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Fosfomycin Resistance in Clinical Isolates of *Escherichia coli* from Urinary Tract Infections in a Tertiary Care Hospital

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ABSTRACT

Background: Fosfomycin is called an old-new antibiotic, because it was reintroduced for the treatment of urinary tract infections. The rampant use of antibiotics has led to Fosfomycin resistance reported from different parts of the world. The purpose of this study was to find out the frequency of Fosfomycin resistance in clinical isolates of *Escherichia coli* (*E. coli*) from urinary tract samples.

Methods: This cross-sectional study, including n=314 patients, was conducted in the Microbiology laboratory at Pakistan Naval Ship SHIFA Hospital, Pakistan from January to June 2022. Urine samples were inoculated on agar at 37±2°C for 24-48hrs. The growth of *E. coli* was confirmed by API 10S as per recommended Clinical Laboratory Standard Institute (CLSI) guidelines. Susceptibility testing was performed by the standard Kirby Bauer Disc Diffusion method. The Chi-square test was applied to categorical variables. *p*-value ≤ 0.05 was considered statistically significant.

Results: Out of 314 clinical isolates, 171 (54.5%) were females and 143 (45.5%) were males (mean age 49±10.3 (6-81) years). Sixty-six (21%) isolates were found resistant to Fosfomycin while 248 (79%) were sensitive. A significant difference was found between the gender (*p*=0.035), whereas, 29 (17.0%) females and 37 (25.9%) males were found resistant to Fosfomycin.

Conclusion: The resistance to Fosfomycin is increasing and it is an impending threat as oral treatment options are limited in urinary tract infections. However, improved surveillance may help in controlling nosocomial infection along with the rational use of antibiotics can prevent and reduce its spread.

Keywords: Fosfomycin; Culture; Susceptibility; Urinary Tract Infections (UTIs).

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INTRODUCTION

Urinary tract infections (UTIs) are the most widespread community and hospital-acquired infections, which need certain treatment measures[1]. Gram-negative rods like *E. coli* are the leading reported pathogen involved in UTIs [2]. There has been a substantial rise in multidrug-resistant *Escherichia coli* strains including those producing β-lactamases and carbapenemases in the last decade, leading to limiting therapeutic options in hand to treat urinary tract infections and UTIs [3]. WHO GLASS (Global Antimicrobial Resistance and Use Surveillance System) report of 2017-2018 points out resistance of >70% towards ceftriaxone and ciprofloxacin in *E. coli* isolated from urinary tract infection in Pakistan[4]. In Pakistan resistance to Fosfomycin is low as there was only one reported case in the Bullens et al. study in an isolate of *E. coli*[5]. Resistance to Fosfomycin from other parts of the world is reported as 8.3% in Italy, 4.6% in Spain, 2.9% in the UK (United Kingdom), and 1.9% from Belgium and Russia[6].

Fosfomycin was first discovered in Spain in 1969 and was commenced in Europe in a period of 1970s [7]. Fosfomycin is a bactericidal antibiotic, which is suitable to treat diseases by MDR (multi-drug resistant) pathogens. Its mode of action is by blocking the transferase enzyme UDP-N-acetylglucosamine enolpyruvyl (MurA), which is involved in stopping the production of murein peptidoglycan. Peptidoglycan biosynthesis is also an important regulator of bacterial cell division[8]. Mechanisms of Fosfomycin resistance are mediated towards *E. coli* by the ability of a pathogen to reduce the uptake of the drug and elaborate enzymes are inactivated and alter the target receptors of the drug, thus rendering its effectiveness[9]. Among the mechanism, the reduced drug uptake occurs most frequently mediated by a mutation in chromosomes like point mutations, base insertions, and base deletions which alter the transport mechanism of the drug reducing its uptake[10].

Enzymes that modify the configuration of Fosfomycin are mediated by plasmids carrying genes (*fosA*-like genes with K and Mn^{2+}-dependent glutathione S-transferase; *fosB*-like genes Mg^{2+}-dependent thiol S-transferase and; *fosX with* Mn^{2+}-dependent epoxide hydrolase) [11]. Rarely resistance towards Fosfomycin can be carried out by modifications of enzyme MurA. Two cases of this kind of resistant isolates were detected in Japan while global expression of MurA is not a phenomenon commonly reported so far in *E. coli* [12,13]. In uncomplicated UTIs, this drug is recommended and can be used as a 3-grams single dose therapy against *E. coli* and *Enterococcus faecalis*. Its activity against both Gram-negative and Gram-positive bacteria is fairly good with little cross-resistance with other antibiotics. Different cases of Fosfomycin resistance from different parts of the world were reported, as a result of the rampant use of antibiotics. Therefore, this study aimed to know the frequency of Fosfomycin resistance in *E. coli* isolated from suspected UTI patients in a tertiary care hospital.

METHODS

This was a descriptive cross-sectional study conducted in the Microbiology laboratory at Naval Ship SHIFA Hospital, Pakistan from January 2022 to July 2022. All the urine samples with pus cells more than 4-5/HPF under the microscope were included in the study. Both genders, outdoor patients and in-door patients were included in the study. Repeat specimens, with broken or damaged containers, and specimens with contaminated and mixed growth were excluded from the study. Patients were informed about the study and took written consent. The ethical study was approved ethical review board of PNS Shifa hospital.

In the protocol, all the urine samples were inoculated on cysteine lactose electrolyte deficient (CLED) agar (Oxoid, UK) by strip method for obtaining a semi-quantitative bacterial count. Inoculated plates were incubated for 24-48 hours at 35°C–37°C in ambient air. The growth of *E. coli* was confirmed by using API (analytical profile index) 10S (bioMérieux, France). A suspension of the organism to be tested was matched with a turbidity of a 0.5 McFarland standard 1×10^8 CFU (colony forming unit)/ml). Antibiotic susceptibility testing of *E. coli* towards Fosfomycin was done on Muller-Hinton Agar (Oxoid, UK) by applying the modified Kirby-Bauer disk diffusion method which is the recommended method by CLSI (Clinical Laboratory Standard Institute) guidelines[14,15]. Fosfomycin resistance was noted by using a Fosfomycin disc 50 µg (Oxoid, UK). *E. coli* ATCC 25922 was used as the control strain. CLSI breakpoints for *E. coli* were used that are more than or equal to 15mm susceptible and less than 15mm resistant.

Statistical analysis was done by SPSS version 25. Mean and Standard deviation was calculated for variables like age and gender. Frequency and percentages were calculated for all categorical variables. Independent sample *t*-test was applied between age and Fosfomycin-resistant *E. coli*. The Chi-square test was applied to see the significance between two categorical variables. *p*-value ≤ 0.05 is considered to be statistically significant.

RESULTS

A total of 314 isolates of *E. coli* were isolated, according to the Open Epi calculator and included in the study. Out of 314 clinical isolates, 171 (54.5%) were from females and 143 (45.5%) were from the male. Outdoor patients were included in the study more than hospitalized. Fosfomycin resistance was noticed in 21% patients as shown in Table 1.

Table 1: Frequency of Fosfomycin resistance concerning to gender, inpatient/outpatient data.

Variables	Frequency n (%)	
Gender (n=314)	**Female**	**Male**
	171(54.5)	143(45.5)
Inpatient/Outpatient	**Indoor**	**Outdoor**
	91(29.0)	223(71.0)
Fosfomycin Resistant *E. coli*	**Resistant**	**Sensitive**
	66(21.0)	248(79.0)

The mean age of the patients was 49± 10.3 (6-81) years. Out of a total of 314 clinical isolates, 29 (17.0%) in females, 37 (25.9%) in males were found resistant and 248 (79%) were sensitive to Fosfomycin (Table 2). A significant difference was found between the gender (*p*=0.035). Out of 314 isolates, 223 isolates were from outdoor patients while 91 were from indoor patients (Table 2). The outdoor patients were found more sensitive 179(80.3%) to Fosfomycin than indoor patients 69 (75.8%) *p*=0.445). The resistance and sensitivity of bacteria to Fosfomycin are shown in Figure 1.

Table 2: Relationship of Fosfomycin with gender and indoor /outdoor patients.

Fosfomycin	Gender Frequency n (%)		Total Frequency n (%)	*p*-Value	Setting Frequency n (%)		Total Frequency n (%)	*p*-Value
	Female	Male			Outdoor	Indoor		
Resistant	29 (17.0%)	37 (25.9%)	66 (21.0%)	0.035	44 (19.7%)	22 (24.2%)	66 (21%)	0.445
Sensitive	142 (83%)	106 (74.1%)	248 (79%)		179(80.3%)	69 (75.8%)	248 (79%)	
Total	171 (100%)	143(100%)	314(100%)		223(100%)	91 (100%)	314 (100%)	

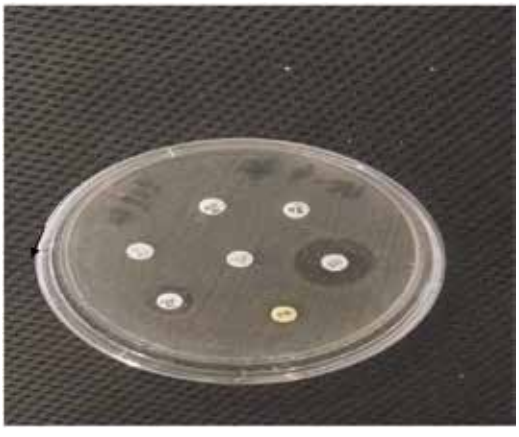

Figure 1: Culture medium showed Fosfomycin resistance.

DISCUSSION

In this study 314 cases of UTI, were studied for sensitivity and resistance pattern of *E. coli* isolates against Fosfomycin and the result showed 21% resistance while 79% were sensitive. Male and outdoor patients outnumbered females in exhibiting symptoms of urinary tract infection and resistance. The growing resistance of pathogens towards multiple antibiotics limiting therapeutic options has created panic among the health care communities[16].

Among different common infections, urinary tract infections are considered the most common infections. Fosfomycin an old drug has very good activity in uncomplicated UTIs. Nitrofurantoin and Fosfomycin are recommended for first-line remedies because of the sensitivity of *E. coli* to both these agents. There is less clinical, epidemiological, or molecular data on *E. coli* resistant to Fosfomycin detected from UTIs. Unfortunately, resistance to this drug is also increasing and reports of resistance from various parts of the world have been reported[17,18].

Fosfomycin is the antibiotic of choice for treating pathogens with resistance to main-line antibiotics (MDR) and extensively drug-resistant (XDR) infections. Therefore, rising resistance towards this drug drastically diminishes accessible therapy options. According to our study, urinary tract infections were more common in females (54.5%) compared to males (45%) which is in association with another study done by Ganesh et al. in 2019 [19]. This fact is

collaborated by another study done by Medina et al. in 2019 as well[20]. This is also detected by a study conducted in Singapore. Their study revealed that urinary tract infection is the most common infection among females, especially from the community[21]. This can be endorsed to the truth that females have a short urethra and decreased level of estrogen. Lower levels of estrogen lead to a reduction of normal vaginal flors[22]. We observed that outdoor patients are more prone to get UTIs than indoor patients, this fact collaborates with another study by Khan et al. 2014[23]. The community-acquired urinary tract is common compared to hospitalized, also following a study in Poland[24]. This is a reflection of illiteracy, low socioeconomic status and poor hygiene. Our study detected 21% Fosfomycin-resistant cases in *E. coli* isolates from urinary samples, this figure is in close association with a study conducted by Michalopoulos et al. in 2022 [25]. Similar results were observed in a study in Israel that urinary tract infections are frequently detected in the community and Fosfomycin resistance is around 23% [26].

Fosfomycin resistance (7.8%) is also reported in a study by Li et al. in 2015, in China., although this antimicrobial agent is not commonly prescribed in China [27] as shown in Table 3. However, delineated Fosfomycin resistance (14.1%) in isolates of *E. coli* in 2020 was found in association with our study[28]. A study was conducted at Rawalpindi and Islamabad, which detected resistance of 3% which was lower than the current study. Therefore, this indicated that it is difficult to use Fosfomycin as empirical therapy[29]. The escalating resistance of Fosfomycin has become a serious public-health trepidation as it limits the alternative antimicrobial agents available for therapy. A study from, Czech Republic reported the very high susceptibility to Fosfomycin trometamol of urinary tract infection pathogens, particularly Gram-negative rods including those producing β-lactamase[30] as shown in Table 3.

Table 3: Fosfomycin resistance and sensitivity rates among different populations.

Population	Resistance (%)	Sensitivity (%)	Studies
Pakistani (Karachi)	21%	79%	Current Study
Israeli	23%	77%	Peretz et al.[26]
Chinses	7.8%	92.2%	Li et al.[27]
Pakistani (Rawalpindi and Islamabad)	3%	97%	Malik et al.[29]
Czech Republic	4.2%	95.8%	Fajfr et al.[30]

The limitations of this study were a single-centered study with a small sample size. Due to time and constriction of finance, the tested strain was only detected on basis of biochemical identification using API 20E. Molecular identification on basis of resistant genes like mcr-1, mrgB, or other mutations was not detected. Hence further studies are required to identify genetic mutations related to Fosfomycin resistance.

CONCLUSION

The study found that Fosfomycin resistance to the most prevalent infection, *E. coli*, is rising in our setup of Pakistan. This highlights the significance of strict infection control measures, strict guidelines on the use of this drug empirically and promoting culture and sensitivity in all the cases of suspected UTIs. These strategies will limit the use of this precious drug and decrease resistance. Furthermore, more studies are required at the multicenter level with a larger sample size to know the exact Fosfomycin resistance.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

ETHICS APPROVAL

This study was approved by the Ethics Review Committee of PNS Shifa Hospital.

PATIENT CONSENT

Written consent was signed by concerned patients.

AUTHORS' CONTRIBUTION

All authors equally contributed to this research write-up.

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