ORIGINAL ARTICLE

Infect Dis Clin Microbiol 2022; 4(4): 252-7

Prevalence of Fosfomycin Resistance Among Enterobacterales Isolates in A Tertiary Care Hospital from Turkey

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ABSTRACT

Objective: Urinary tract infections are one of the most common causes of morbidity around the world. Fosfomycin is a specific broad-spectrum antibiotic used to treat these infections. However, in recent years, many studies have reported increased fosfomycin resistance in Enterobacterales isolates. Therefore, this study aimed to evaluate the distribution of pathogens isolated from urine samples and find the fosfomycin resistance rates over nine years (2012-2020).

Materials and Methods: A total of 18,884 uropathogenic Enterobacterales isolates were included in the study between 2012 and 2020. The isolates were identified by VITEK® 2 Compact (bioMérieux, Marcy l'Etoile, France), and the antimicrobial susceptibilities of the isolates were also evaluated using the VITEK® MS automated system (bioMérieux, Marcy l'Etoile, France).

Results: *Escherichia coli* (64.04%) was the most common bacteria among Enterobacterales. Fosfomycin resistance rates were 1.98%, 21.64%, and 10.36% in E. coli, *Klebsiella pneumoniae*, and all bacteria, respectively. The 34.97% of isolates were extended-spectrum β-lactamase (ESBL)-producing Enterobacterales, and the fosfomycin resistance rate was 13.08% in these isolates. In addition, fosfomycin resistance rates were found as 3.06% and 23.84% in ESBL-producing E. coli and ESBL-producing *K. pneumoniae*, respectively.

Conclusion: Fosfomycin seems a good option for effectively treating UTIs caused by E. coli. On the other hand, we found that fosfomycin resistance tends to increase over the years. Therefore, we recommend further studies to evaluate fosfomycin resistance.

Keywords: fosfomycin resistance, extended-spectrum beta-lactamases producing, Enterobacterales

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Received: May 20, 2022
Accepted: October 14, 2022
Published: December 21, 2022

Suggested citation:
Tanrıverdi-Çaycı Y, Güney DB, Ertokatlı M, Hacıeminoğlu-Ülker K, Birinci A. Prevalence of fosfomycin resistance among Enterobacterales isolates in a tertiary care hospital from Turkey. Infect Dis Clin Microbiol. 2022;4:252-7.

DOI: 10.36519/idcm.2022.163

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INTRODUCTION

Urinary tract infections (UTIs) are seen in all age groups and are among the most frequent bacterial infections. Enterobacterales are the most common bacterial species that cause UTIs (1). *Escherichia coli* is the most common cause of both complicated UTIs (65%) and uncomplicated UTIs (75%). *E. coli* is followed by other Enterobacterales such as *Klebsiella* spp., *Proteus* spp., and *Serratia* spp. (2, 3). Resistance of urinary tract pathogens to antibacterial agents (amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, and ciprofloxacin), widely used for empirical treatment, is gradually increasing worldwide. In European countries, the resistance of *E. coli* to amoxicillin-clavulanic acid, trimethoprim/sulfamethoxazole, and ciprofloxacin has been reported to range from 5.3% to 37.6%, 10.5% to 39.8%, 14.6% to 21.4%, respectively. (4, 5). In addition, some studies have reported that extended-spectrum β-lactamases (ESBL) are increasing among Enterobacterales (6). Current guidelines do not recommend the usage of the antibiotic for empirical therapy if resistance to the antibiotic locally does not exceed 20% (7). Currently, fosfomycin has become more critical in treating UTIs because of increasing resistance to other agents and its limited adverse effects. A single dose of 3 g is recommended for the treatment of uncomplicated UTIs (7, 8).

Fosfomycin is an inhibitor of the MurA enzyme, UDP-N-acetylglucosamine enolpyruvyl transferase; thus, it inhibits the synthesis of peptidoglycan, which is necessary for bacterial cell wall synthesis (9). It has a broad spectrum of activity against most of both Gram-positive and Gram-negative bacteria (10). Because of its unique structure and action mechanism, cross-resistance is uncommon. However, mutations in MurA cause inherent resistance to fosfomycin in some bacteria, such as *Chlamydia* spp., *Mycobacterium tuberculosis*, and *Vibrio fischeri*. Fosfomycin-susceptible bacteria like *E. coli* usually develop resistance with mutations in the uptake systems. The susceptibility of ESBL-producing *E. coli* isolates was reported as 81%-100% and 15% -100% in ESBL-producing *K. pneumoniae* isolates (12). A study conducted in Germany, Belgium, and Spain showed that only <1.5% of *E. coli* were resistant to fosfomycin (13). In another study conducted in Switzerland, fosfomycin resistance among ESBL-producing *E. coli* was reported as 1.4% (14).

This study aims to evaluate the distribution of pathogens isolated from urine samples in our center to find the frequency of resistance to fosfomycin in both inpatients and outpatients and to find alterations in the frequency of fosfomycin resistance over time.

MATERIALS AND METHODS

In this retrospective study, all urinary samples from both outpatients and inpatients sent to the microbiology laboratory of Ondokuz Mayıs University Medical Center from January 2012 to December 2020 were enrolled. In addition, demographic data, such as patient age and gender, and laboratory data were extracted from the hospital records. Only one urinary culture from each patient was included in the study.

Urine samples were routinely inoculated onto 5% sheep blood agar (bioMérieux, Marcy l'Etoile, France) and eosin methylene blue agar (bioMérieux, Marcy l'Etoile, France) and the plates were then incubated for 20-22 hours at 35 °C. The VITEK® MS automated system (bioMérieux, Marcy l'Etoile, France) was used for identification. The antimicrobial susceptibility and ESBL production of the isolates was determined using the VITEK® 2 Compact (bioMérieux, Marcy l'Etoile, France) and evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Guideline specific to that year.

HIGHLIGHTS

- Fosfomycin is a frequently preferred agent, especially in uncomplicated urinary tract infections.
- In recent years, an increase in resistance to fosfomycin has been reported.
- In this study, fosfomycin resistance was high in *K. pneumoniae* isolates.
- Fosfomycin resistance was reported to be higher in ESBL-positive isolates.

The study was approved by Ondokuz Mayıs University Medical Faculty Ethics Committee (B.30.2.ODM.0.20.08/498).

Statistical Analysis

The association between years and fosfomycin resistance rates was analyzed with the ANOVA test, and the association between outpatients/inpatients and fosfomycin resistance rates was analyzed with Pearson's $\chi 2$ test. The statistical significance was set as $p<0.05$.

RESULTS

A total of 18,884 urine cultures were tested between 2012 and 2020; 12,063 (63.88%) cultures were from female patients. The mean age was 48.74 years (2-99). The total rate of fosfomycin-resistant isolates was 2.86% in 2012. The rate increased between 2012-2016; it was 13.47% in 2016. However, it was 9% in 2017 and 14% in 2020; the trend was statistically significant (95% confidence interval [CI], $p<0.05$). The alterations in fosfomycin resistance over the years are shown in Table 1.

E. coli was isolated as the most common urinary pathogen (64.04%). *Klebsiella* spp. was the second most common causative agent over the years (23.32%). In total, the fosfomycin resistance rate in *E. coli* was 1.98%, 21.64% in *K. pneumoniae*, 35.73% in *Enterobacter* spp., and 16.67% in *Proteus* spp. The resistance ratio was 0% in *Salmonella* spp. (7 isolates over 9 years). The frequency and fosfomycin resistance rates of bacterial species are shown in Table 2.

29.31% of total isolates (n=5,534) were from outpatient clinics, and 70.69% (n=13,350) were from inpatient clinics. Fosfomycin resistance was 9.36% from isolates of outpatient clinics (n=518) and 10.78% of isolates from inpatient clinics (n=439). The results were statistically significant (95% CI, $p<0.05$). Fosfomycin resistance rates according to clinics are shown in Table 3.

Total of 18,884 isolates, 17,398 (92.13%) were tested for ESBL with VITEK® 2 Compact (bioMérieux, Marcy l'Etoile, France). Moreover, 34.97% of the total isolates (n=6,604) were ESBL-producing. The fosfomycin resistance rate of ESBL-producing isolates was 13.08%, and fosfomycin-resistant non-ESBL-producing isolates were 7.64%. The fosfomycin resis-

Table 1. Number of isolates and fosfomycin resistance rates over the years.

Year	Total	Fosfomycin resistant
	n (%)	n (%)
2012	1503 (7.96)	43 (2.86)
2013	2031 (10.76)	145 (7.14)
2014	1945 (10.3)	141 (7.25)
2015	2375 (12.57)	298 (12.55)
2016	2309 (12.23)	311 (13.47)
2017	2374 (12.57)	213 (9)
2018	2356 (12.48)	285 (12.10)
2019	2324 (12.3)	287 (12.35)
2020	1667 (8.83)	234 (14)
Total	18,884 (100)	1957 (10.36)

Table 2. Frequency and fosfomycin resistance rates of bacterial species.

Bacteria	Total	Fosfomycin resistance
	n (%)	n (%)
Escherichia coli	12,094 (64.04)	240 (1.98)
Klebsiella spp.	4403 (23.32)	953 (21.64)
Enterobacter spp.	778 (4.12)	278 (35.73)
Proteus spp.	648 (3.43)	108 (16.67)
Providencia rettgeri	280 (1.48)	100 (35.71)
Morgenella spp.	273 (1.45)	236 (86.45)
Citrobacter spp.	197 (1.04)	6 (3.05)
Serratia spp.	179 (0.94)	22 (12.29)
Raoultella ornitholytica	12 (0.06)	7 (58.33)
Salmonella spp.	7 (0.04)	-
Pantoea agglomerans	6 (0.03)	4 (66.67)
Hafnia alvei	6 (0.03)	3 (50)
Escherichia fergussoni	1 (0.01)	-

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tance was 3.06% among ESBL-producing *E. coli* and 1.45% among non-ESBL-producing *E. coli*. The fosfomycin resistance among ESBL-producing *Klebsiella* spp. and non-ESBL-producing *Klebsiella* spp. was 23.84% and 18.93%, respectively. The relationship between ESBL production and the fosfomycin resistance of all bacterial species is shown in Table 4.

DISCUSSION

Recent guidelines recommend fosfomycin for empirical treatment of uncomplicated UTIs (7, 8). However, this situation may lead to an increase in fosfomycin resistance. In addition, fosfomycin may be a choice for multidrug-resistant bacteria such as vancomycin-resistant enterococci (VRE), methicillin-resistant *S. aureus* (MRSA), and ESBL–producing Gram-negative bacilli (7). Therefore, this study investigated the frequency of bacterial species, fosfomycin resistance rates, and the relationship between ESBL-producing and fosfomycin resistance in uropathogenic Enterobacterales isolates.

Our study found the most common urinary pathogen as *E. coli* (64.04%), followed by *Klebsiella* spp. (23.32%) inconsistent with previous studies (2, 3). The majority of the cultures were from females (63.88%), similar to previous studies (15). Our results showed statistically significant increases in fosfomycin antibiotic resistance rates between 2012 and 2020 (from 2.86% to 14%, $p<0.05$). This significant trend may be related to the use of fosfomycin effectively for more than 30 years (16). Our results

Table 3. Fosfomycin susceptibility and resistance in isolates from inpatients and outpatients' clinics.

Clinics	Fosfomycin susceptible	Fosfomycin resistant
	n (%)	n (%)
Outpatients	5016 (90.64)	518 (9.36)
Inpatients	11,911 (89.22)	1439 (10.78)
Total	16,927 (89.64)	1957 (10.36)

Table 4. The associations between ESBL and fosfomycin resistance among all bacterial species.

Bacteria species	ESBL-Producing isolates		Non-ESBL-Producing isolates	
	Total	Fosfomycin resistant	Total	Fosfomycin resistant
		n (%)		n (%)
Escherichia coli	3849	118 (3.06)	7572	110 (1.45)
Klebsiella spp.	2156	514 (23.84)	2049	388 (18.93)
Enterobacter spp.	201	72 (35.82)	389	122 (31.36)
Proteus spp.	60	24 (40)	389	56 (14.39)
Providencia rettgeri	215	71 (33.02)	30	12 (40)
Morgenella spp.	59	56 (94.91)	134	116 (86.56)
Citrobacter spp.	41	2 (4.87)	104	2 (1.92)
Serratia spp.	19	5 (26.31)	107	12 (11.21)
Raoultella ornitholytica	2	1 (50)	7	3 (42.85)
Salmonella spp.	-	-	6	-
Pantoea agglomerans	-	-	4	3 (75)
Hafnia alvei	1	1 (100)	3	1 (33.33)
Escherichia fergussoni	-	-	1	-
Total	**6603**	**864 (13.08)**	**10,795**	**825 (7.64)**

are also consistent with a study that found an increase in fosfomycin resistance in ESBL-producing *E. coli* from 4.4% in 2005 to 11.4% in 2009 (17).

We found the fosfomycin resistance of *E. coli* as 1.98%, similar to a previous study from Turkey, which found 2% (18). In our study, fosfomycin resistance of *Morganella* spp. was found to be 86.45%. This result was lower than previous studies from Turkey and Iran, which both found 100% (18, 19); the cause may be the relatively low sample number of previous studies. The high sample size in our study allowed a more optimal assessment of fosfomycin resistance to rare bacteria. Fosfomycin resistance of *Klebsiella* spp. was found at 21.64%. This result is markedly higher than a study from Turkey, which found 6.9%, but in contrast, lower than a study from Switzerland, which found 40.6% in 2009 and 23.2% in 2016 (18, 20). Our study's high rates of resistance may result from the fact that most patients were inpatients (70.69%).

In our study, a statistically significant difference in fosfomycin resistance between inpatients and outpatients was observed, which were 10.78% and 9.36%, respectively ($p<0.05$). The fact that fosfomycin resistance is significantly higher in inpatients is an expected result since these patients have more resistant bacteria and nosocomial infections than outpatients. Furthermore, these findings are consistent with a study that compares inpatient and outpatient fosfomycin resistance of *E. coli*, *K. pneumoniae*, and *Proteus mirabilis* (20).

In Greece, India, and Turkey, fosfomycin resistance among ESBL-producing Enterobacterales was reported at 1.9%, 8%, and 8.8%, respectively (18, 21, 22). In our study, fosfomycin resistance among ESBL-producing Enterobacterales isolates was higher (13.08%) than the results of the studies above. Also, a statistically significant difference in fosfomycin resistance between ESBL-producing and non-ESBL-producing isolates ($p<0.05$) was determined, especially in ESBL-producing *Klebsiella* spp. (23.84%) and ESBL-producing *Enterobacter* spp. (35.82%) compared to ESBL-producing *E. coli* (3.06%). Similar to our results, a study from Switzerland showed that 17 of 1225 (1.38%) ESBL-producing *E. coli* were resistant to fosfomycin (14). This may result from the fact that non-*E. coli* Enterobacterales, especially *Klebsiella* spp., tend to be higher resistant to fosfomycin (21). On the other hand, a study from Turkey showed that ESBL-producing *Klebsiella* spp. (73.3%) had a lower fosfomycin susceptibility rate than ESBL-producing *E. coli* (94.1%) (18).

Our study has some limitations. First, since it is single-centered, it is insufficient to represent the whole country. Furthermore, it is retrospective, and some data, such as the clinical status of the patients, prior antibiotic use, risk factors for infection with resistant bacteria, and whether the patients had asymptomatic bacteriuria, uncomplicated UTIs, or complicated UTIs, were not available and not included in the study.

In conclusion, fosfomycin seems a good option for effectively treating UTIs caused by *E. coli*. On the other hand, we found that fosfomycin resistance tends to increase over the years. Therefore, these findings should be considered, and further studies should be conducted to evaluate fosfomycin resistance.

Ethical Approval: The Clinical Research Ethics Committee of Ondokuz Mayıs University approved the study on August 02, 2022 with the decision number of OMU KAEK 2022/355.

Informed Consent: N/A

Peer-review: Externally peer-reviewed

Author Contributions: Concept – Y.T.Ç.; Design – Y.T.Ç.; Supervision – YTC, K.H.Ü, A.B.; Fundings – Y.T.Ç.; Materials – Y.T.Ç., A.B.; Data Collection and/or Processing – D.B.G., M.E., K.H.Ü; Analysis and/or Interpretation – Y.T.Ç., K.H.Ü; Literature Review – D.B.G., M.E., K.H.Ü, Y.T.Ç.; Writer – DBG, M.E.; Critical Reviews – Y.T.Ç., K.H.Ü, A.B.

Conflict of Interest: The authors declare no conflict of interest.

Financial Disclosure: The authors declared that this study has received no financial support.

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