Lysine Electrolyte Deficient (CLED) medium in a semi-quantitative method. The microscopy of urine samples was done by Gram stain to look for pus cells and microorganisms. Isolation and identification of *E. coli* were done and antimicrobial susceptibility was done as per the Clinical and Laboratory Standards Institute (CLSI) guidelines by the Kirby Bauer disc diffusion method.

Ampicillin(10µg), Amikacin (30µg), Cefoxitin (30 µg), Cefotaxime (30 µg), Cefuroxime (30 µg), Ceftazidime (30 µg), Ciprofloxacin (5 µg), Cotrimoxazole (1.25/23.75 µg), Piperacillin-Tazobactam (100/10 µg), Gentamicin (10 µg), Imipenem (10 µg), Meropenem (10 µg), Nitrofurantoin (300 µg), Norfloxacin (10 µg), Fosfomycin (200 µg + 50 µg glucose-6-phosphate) discs were tested by disc diffusion method.

A suspension of 3-5 colonies in sterile peptone water was made and incubated at 35-37°C for approximately half an hour and matched with 0.5 McFarland's barium sulfate standard. Once the suspension is matched with 0.5 McFarland's standard, a sterile swab is taken and dipped in the inoculated suspension & rotated firmly several times against the upper inside wall of the tube to remove the excess suspension. A lawn culture was done on Muller Hinton agar. The discs were applied aseptically, at least 24 mm apart [Figure 1]. The lid was closed and the plates were incubated at 37ºC for 18 – 24 hours following which the zone diameter was measured and interpreted according to ti the CLSI guideline. The susceptibility zone for fosfomycin was considered to be ≥ 16mm, the intermediate zone was 13-15 mm, and the resistant zone was considered to be ≤12mm.

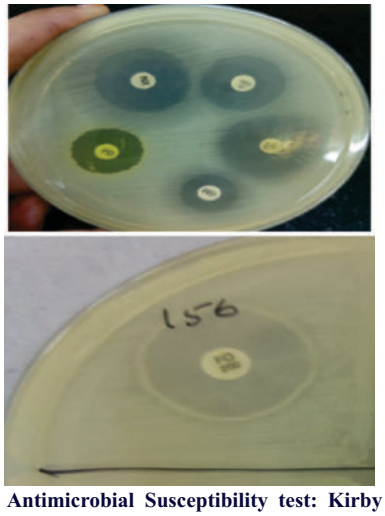

Figure 1: Antimicrobial Susceptibility test: Kirby Bauer Disc diffusion Method

Statistical software: Analysis of data was done by SPSS version 21. Graphs have been generated using Microsoft Word and Excel.

RESULTS:

The study was performed on 87 isolates of E. *coli* from the urine samples.

Out of the 87 isolates, 29 (33.3%) were isolated from male patients, whereas 58 (66.7%) were from female patients.

All the isolates were tested for the above antibiotics and it was noted that 100% were susceptible to fosfomycin, 90.8% were susceptible to nitrofurantoin, followed by meropenem which was 81.6%, and Piperacillin-tazobactam 71.3% [Figure 2].

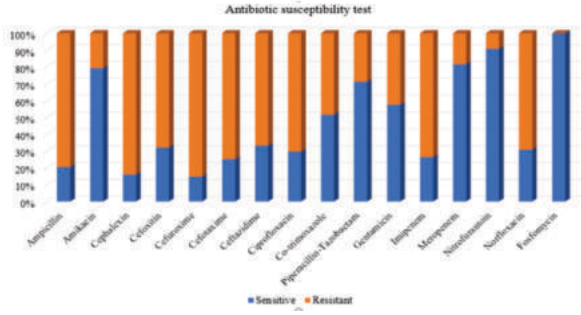

Figure 2: Antibiotic susceptibility test

DISCUSSION:

In a person with a good immune system, immunocompromised hosts, and uncomplicated urinary tract infections *E. coli* is one of the etiological agents isolated. There is an emergence of resistance to multi drugs in the hospital as well as in the general public community which is either transmissible resistance or inherited to various antibiotics like fluoroquinolones, cephalosporins, and carbapenems.[10] Recently cross-resistance to aminoglycosides is seen by some of the resistant strains of *E. coli*. [11] This is usually seen because of an increase in the usage of highly effective parenteral antibiotics such as 3rd generation cephalosporins or carbapenems in urinary tract infection treatment, which also often require hospitalization. This impacts the selection of suitable antimicrobial drugs for this resistant *E. coli.* In this study, it was observed that few of the isolates were isolated from patients who had comorbidities like diabetes mellitus and hypertension. In this study, the susceptibility of fosfomycin was tested on E. coli from a urine sample from different wards and outpatient departments. We observed that almost all the isolates included in the study showed variable but high resistance to most of the antibiotics especially Ampicillin (79.3%), Cephalosporins (Cephalexin – 83.9%, Cefuroxime – 85.1%, Cefotaxime – 74.7%), Ciprofloxacin (70.1%), Imipenem (73.6%) while 100% remained sensitive to fosfomycin.

Our study corroborates with Gupta V et al, Mittal S et al study which indicates 100% sensitivity to fosfomycin with varied resistance to other antibiotics, 52.6% of isolates showed ESBLs production, 8% of isolates in Gupta V et al study showed Amp C production, Mittal S et al study, 29% isolates were found to be multidrug-resistant (MDR). [12, 13]

However, few studies showed *E. coli* isolates to have varied sensitivity to fosfomycin like Fajfr M et al., Banerjee S et al., and Sahni RD et al. indicated 95.8%, 95.18%, and 83% of isolates were susceptible to fosfomycin respectively. [14,15,16]

A study done by Jain et al observed that out of a total of 145 E.coli isolate94.48% were susceptible to fosfomycin, 87.58 % to tigecycline, and 77.24 % to nitrofurantoin. 85.51% of isolates were multidrug resistant in their study which was comparable to our study of multidrug-resistant *E.coli.*[17]

In a study done by Hareendranath G et al, their observations for 150 multidrug urinary isolates of *E.coli* states that 129 (86 %) isolates were resistant to cefotaxime and 75 % of these were ESBL producing. All the ESBL-producing isolates of *E.coli* were 100% susceptible to fosfomycin. Amongst the multidrug-resistant *E.coli* according to their study, 120 (80 %) were susceptible to nitrofurantoin and 98 % were susceptible to fosfomycin.[18]

Recently, it is observed that Fosfomycin an old antimicrobial drug has a broad range of activity against both Gram-negative and Gram-positive organisms and can be used as an alternate option for organisms with resistance to many drugs and has very little chance of cross-resistance due to its structural indifference with any other antimicrobial drug.[19]

However, there can be the development of resistance by reduced permeability of fosfomycin related to GlpT and UhpT, and MurA related to target modification along with drug inactivation which can occur due to the acquisition of fos genes mostly by the plasmid. [4, 20] *Fosfomycin has a long* half-life (mean half-life-SD, 5.7-2.8 h) with better penetration to various tissues and *is by far mostly an under-prescribed antimicrobial agent though it has an excellent in vitro susceptibility in* India. As it has a high urinary concentration and very few side effects it can be considered one of the therapeutic options in complicated UTI cases and also for Multidrug-resistant organisms and Carbapenem-resistant Enterobacterales (CRE). [21, 22]

Limitations:

This was a study done for a short duration of 3 months on only 87 isolates of E.coli & antimicrobial susceptibility was done by conventional Kirby Bauer disc diffusion test. These isolates were not tested on an automated system like VITEK. This study had not included other members of the family *Enterobacteriaceae* & *Enterococcus faecalis*.

CONCLUSION:

Fosfomycin which can be given as an oral drug is dosage friendly, with minimal side effects, and can be used for multidrug-resistant organisms because of the non-availability of new drugs. Although it can be used as monotherapy because of its very little cross-resistance with other antibiotics it can be used as a combination drug that makes synergistic interactions benefit from an increase in the bactericidal effect and prevention of antimicrobial resistance.

Conflict of Interest: None declared.

Acknowledgment: Nil

REFERENCES:

1. Medina M, Castillo-Pino E. An introduction to the epidemiology and burden of urinary tract infections. *Ther Adv Urol*. 2019;11:1756287219832172. Published 2019 May 2. doi:10.1177/1756287219832172.
2. Ahmed SS, Shariq A, Alsalloom AA, Babikir IH, Alhomoud BN. Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. *Int J Health Sci (Qassim)*. 2019;13(2):48-55.
3. Lee DS, Lee SJ, Choe HS. Community-Acquired Urinary Tract Infection by *Escherichia coli* in the Era of Antibiotic Resistance. *Biomed Res Int*. 2018:7656752. Published 2018 Sep 26. doi:10.1155/2018/7656752
4. Catherine G Derington, Nancy Benavides, Thomas Delate, Douglas N Fish, Multiple-Dose Oral Fosfomycin for Treatment of Complicated Urinary Tract Infections in the Outpatient Setting, *Open Forum Infectious Diseases*. 2020:7(2); ofaa034 https:// doi.org/10.1093/ofid/ofaa034
5. Mothibi LM, Bosman NN, Nana T. Fosfomycin susceptibility of uropathogens at Charlotte Maxeke Johannesburg Academic Hospital. S Afr J Infect Dis. 2020 Oct 26;35(1):173. doi: 10.4102/sajid.v35i1.173. PMID: 34485478; PMCID: PMC8377994
6. Raz R. Fosfomycin: an old-new antibiotic.Clin Microbiol Infect.2012 Jan;18(1)4-7. Doi:10.1111/j.1469-0691.2011.03636.x.Epub 2011 Sep 13.PMID:21914036.
7. Gardiner BJ, Stewardson AJ, Abbott IJ, Peleg AY. Nitrofurantoin and fosfomycin for resistant urinary tract infections: old drugs for emerging problems. Aust Prescr. 2019 Feb;42(1):14-19. doi: 10.18773/austprescr.2019.002. Epub 2019 Feb 1. PMID: 30765904; PMCID: PMC6370609
8. Falagas ME, Vouloumanou EK, Samonis G, Vardakas KZ. Fosfomycin. *Clin Microbiol Rev*. 2016;29(2):321-347. doi:10.1128/CMR.00068-15
9. Ruiz Ramos J, Salavert Lletí M. Fosfomycin in infections caused by multidrug-resistant Gram-negative pathogens. *Rev Esp Quimioter*. 2019;32 Suppl 1(Suppl 1):45-54.
10. Falagas ME, Polemis M, Alexiou VG, Marini-Mastrogiannaki A, Kremastinou J, Vatopoulos AC. Antimicrobial resistance of Escherichia coli urinary isolates from primary care patients in Greece. *Med Sci Monit*. 2008;14(2):CR75-CR79.
11. Tsukamoto N, Ohkoshi Y, Okubo T, Sato T, Kuwahara O, Fujii N, Tamura Y, Yokota S. High prevalence of cross-resistance to aminoglycosides in fluoroquinolone-resistant Escherichia coli clinical isolates. Chemotherapy. 2013;59(5):379-84. doi 10.1159/000361011. Epub 2014 May 13. PMID: 24852043.
12. Gupta V, Rani H, Singla N, Kaistha N, Chander J. Determination of Extended-Spectrum β-Lactamases and AmpC Production in Uropathogenic Isolates of Escherichia coli and Susceptibility to Fosfomycin. *J Lab Physicians*. 2013;5(2):90-93. doi:10.4103/0974-2727.119849
13. Mittal S, Sharma M, Chaudhary U. Fosfomycin use in multi drug resistant uropathogenic Escherichia coli. *Infect Disord Drug Targets*. 2015;15(3):196-201. doi:10.2174/1871526515666150916141907.
14. Fajfr M, Louda M, Paterová P, et al. The susceptibility to fosfomycin of Gram-negative bacteria isolates from urinary tract infection in the Czech Republic: data from a unicentric study. *BMC Urol*. 2017;17(1):33. Published 2017 Apr 26. doi:10.1186/s12894-017-0222-6.
15. 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):149-154. doi:10.4103/iju.IJU_285_16.
16. Sahni RD, Balaji V, Varghese R, et al. Evaluation of fosfomycin activity against uropathogens in a fosfomycin-naive population in South India: a prospective study. Future Microbiology. 2013 May;8(5):675-680. DOI: 10.2217/fmb.13.31. PMID:

23642120.
17. Jain R, Pal N, Hooja S. Fosfomycin susceptibility among multidrug resistant and extended spectrum ß-Lactamase producing uropathogenic Escherichia coli isolates at a tertiary care hospital of Western India. CHRISMED J Health Res 2022;9:125-9.
18. Hareendranath G, Mukundan A, Kunjappan SP. Fosfomycin susceptibility in multidrug resistant urinary Escherichia coli isolates. J Evolution Med Dent Sci 2021;10(07):414-418, DOI: 10.14260/jemds/2021/92
19. 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 (Basel)*. 2020;9(3):112. Published 2020 Mar 6. doi:10.3390/antibiotics9030112
20. Takahata S, Ida T, Hiraishi T, et al. Molecular mechanisms of fosfomycin resistance in clinical isolates of Escherichia coli. *Int J Antimicrob Agents*. 2010;35(4):333-337. doi:10.1016/j.ijantimicag.2009.11.011
21. Sultan A, Rizvi M, Khan F, Sami H, Shukla I, Khan HM. Increasing antimicrobial resistance among uropathogens: Is fosfomycin the answer? *Urol Ann*. 2015;7(1):26-30. doi:10.4103/0974-7796.148585.
22. Falagas ME, Kastoris AC, Kapaskelis AM, Karageorgopoulos DE. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum beta-lactamase producing, Enterobacteriaceae infections: a systematic review. *Lancet Infect Dis*. 2010;10(1):43-50. doi:10.1016/S1473-3099(09)70325-1