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Original Article

In Vitro Efficacy Of Fosfomycin Against *E. Coli* And Prevalence Of MDR And XDR *E. Coli* Isolates From UTI Patients

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Abstract

Objective: The efficacy of Fosfomycin against ESBL and/or carbapenem-resistant *E. coli* isolated from urine samples was determined.

Methodology: Three hundred fifty (350) urine samples were collected from the patients having UTI who visited the Department of Urology, JPMC, Karachi. The CLED agar was used for the primary isolation of uropathogens. Regular antimicrobial sensitivity testing was conducted in accordance with CLSI standards, and the minimum inhibitory concentration (MIC) of Fosfomycin was assessed using E-strips.

Results: Out of 350 urine samples 213 (60.85%) were *E. coli*. Patients with *E. coli* had an average age of 38.75 and 15.01 years. Females are more prone to have UTI 146(68.54%). *E. coli* was highest among uropathogens having a frequency of 213(60.85%). *E. coli* manifest highest resistance to ampicillin 187(87.79%) and low resistance to meropenem 12(5.63%), imipenem 15(7.51%) and Fosfomycin 21(9.85%). The overall carbapenem-resistant *E. coli* was 9(6.4%) and for the majority of (61.5%) Fosfomycin-resistant *E. coli*, MIC value was >1024µg/ml. Isolates were categorized in the non-MDR, MDR and XDR. Most of the isolates were MDR (53%), followed by non-MDR (35%) and XDR (11%).

Conclusion: In conclusion, the present study suggests that Fosfomycin is still effective against *E. coli*. More than 50% *E. coli* isolates were MDR and it's an alarming situation for urologists.

Keywords: Carbapenem-resistant, Extensively Drug Resistant, Fosfomycin, Multidrug-resistant *E. coli*, Urinary tract infection

Introduction

A phosphonic acid derivative is Fosfomycin, water-soluble and available in Fosfomycin tromethamine, Fosfomycin calcium salts, and Fosfomycin disodium.[1] It combats both Gram-negative and Gram-positive bacteria, as well as extended-spectrum β-lactamases (ESBLs)-producers including *E. coli* and other members of Enterobacteriaceae.[2] Fosfomycin is bactericidal and inhibits cell wall synthesis by hindering phosphoenolpyruvate transferase.[3] Its distinct mode of action works in concert with other antibiotics, such as aminoglycosides, beta-lactams, and fluoroquinolones, to treat infections.[4] Fosfomycin was obsoleted because of its therapeutic failure and some discrepancies.[5] Later on the pharmacokinetic and pharmacodynamic characteristics are being arbitrated again due to the rapidly emerging ESBL and/or carbapenem-resistant (CR) uropathogens and recommended for UTI. [6]

Resistance to Fosfomycin is mainly by the alterations in the gene (MurA) encoding the target site for Fosfomycin and/or mutation in the transporter proteins. Both are chromosomal-mediated and present at lower frequencies. These mechanisms are normally not associated with other resistant mechanisms of bacteria.[7] Therefore it is recommended for the UTI, particularly of ESBL-producing and/or carbapenem-resistant *E. coli* and other multi-drug resistant (MDR) uropathogens including *Klebsiella*, *Enterococcus faecalis* and *Staphylococcus aureus*.[8] The resistance to Fosfomycin is lower (0.7%) in uropathogens reported from Canada and Europe.[9] Similarly it is also lower (4.2%) in carbapenem-resistant Enterobacteriaceae (CRE).[10] In Pakistan before 2005 resistance to Fosfomycin was at a lower level and not reported. Subsequently, resistance to Fosfomycin was reported at 5.8% to 12.3% in carbapenem-resistant *E. coli*.[11,12] Numerous distinctive classifications for multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) categories are exploited within the medicinal and antibacterial resistance literature. These definitions are independently characterized for each bacterium or a bunch of microscopic organisms for open understanding, not for clinical judgment.[13] In specific, many *E. coli* strains have developed multidrug-resistant, multidrug-resistant, or pan-drug-resistant (MDR, XDR, or PDR) types, posing significant challenges to infectious disease therapies.[14]

There is a need for continuous monitoring of antimicrobial-resistant patterns locally. The data regarding the efficacy of Fosfomycin against multidrug-resistant uropathogens *E. coli* is limited from this hospital. Therefore, the present study was designed to determine the efficacy of Fosfomycin against *E. coli* and the prevalence of MDR and XDR *E. coli* isolates from UTI Patients.

Materials and Methods

This cross-sectional descriptive research was carried out in the Department of Microbiology, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi in collaboration with the Department of Urology, JPMC, Karachi, from September 2018 to April 2019.

Ethical approval: The present study was submitted to the Institutional Review Board of JPMC, Karachi for ethical consideration and this was approved (No. F.2-81/2018-GEN/1919/JPMC).

Sample size:

The sample size for this study was calculated by Open Epi software using the reference study.[15] According to the calculation of sample size 350 urine specimens were collected from the patients having signs and symptoms of UTI who visited the department of urology, JPMC, Karachi

Microbial assay:

The urine was collected by clean-catch technique in sterilized disposable containers by educating the patients. Urine samples were inoculated on cystine lactose electrolyte deficient (CLED) agar by using a sterilized and calibrated wire loop. Inoculated plates were incubated at 37˚C for 24 to 48 hrs. After the incubation period colonies of the desired pathogen were calculated and interpreted as previously described.[16,17] Bacterial identification especially *E. coli* was performed by the manual traditional method, using macroscopic, microscopic characteristic and biochemical tests.

Antimicrobial susceptibility testing (AST):

This was performed by the disc diffusion technique adapted from the CLSI, (2018) [18]. *E. coli* ATCC (25922) strain was used for quality control for biochemical and routine AST. Fosfomycin-resistant *E. coli* isolates by disc diffusion test were confirmed by minimum inhibitory concentration (MIC) using Fosfomycin E-strip and results were interpreted in accordance with CLSI.[18]

Categorization of resistant *E. coli*:
Criteria for the categorization of multi-drug resistant (MDR), extensively drug-resistant (XDR), and pan-drug resistant (PDR) *E. coli* was adopted from Magiorakos et al (2012).[13]

Statistical analysis: The data was entered on the Excel sheet and was imported to SPSS version 22.0. The frequencies of the categorical variables were represented in percentages. The age of the study population was represented in mean age. The statistically significant differences were measured in categorical values determined by the Chai squire test and ≤0.05 P value was considered as significant.

Results

A total of 213 (60.85%) *E. coli* was isolated from 350 urine samples. Here the results of those patients are presented which were cultures positive for *E. coli*. The mean age of patients with *E. coli* culture positive was 38.75±15.01 years. The prevalence of UTI was higher in female and the most case was symptomatic. Micturition and urgency clinical presentations were common in UTI patients. History of recurrent and calculi was also common (Table.1). *E. coli* showed higher resistance to ampicillin 187(87.79%), ofloxacin 148(69.48%) followed by ciprofloxacin, amoxicillin-clavulanate and ceftriaxone 138(64.78%). *E. coli* revealed lower resistance to fosfomycin, imipenem, and meropenem (Fig.1).

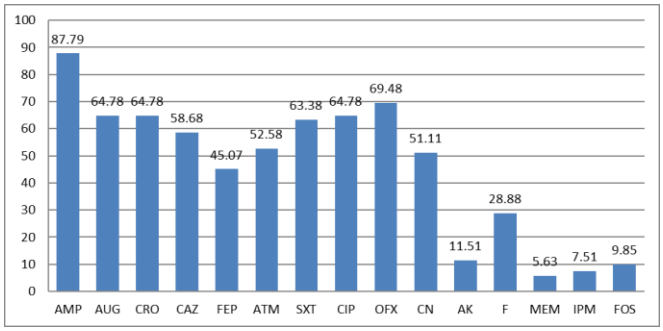

Figure 1. Resistance pattern of *E. coli* isolates from UTI patients (n=213)

Abbreviations: AMP: Ampicillin, AK: Amikacin, ATM: Aztreonam, AUG: amoxicillin-clavulanate, CAZ: Ceftazidime, CIP: Ciprofloxacin, CN: Gentamicin, CRO: Ceftriaxone, F: Nitrofurantoin, FEP: Cefepime, FOS: Fosfomycin, IPM: Imipenem, MEM: Meropenem, OFX: Ofloxacin, SXT: sulfamethoxazole-trimethoprim

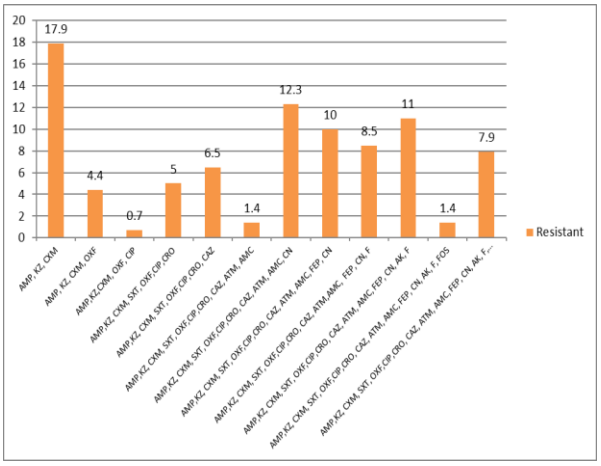

Figure.2 Resistance to ≥ three antimicrobial agents among *E. coli* isolates (n=213)

The resistance pattern of *E. coli* was categorized into 12 categories. The resistance to 3 antimicrobials (AMP, KZ, and CXM) was the highest (17.9%) and lowest (0.7%) to the 5 antimicrobial agents [AMP, KZ, CXM, OFX, and CIP] (Fig. 2). The isolates were also categories in the non-MDR, MDR and XDR. Most of the isolates were MDR, followed by the non-MDR and XDR (Fig. 3). The MDR isolates were significantly higher in ESBL producers as compared to non-ESBL producers (*P* 0.001).

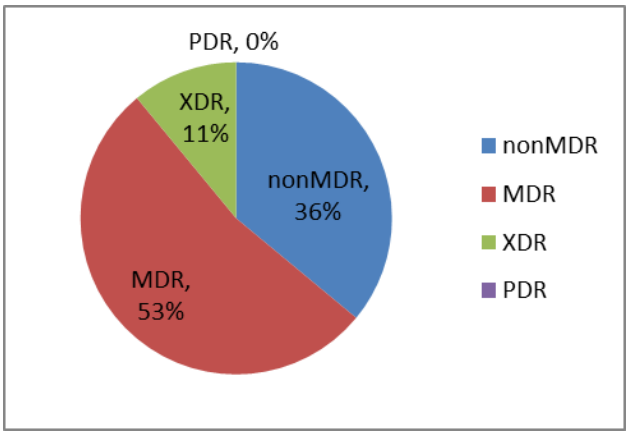

Figure 3. Frequency of non-MDR, multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug resistant (PDR) *E. coli* in the present study.

Fosfomycin-resistant *E. coli* was mostly MDR and XDR isolates. The E-test MIC values of Fosfomycin against *E. coli* revealed that the majority of isolates fell in the sensitive category between 32 to ≤64 µg/ml, followed by 0.25 to <32 µg/ml. The Fosfomycin resistance was higher in ESBL producers than non-ESBL producers

with a *P* value of 0.01. This revealed the significant difference between these two groups.

Discussion

Urinary tract infections (UTIs) increase the financial weight of the healthcare system in respect of its laboratory diagnosis, clinical management, hospital stay, and other factors. UTIs are frequently recurrent, difficult to treat, and can damage the kidney parenchyma, leading to kidney failure and other impediments. Among Enterobacteriaceae *E. coli* is the common uropathogens.[19] Similarly in the present study *E. coli* was the dominant uropathogen. Similar findings are also reported by Tenney *et al*. (2018). [20]

Antimicrobial resistance (AMR) is a worldwide menace to community health and an immense challenge to clinicians.[21] The magnitude of AMR fluctuates geographically, from hospital to hospital and even within hospital areas. In Africa and South Asia, it is at a higher rate. In the present study, the in vitro efficacy of ampicillin, cefazolin, cefuroxime, sulfamethoxazole-trimethoprim, and ofloxacin was poor against *E coli*. Resistance to commonly prescribed 3rd and 4th generation cephalosporin is 64 and 45.5 percent respectively which are commonly used in empirical therapy of UTI. A similar pattern has been reported in a previous study by Parajuli *et al*. (2017).[22]

Uropathogens that are MDR and XDR stance a serious threat to healthcare providers and the adverse outcomes are mortality, morbidity, and increased financial burden.[14] Recently increasing incidences of multidrug-resistant (MDR) *E. coli* has been reported globally. In the present study, 53% *E. coli* were MDR and 11% were XDR and no PDR *E. coli* were isolated. A previous study from Nepal showed a higher (64.9%) prevalence of MDR and a lower (5%) XDR *E. coli* reported.[22] From Mexico MDR *E. coli* are reported from 16.4 to 97%.[15]AMR prevalence is associated with different factors including the type of bacteria, resistance mechanisms, and antibiotics pressure in a particular region. In countries with limited capital, and over-loaded healthcare systems AMR is strenuous to succeed in control of infections caused by highly resistant uropathogens. The advent of multidrug-resistant *E. coli* is startling to clinicians and treatment possibilities are finite. Therefore, an old antibiotic such as Fosfomycin is amending having effective antimicrobial activity against these MDR *E.* coli and other uropathogens.[15,23] This data delivers assessments of the Fosfomycin activity against *E. coli* and the results are promising and it is still effective in MDR *E. coli*. The coexistence of Fosfomycin resistance in *E. coli* was not associated with other agents and our results are in agreement with Linsenmeyer *et al*. (2016) and Patel *et al*. (2017) validate the findings of this study which show overall susceptibility to Fosfomycin 90.6%.[10,24]

Table-1 Demographic and clinical variables of the study population infected with *E. coli* (n=213)

Risk factors	Number N	Percentage %
Age (Means, Years)	38.75±15.01	
Female	146	68.54
Fever	89	41.78
Flank pain	168	87
Burning Micturition	198	92.95
Frequency	172	80.75
Diabetes	89	41.78
Calculi	91	42.72
Previous Hospitalization	72	33.80
Pregnancy	51	23.94
Catheterization	59	27.69
Recurrent	133	62.44

This study had few limitations so more studies are required to investigate the resistance genes. This has been a universal concern; therefore, the timely and continuous monitoring of antibiotic susceptibility patterns is necessary for the prescription of the relevant antibiotics.

Conclusion

In conclusion, the present study suggests that Fosfomycin is still effective against *E. coli*. More than 50% of *E. coli* isolates were MDR and it's an alarming situation for a urologist.

References

1. Ito R, Mustapha MM, Tomich AD, Callaghan JD, Mceiheny CL, Mettus RT, Shanks RM, Sluis Cremer N, Doi Y. Widespread Fosfomycin resistance in Gram negative bacteria attributable to the chromosomal fosA gene. *mBio* 2017; 8(4): e00749-17
2. Derington CG, Benavides N, Delate T, Fish DN. Multiple-Dose Oral Fosfomycin for Treatment of Complicated Urinary Tract Infections in the Outpatient Setting, *Open Forum Infect Dis* 2020;7(2):1-8. https://doi.org/10.1093/ofid/ofaa034

3. Fajfr M, Louda M, Paterova P, Ryskova L, Pacovsky J, Kosina J, Zemlickova H and Brodak M. The susceptibility to fosfomycin of Gram negative bacteria isolates from urinary tract infection in the Czech Republic. *BMC Urol* 2017; 17(1):33.
4. Kaase M, Szabados F, Anders A, Gatermann SG. Fosfomycin susceptibility in carbapenem-resistant Enterobacteriaceae from Germany. *J Clin Microbiol* 2014;52(6):1893-7. doi: 10.1128/JCM.03484-13.
5. Zhanel GG, Walkty AJ, Karlowsky JA. Fosfomycin: A first-line oral therapy for acute uncomplicated cystitis. *Can J Infect Dis Med Microbiol* 2016; 2082693.
6. Keepers TR, Gomez MK, Celeri C, Krause KM, Beik D and Critchley I. Fosfomycin and comparator activity against select Enterobacteriaceae, Pseudomonas and Enterococcus urinary tract infection isolates from United States. *Infect Dis Ther* 2017; 6(2):233-243.
7. Okhoshi y, Sato T, Suzuki Y, Yamamoto S, Shiraishi T, Ogasawara N and Yokota S. Mechanism of reduced susceptibility to fosfomycin in Escherichia coli clinical isolates. *Bio Med Res Intern* 2017; 2017:8 pages.
8. Fransen F, Hermans K, Melchers MJB, Lagarde CCM, Meletiadis J, Mouton JW. Pharmacodynamics of fosfomycin against ESBL- and/or carbapenemase-producing Enterobacteriaceae. *J Antimicrob Chemother* 2017; 72(12):3374-3381
9. Kandil H, Cramp E and Vaghela Tejal. Trend in antibiotic resistance in urologic practice. *Eur Urol Focus* 2016; 2(4):363-373.
10. Linsenmeyer K, Strymish J, Weir S, Berg G, Brecher S and Gupta k. Activity of Fosfomycin against extended spectrum β-lactamase producing uropathogens in patients in the community and hospitalized patients. *Antimicrob Agents Chemother* 2016; 60(2):1134-1136.
11. Habeeb MA, Sarwar Y, Ali A, Salman M and Haque A. Rapid emergence of ESBL producers in E. coli causing urinary and wound infections in Pakistan. *Pak J Med Sci* 2013; 29(2):540-544.
12. Qamar S, Shaheen N, Shakoor S, Farooqi J, Jabeen K and Hasan R. Frequency of colistin and fosfomycin resistance in carbapenem-resistant Enterobacteriaceae from tertiary care hospital in Karachi. *Infect Drug Resist* 2017; 10:231-236.
13. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18(3):268-81. doi: 10.1111/j.1469-0691.2011.03570.x.
14. Wang M, Wang W, Niu Y, Liu T, Li L, Zhang M, Li Z, Su W, Liu F, Zhang X and Xu H. A Clinical Extensively-Drug Resistant (XDR) Escherichia coli and Role of Its β-Lactamase Genes. Front. Microbiol 2020;11:590357. doi: 10.3389/fmicb.2020.590357
15. Ramírez-Castillo, F.Y., Moreno-Flores, A.C., Avelar-González, F.J. et al. An evaluation of multidrug-resistant Escherichia coli isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study. *Ann Clin Microbiol Antimicrob* 2018;17. https://doi.org/10.1186/s12941-018-0286-5
16. Maher PJ, Brown AE and Gatewood M OK. The effect of written posted instructions on collection of clean catch urine specimen in the emergency department. *J Emer Med* 2016; 52(5): 639-644.
17. Coulthard MG. Using urine nitrite sticks to test for urinary tract infection in children aged < 2 years: a meta-analysis. *Pediatr Nephrol* 2019 34(7):1283-1288. doi: 10.1007/s00467-019-04226-6.
18. Clinical & Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 28th informational supplement. M100-S28. Wayne, PA: CLSI, 2018.
19. Sabih A and Leslie SW. Complicated Urinary tract infections. *Statpearls internet* 2018.
20. Tenney J, Hudson N, Alnifaidy H, Cheung Li J and Fung KH. Risk factors for acquiring multidrug resistant organisms in urinary tract infections: A systemic literature review. *Saudi Pharm J* 2018; 26(5): 678-684.
21. Effah, C.Y., Sun, T., Liu, S. et al. *Klebsiella pneumoniae*: an increasing threat to public health. *Ann Clin Microbiol Antimicrob* 2020;9(1). https://doi.org/10.1186/s12941-019-0343-8
22. Parajuli, N.P., Maharjan, P., Parajuli, H. et al. High rates of multidrug resistance among uropathogenic Escherichia coli in children and analyses of ESBL producers from Nepal. *Antimicrob Resist Infect Control* 2017;6(9). https://doi.org/10.1186/s13756-016-0168-6
23. Simsek M. Sensitivity of extended spectrum of β-lactamase producing *Escherichia coli* and *Klebsiella* species to Fosfomycin. *J Pak Med Assoc* 2020, 70: 1187-1192. https://doi.org/10.5455/JPMA.32169
24. Patel B, Patel K, Shetty A, Soman R and Rodrigues C. Fosfomycin susceptibility in urinary tract Enterobacteriaceae. *J Assoc Physi India* 2017; 65(9):14-16.