

Research Article

The Detection of Fosfomycin-Modifying Enzymes (fos) in Uropathogenic Enterobacterale, Azerbaijan, Iran

Aidin Lalezadeh ¹, Pardis Ghotaslou ², and Reza Ghotaslou ³

¹Department of Microbiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

²Students' Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

³Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Correspondence should be addressed to Reza Ghotaslou; rzgottaslo@yahoo.com

Received 9 July 2022; Revised 4 April 2023; Accepted 11 May 2023; Published 19 May 2023

Academic Editor: Divakar Sharma

Copyright © 2023 Aidin Lalezadeh et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Enterobacteriaceae is the most common agent of urinary tract infections (UTIs). Multidrug resistant (MDR) and XDR (extensively drug-resistant) *Enterobacteriaceae* in UTIs have increased in the world. The present study aimed to study the fosfomycin resistance frequency and the fosfomycin resistance genes among *Enterobacteriaceae* isolated from UTIs. The urine was collected and cultured in the standard protocol. To determine the susceptibility testing to fosfomycin in 211 isolates, agar dilution and disk agar diffusion methods were used. MDR was nonsusceptibility to at least one agent in three or more antimicrobial categories. The fosfomycin resistance genes were also evaluated by PCR. The frequency of resistance to fosfomycin was in 14 (6.6%) and 15 (7.1%) isolates by the disk agar diffusion and MIC assays, respectively. However, the MIC₅₀ and MIC₉₀ existed at 8 µg/mL and 16 µg/mL, respectively. The MDR was found in 80%. The frequencies of fosfomycin resistance genes were 5 (33.3%), 3 (20%), 2 (13.3%), 1 (6.6%), and 1 (6.6%) for *fosC*, *fosX*, *fosA3*, *fosA*, and *fosB2*, respectively. The *fosB* and *fosC2* were not found. A low resistance rate to fosfomycin is observed. Fosfomycin is still one of the most effective and valuable alternative antibiotics against MDR *Enterobacteriaceae* isolated from UTIs in our region.

1. Introduction

The most common cause of urinary tract infections (UTIs) is *Enterobacteriaceae*. Recently, the occurrence of resistance to antimicrobial agents among *Enterobacteriaceae* and multidrug resistant (MDR) and extensively drug-resistant (XDR) isolates have become a worldwide concern. The increased resistance rate to antibiotics has directed to a decrease in the successful management of UTIs due to *Enterobacteriaceae* [1]. A few of the causes of the increase in MDR or XDR pathogens are empirical antibiotic prescription without urine culture and susceptibility testing and ineffective UTI treatment as well as the development of persistent or recurrent infections [2]. *Enterobacteriaceae* as heterogeneous groups cause numerous infections mainly UTIs, bacteremia, hospital infections, and gastroenteritis [3, 4]. UTIs are considered one of the most common bacterial infections

seen in primary health care and are very common especially in females [5]. In the United States, UTIs are considered a huge economic problem that costs about \$6 billion per year [6].

During the past decade, the introduction of novel antimicrobial agents has been restricted and physicians sometimes have to use some old antimicrobial agents that have come out of the clinical practice. Nevertheless, fosfomycin rests on an actual antimicrobial agent against the UTIs caused by *Enterobacteriaceae* [7, 8]. Fosfomycin is a bactericidal antibiotic that inhibits the production of bacterial peptidoglycan (inhibits UDP-N-acetylglucosamineolpyruvyl transferase) [9]. Fosfomycin has an exceptional mechanism [10] and is effective on Gram-negative and Gram-positive bacteria [11]. The use of fosfomycin is approved in uncomplicated UTIs by the FDA [12]. There are three forms of fosfomycin including

tromethamine as a soluble salt, calcium for oral use in UTI treatment, and disodium for intravenous usage [13]. A previous study stated high susceptibility to fosfomycin in MDR and XDR Enterobacteriaceae [1]; on the other hand, Oteo et. al reported resistance to fosfomycin among Enterobacteriaceae isolates [14]. Resistance to fosfomycin has often been prompted by plasmid genes, including fosfactors [15]. In the present study, the resistance frequency of Enterobacteriaceae to fosfomycin and the fosfomycin resistance genes were studied.

2. Materials and Methods

2.1. Bacterial Isolates. Two hundred and eleven Enterobacteriaceae isolated from UTIs were collected from hospitalized patients from June to December 2021 in Azerbaijan, Iran. The samples were collected from patients who had signs and symptoms suggestive of UTIs as decided by the physician. Patients were recommended to collect midstream urine samples into a sterile wide-mouth container with all aseptic measures. Samples from catheterized patients were occupied with aseptic measures. The collected urine was tested within an hour of sampling. The urine samples were cultured by a semiquantitative method on blood agar and MacConkey agar (Merck, Germany) and incubated at 37°C for 24 hours. Only those samples which grew significant bacteriuria were included in the study. A sample was considered positive for UTIs if a single organism was cultured at a concentration of 10^5 cfu/mL. Finally, bacterial identification was performed based on Gram staining, culture, and standard biochemical tests such as TSI, H₂S, citrate, indole, urea, lysine, SIM, MR-VP, and gas production [16, 17]. All fungal pathogens other than Enterobacteriaceae isolates were excluded. The patients have taken antibiotics within the last two weeks before this study, the patients who did not give their consent for participation, women who were in their menstrual period, and those people who did not have the conditions to enter the study were excluded. The current study was approved by the Ethics Committee of Tabriz University of Medical Sciences (Ir.Tbzmed.Rec.1397.579).

2.2. The Susceptibility Testing. The susceptibility testing was done by the disk agar diffusion method concerning the Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. The antibiotic disks were trimethoprim/sulfamethoxazole, nitrofurantoin, fosfomycin, amoxicillin-clavulanic acid, imipenem, cefixime, ampicillin, cefazolin, amikacin, gentamicin, nalidixic acid, ciprofloxacin, and levofloxacin (Mast, UK). The fosfomycin disk (200 µg) enclosed 50 µg (glucose-6-phosphate). The MICs (minimum inhibitory concentrations) of fosfomycin were carried out via the agar dilution assay supplemented with 25 µg/mL of glucose-6-phosphate. The analysis of MIC results was prepared as stated by the CLSI guiding principles [18]. The agar dilution method (Mueller–Hinton Broth) was used to measure MIC under defined test conditions; MIC is defined as the lowermost concentration of an antibacterial agent that

inhibits the growth of bacteria. The method described in this research was planned for the testing of pure cultures of bacteria that are easily grown by overnight incubation. The antimicrobial powders (Merck, Germany) were prepared by the manufacturer. The MIC of fosfomycin was done by supplementation with 25 mg/L glucose-6-phosphate. To control the susceptibility testing, we used *E. coli* ATCC 25922. Multiple drug resistance (MDR) is resistance to at least one antibiotic in three or more antimicrobial classes (1). XDR was defined as no susceptibility to at least one agent in wholly but two or fewer antimicrobial groups (i.e., bacterial isolates persist susceptible to only one or two antimicrobial categories) (8).

2.3. The PCR. The DNA extraction of isolates was carried out by the boiling method [19]. To extract the DNA, samples were incubated at 99°C for 15 min, with adding 0.5% Tween 20, and immediately cooled on ice. The fos genes were detected by PCR. PCR assays were achieved on a 96-well Thermal Cycler (Eppendorf, UK). PCR was carried out in the reactions with a last volume of 20 µL including 5 µL of Master Mix IX (SinaClon Co., Iran) having Taq DNA polymerase, MgCl₂, and dNTPs. Primers and conditions were used as previously described by Bi et al. [20]. Resistance to fosfomycin has been frequently encouraged by plasmid genes, including fos factors [14]. PCR products were examined via electrophoresis on a 1% agarose gel [21]. The gels were marked with the DNA-safe stain and were imaged underneath ultraviolet light; the size of the products was measured by judgment with a 100 bp molecular size marker (SinaClon Co., Iran). In the current study, 22 randomly designated fosfomycin-sensitive isolates were used as controls. Furthermore, each of the positive-fosfomycin resistance gene strains in our laboratory was used as a positive control. One negative control was also involved in each PCR run, where DNA was substituted by an equal volume of sterilized water.

2.4. Statistical Analysis. The data was analyzed using descriptive-analytic assays in SPSS software version 22 (Washington, the USA). Qualitative data were assessed by using Chi-square and Fisher's test and *P* values ≤ 0.05 were considered statistically significant.

3. Results

In the present study, 211 nonduplicates, Enterobacteriaceae, were isolated from 67 males (31.8%) and 142 females (68.2%). The mean age of patients was 41 ± 20 years. The isolates were collected from the various hospital wards. In the present study, 158 (74.9%) of isolates were *Escherichia coli* (*E. coli*), followed by *Klebsiella pneumoniae* (*K. pneumoniae*) 39 (18.5%), *Enterobacter agglomerans* 4 (1.9%), *Enterobacter cloacae* 2 (0.9%), *Klebsiella oxytoca*, 2 (0.9%), *Proteus vulgaris* 2 (0.9%), *Morganellamorganii* 2 (0.9%), and *Proteus mirabilis* 2 (0.9%).

The highest rate of resistance was detected to ampicillin (95.1%), followed by cotrimoxazole (77.3%), nitrofurantoin

TABLE 1: The resistance rate of bacterial species to antimicrobial agents.

Bacteria	Fosfomycin (%)	Gentamicin (%)	Amikacin (%)	Ciprofloxacin (%)	Ampicillin (%)	Cotrimoxazol (%)	Nalidixic acid (%)	Tetracycline (%)	Cefazolin (%)	Imipenem (%)
<i>Escherichia coli</i>	3.1	46.2	15.9	57.6	100	76.3	73.7	67.5	45.2	2.2
<i>Klebsiella pneumoniae</i>	17.9	40	20.6	40	100	85.3	55.6	57.9	44.7	27.6
<i>Enterobacter agglomerance</i>	12	50	25	66.7	99	75	75	50	100	33.3
<i>Enterobacter cloacae</i>	0	50	0.0	50	100	50	75	55	0.0	0.0
<i>Klebsiella oxytoca</i>	0.0	100	50	50	98	50	50	0.0	0.0	0.0
<i>Morganellamorganii</i>	50	0.0	0.0	0.0	0.0	0	90	65	0.0	0.0
<i>Proteus vulgaris</i>	0.0	90	0.0	0.0	96	100	100	65	0.0	0.0
<i>Proteus mirabilis</i>	0.0	100	0.0	0.0	100	100	90	70	0.0	100

TABLE 2: The range of fosfomycin MIC in different *Enterobacteriaceae*.

Bacteria	MIC8, (n)	MIC16, (n)	MIC32, (n)	MIC64, (n)	MIC128, (n)	MIC256, (n)	MIC1024, (n)
<i>Escherichia coli</i> , 158 isolates	100	23	23	5	2	0	2
<i>Klebsiella pneumoniae</i> , 39 isolates	22	2	5	2	1	1	2
<i>Enterobacter agglomerance</i> , 4 isolates	3	0	1	0	0	0	0
<i>Enterobacter cloacae</i> , 2 isolates	1	1	0	0	0	0	0
<i>Klebsiella oxytoca</i> , 2 isolates	1	0	1	0	0	0	0
<i>Morganell amorganii</i> , 2 isolates	0	2	0	0	0	0	0
<i>Proteus vulgaris</i> , 2 isolates	1	0	1	0	0	0	0
<i>Proteus mirabilis</i> , 2 isolate	0	0	1	0	0	0	0

n, number.

(75%), nalidixic acid (70%), amoxicillin-clavulanic acid (70%), cefixime (65%), cefazolin (60%), ciprofloxacin (50%), levofloxacin (44%), gentamicin (43.5%), amikacin (17.3%), imipenem (9.2%), and fosfomycin (6.6%) (Table 1). The MDR was found in 80%. The frequency of resistance to fosfomycin was 14 (6.6%) by the disk agar diffusion assay. The resistance rate to fosfomycin was found in 15 isolates (7.1%) by MIC (9 *E. coli* and 6 *K. pneumoniae* isolates). The MIC range was 1–256 µg/mL and the MIC₅₀ and MIC₉₀ were 8 µg/mL and 16 µg/mL, respectively (Table 2). The resistance to fosfomycin among MDR isolates was higher than non-MDR, 10% and 6%, respectively ($P \leq 0.05$). The rate of fosfomycin resistance genes in fosfomycin-resistant isolates was 5 (33.3%), 3 (20%), 2 (13.3%), 1 (6.6%), and 1 (6.6%) for fosC, fosX, fosA3, fosA, and fosB2, respectively. The fosB and fosC2 were not identified (Table 3 and Figure 1).

4. Discussion

Enterobacteriaceae is the most common causative agent of UTIs, and the rate of antibiotic resistance and MDR has increased [22]. In the current study, the MDR was high among Enterobacteriaceae isolated from UTIs. Fosfomycin is one of the most effective and useful drugs in the treatment of infections due to MDR Enterobacteriaceae.

Fosfomycin has widely distributed in many human organs, chiefly the urinary tract [23]. In the current research, the overall resistance rate to fosfomycin among Enterobacteriaceae was 7.1% by MICs. Falagas et al. in Greece showed that 8.2% of MDR Enterobacteriaceae isolates were resistant to fosfomycin [1]. These similar findings are also reported in different countries and Iran [8, 10, 23–26]. In the present study, the most fosfomycin-resistant isolates belonged to *K. pneumoniae* (23%) and *E. coli* (6.9%). The difference in the rate of resistance to fosfomycin may be due to the amount of antibiotic usage in the different are as depending on a diverse policy. However, one of the reasons for the increase in drug resistance is an inadequate empirical antibiotic prescription without urine culture and susceptibility testing. The use of prophylactic antimicrobial therapy is also considered the main risk factor in the development of antibiotic resistance.

In the current study, the highest resistance rate is observed to ampicillin (95.1) followed by trimethoprim/sulfamethoxazole (77.3). A high resistance rate to antimicrobial agents has already been described [27–29]. The great usage

TABLE 3: Different fos genes per species in this study.

Bacteria	fosC	fosX	fosA3	fosA	fosB2
<i>Escherichia coli</i>	3	2	2	0	0
<i>Klebsiella pneumoniae</i>	2	1	0	1	1
<i>Enterobacter agglomerance</i>	0	0	0	0	0
<i>Enterobacter cloacae</i>	0	0	0	0	0
<i>Klebsiella oxytoca</i>	0	0	0	0	0
<i>Morganell amorganii</i>	0	0	0	0	0
<i>Proteus vulgaris</i>	0	0	0	0	0
<i>Proteus mirabilis</i>	0	0	0	0	0

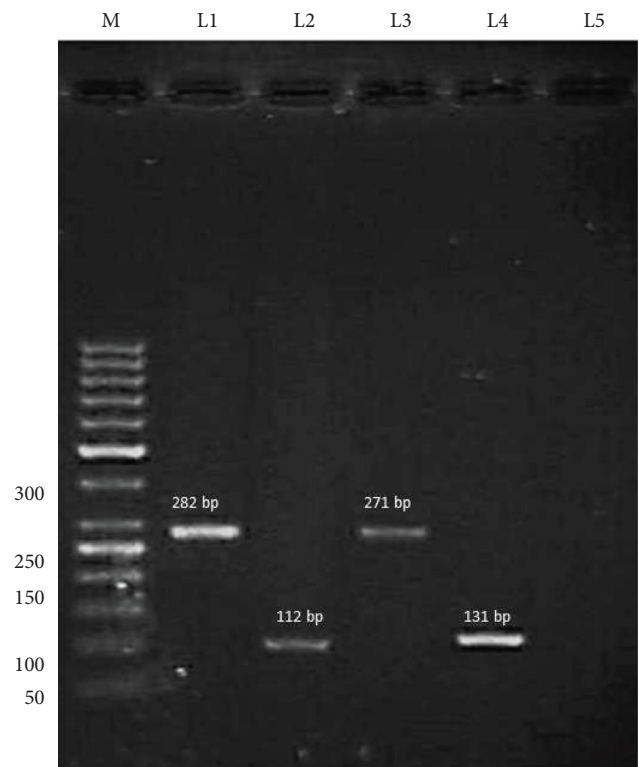


FIGURE 1: The gel electrophoresis of fosfomycin resistance genes in 1% agarose gel. Lane M DNA size marker (50 bp); L1: fosA3 gene (282 bp), L2: fosC gene (112 bp), L3: fosA gene (271 bp), L4: fosX gene (131 bp), and L5: negative.

of antimicrobial agents in experimental practice leads to resistance of bacteria to antibiotics. Bacteria usually have two resistance mechanisms to antibiotics named innate and

acquired. Acquired resistance gets up from chromosomal mutations, but resistance to antimicrobial agents is usually related to gene transfer (plasmids, transposons, and integrons or a combination of these mechanisms). Efflux pumps are also accepted as the chief cause of the MDR mechanism in bacteria. Enterobacteriaceae species are opportunistic pathogens in hospitals, and due to Enterobacteriaceae resistance to antibiotics, they are described as one of the leading reasons for resistant nosocomial infection [28].

The *fosA3* gene was distinguished in 9% of isolates in China, whereas *fosC2* and *fosA* genes were not found [30]. The *fosA3* gene was also reported as the most common resistance gene among *E. coli* isolates in Hong Kong and China [30, 31]. Villa et al. found that *fosA3* is a plasmid-mediated gene based on sequencing data, which is common in Asia and rare in European countries [32]. Zaniani et al. from Iran reported that the *fosC2* and *fosA3* genes were not detected in *E. coli* isolates [33]. However, there are several reports of fosfomycin resistance genes in Iran [28, 34–37]. In the current study, the rate of the *fosA3* (0.9%) is not consistent with mentioned studies. The main fosfomycin resistance genes in the current study were *fosC* and *fosX*. The results of this study identified *fosX* gene as an important issue because this gene has been reported rarely in previous investigations.

The mobility of these genes (encoded on a conjugated plasmid, transposons, or within integrons) may accelerate the dissemination of fosfomycin resistance around our region. Mobile fosfomycin-resistant genes have also been distinguished in human, animal, food, and environmental source which initiate a growing worry regarding the risk of the spread of such bacteria, especially *E. coli*, at the human-animal-environment crossing point. The reason for the presence of *fosC* and *fosX* genes in the present study isolates as compared to their absence or low rate in previous studies may be due to the hospital setting, patient's population, geographic variation, sample size, the mechanism of antimicrobial resistance, kind of bacteria, and source of infections.

Today, it is suggested to use fosfomycin for the management of UTIs, particularly in severe UTIs in women and MDR Enterobacteriaceae [12, 38, 39]. Fosfomycin is an intensely effective drug against the uropathogenic MDR Enterobacteriaceae [40, 41]. We must be aware that due to the wide spreading of resistant *Enterobacteriaceae* isolates to antibiotics in different places (animals, humans, food, and environment), as well as fosfomycin usage in veterinarians, *Enterobacteriaceae* is readily transferred to new places and other bacteria [30, 42]. Furthermore, considering the determinant locations of mobile genetic elements (for example, plasmids), even the minor recognized resistance cases are important. So, attributable to a high chance of resistance agents spreading, the prevention is chief strategy. One of the most main options for the prevention of resistance spreading to antibiotics may be combination therapy. The significant increase in MDR uropathogenic *Enterobacteriaceae* is considered a big challenge in the treatment of UTIs. On the other hand, high fosfomycin susceptibility among MDR isolates observed in the current study gives hope in using this

drug, rather than using other nephrotoxic drugs such as colistin. Owing to the unique mechanism of fosfomycin action, low incidence of resistance, oral availability with single-dose administration, and less propensity to display cross-resistance to other antibiotics, fosfomycin is a good choice for the treatment of UTIs. It is still possible to prescribe fosfomycin in this area, but monitoring is necessary to understand the evolution of the dispersal of fos genes or other resistance mechanisms using advanced techniques such as Next Generation Sequencing.

To validate the results of the current study, it recommends doing a larger study targeting geographically and in diverse isolates. Our study also showed that disc diffusion agar is a good assay for fosfomycin susceptibility testing. Continuous monitoring of fosfomycin susceptibility is recommended to keep a check on any increase in resistance pattern, prevent its spread, and further aid in its clinical application. Despite our promising results, this study had some limitations such as the lack of examining resistance genes other than fos genes, the plasmid content, and the genetic background of the fos genes.

5. Conclusion

The MDR is worrying among *Enterobacteriaceae* isolated from UTIs, whereas the fosfomycin resistance rate is still low. The most common fosfomycin resistance genes belonging to *K. pneumoniae* and *E. coli* were *fosC*, *fosX*, *fosA3*, *fosA*, and *fosB2*.

Data Availability

The data that support the findings of this study are available within the article.

Ethical Approval

The current study was settled by the Ethics Committee of Tabriz University of Medical Sciences (Ir.Tbzmed.Rec.1397.579).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The present project was supported by the Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, under Grant no. 579.

References

- [1] M. E. Falagas, S. Maraki, D. E. Karageorgopoulos, A. C. Kastoris, E. Mavromanolakis, and G. Samonis, "Antimicrobial susceptibility of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Enterobacteriaceae isolates to fosfomycin," *International Journal of Antimicrobial Agents*, vol. 35, no. 3, pp. 240–243, 2010.
- [2] W. Adamus-Białek, A. Baraniak, M. Wawszczak et al., "The genetic background of antibiotic resistance among clinical

- uropathogenic *Escherichia coli* strains,” *Molecular Biology Reports*, vol. 45, no. 5, pp. 1055–1065, 2018.
- [3] E. Sheykhsaran, H. Bannazadeh Baghi, M. H. Soroush Barhaghi et al., “The rate of resistance to tetracyclines and distribution of tetA, tetB, tetC, tetD, tetE, tetG, tetJ and tetY genes in Enterobacteriaceae isolated from Azerbaijan, Iran during 2017,” *Physiology and Pharmacology*, vol. 22, no. 3, pp. 205–212, 2018.
 - [4] R. Ghotaslou, F. Yeganeh Sefidan, M. T. Akhi, M. Asgharzadeh, and Y. Mohammadzadeh Asl, “Dissemination of genes encoding aminoglycoside-modifying enzymes and *armA* among *Enterobacteriaceae* isolates in northwest Iran,” *Microbial Drug Resistance*, vol. 23, no. 7, pp. 826–832, 2017.
 - [5] S. Farajnia, M. Y. Alikhani, R. Ghotaslou, B. Naghili, and A. Nakhband, “Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran,” *International Journal of Infectious Diseases*, vol. 13, no. 2, pp. 140–144, 2009.
 - [6] R. Mann, D. G. Mediati, I. G. Duggin, E. J. Harry, and A. L. Bottomley, “Metabolic adaptations of uropathogenic *E. coli* in the urinary tract,” *Frontiers in Cellular and Infection Microbiology*, vol. 7, p. 241, 2017.
 - [7] S. Liu, N. Huang, C. Zhou et al., “Molecular mechanisms and epidemiology of carbapenem-resistant *Enterobacter cloacae* complex isolated from Chinese patients during 2004–2018,” *Infection and Drug Resistance*, vol. 14, pp. 3647–3658, 2021.
 - [8] C. Beuk, C. Hill, S. Whitehead, E. Blondel-Hill, K. Wagner, and N. Cheeptham, “Determination of susceptibility to fosfomycin and tigecycline of Enterobacteriaceae, particularly *Escherichia coli* isolates, producing extended-spectrum β -lactamases from multiple regional Canadian hospitals,” *The Canadian Journal of Infectious Diseases and Medical Microbiology*, vol. 24, no. 3, pp. e80–e82, 2013.
 - [9] M. E. Falagas, K. P. Giannopoulou, G. N. Kokolakis, and P. I. Rafailidis, “Fosfomycin: use beyond urinary tract and gastrointestinal infections,” *Clinical Infectious Diseases*, vol. 46, no. 7, pp. 1069–1077, 2008.
 - [10] S. Y. Lee, Y. J. Park, J. K. Yu et al., “Prevalence of acquired fosfomycin resistance among extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates in Korea and IS 26-composite transposon surrounding *fosA3*,” *Journal of Antimicrobial Chemotherapy*, vol. 67, no. 12, pp. 2843–2847, 2012.
 - [11] M. Woodward, G. W. Coombs, S. Mowlaboccus, and D. Daley, “Fosfomycin susceptibility of *Escherichia coli* urinary tract infection isolates from Australia,” *Australasian Journal on Ageing*, vol. 40, no. S1, p. 64, 2021.
 - [12] U.S. Food and Drug Administration, “Drugs@FDA: FDA-approved drugs,” 2021, <https://www.accessdata.fda.gov/scripts/cder/daf/>.
 - [13] A. S. Michalopoulos, I. G. Livaditis, and V. Gougoutas, “The revival of fosfomycin,” *International Journal of Infectious Diseases*, vol. 15, no. 11, pp. e732–e739, 2011.
 - [14] J. Oteo, V. Bautista, N. Lara et al., “Parallel increase in community use of fosfomycin and resistance to fosfomycin in extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli*,” *Journal of Antimicrobial Chemotherapy*, vol. 65, no. 11, pp. 2459–2463, 2010.
 - [15] J. L. Cottell and M. A. Webber, “Prevalence, mechanisms and comparison of detection methods of fosfomycin resistance in *E. coli* from urinary tract infections,” *BioRxiv*, vol. 14, Article ID 234435, 2017.
 - [16] S. Indrajith, A. K. Mukhopadhyay, G. Chowdhury et al., “Molecular insights of Carbapenem resistance *Klebsiella pneumoniae* isolates with focus on multidrug resistance from clinical samples,” *Journal of Infection and Public Health*, vol. 14, no. 1, pp. 131–138, 2021.
 - [17] A. Rohman, F. Ijong, and I. Suwetja, “Viability of *Edwardsiella tarda* and *Escherichia coli* preserved with glycerol-tryptone soy broth (TSB) kept at freezing temperature,” *AQUATIC SCIENCE and MANAGEMENT*, vol. 1, no. 2, pp. 154–159, 2013.
 - [18] M. P. Weinstein, *Performance Standards for Antimicrobial Susceptibility Testing*, Clinical and Laboratory Standards Institute, Pittsburgh, PA, USA, 2021.
 - [19] K. Jangpatarapongsa, K. Saimuang, D. Polpanich et al., “Increased sensitivity of enterotoxigenic *Escherichia coli* detection in stool samples using oligonucleotide immobilized-magnetic nanoparticles,” *Biotechnology Reports*, vol. 32, Article ID e00677, 2021.
 - [20] W. Bi, B. Li, J. Song et al., “Antimicrobial susceptibility and mechanisms of fosfomycin resistance in extended-spectrum β -lactamase-producing *Escherichia coli* strains from urinary tract infections in Wenzhou, China,” *International Journal of Antimicrobial Agents*, vol. 50, no. 1, pp. 29–34, 2017.
 - [21] Â. Costa, A. O. Santos, J. Sousa et al., “Improved method for the extraction of high-quality DNA from lignocellulosic compost samples for metagenomic studies,” *Applied Microbiology and Biotechnology*, vol. 105, no. 23, pp. 8881–8893, 2021.
 - [22] D. T. Davies and M. Everett, “Designing inhibitors of β -lactamase enzymes to overcome carbapenem resistance in Gram-negative bacteria,” *Accounts of Chemical Research*, vol. 54, no. 9, pp. 2055–2064, 2021.
 - [23] J. C. Ontong, N. F. Ozioma, S. P. Voravuthikunchai, and S. Chusri, “Synergistic antibacterial effects of colistin in combination with aminoglycoside, carbapenems, cephalosporins, fluoroquinolones, tetracyclines, fosfomycin, and piperacillin on multidrug resistant *Klebsiella pneumoniae* isolates,” *PLoS One*, vol. 16, no. 1, Article ID e0244673, 2021.
 - [24] J. T. Seroy, S. A. Grim, G. E. Reid, T. Wellington, and N. M. Clark, “Treatment of MDR urinary tract infections with oral fosfomycin: a retrospective analysis,” *Journal of Antimicrobial Chemotherapy*, vol. 71, no. 9, pp. 2563–2568, 2016.
 - [25] T. R. Keepers, M. Gomez, C. Celeri, K. M. Krause, D. Biek, and I. Critchley, “Fosfomycin and comparator activity against select Enterobacteriaceae, Pseudomonas, and Enterococcus urinary tract infection isolates from the United States in 2012,” *Infectious diseases and therapy*, vol. 6, no. 2, pp. 233–243, 2017.
 - [26] S. Malekpour Kolbadezhad and L. Fozouni, “Molecular monitoring of fosfomycin resistance in *Escherichia coli* strains isolated from patients with urinary catheters in north-east of Iran,” *Journal of Medical Microbiology and Infectious Diseases*, vol. 6, no. 4, pp. 112–117, 2018.
 - [27] L. Huang, C. Huang, Y. Yan, L. Sun, and H. Li, “Urinary tract infection etiological profiles and antibiotic resistance patterns varied among different age categories: a retrospective study from a tertiary general hospital during a 12-year period,” *Frontiers in Microbiology*, vol. 12, Article ID 813145, 2021.
 - [28] M. R. Sadeghi, R. Ghotaslou, M. T. Akhi, M. Asgharzadeh, and A. Hasani, “Molecular characterization of extended-spectrum β -lactamase, plasmid-mediated AmpC cephalosporinase and carbapenemase genes among Enterobacteriaceae isolates in five medical centres of East and West Azerbaijan, Iran,” *Journal of Medical Microbiology*, vol. 65, no. 11, pp. 1322–1331, 2016.

- [29] A. Taherpour and A. Hashemi, "Detection of OqxAB efflux pumps, OmpK35 and OmpK36 porins in extended-spectrum- β -lactamase-producing *Klebsiella pneumoniae* isolates from Iran," *Hippokratia*, vol. 17, no. 4, pp. 355–358, 2013.
- [30] J. Hou, X. Huang, Y. Deng et al., "Dissemination of fosfomycin resistance gene fosA3 with CTX-M β -lactamase genes and rmtB carried on IncFII plasmids among *Escherichia coli* isolates from pets in China," *Antimicrobial Agents and Chemotherapy*, pp. 05104–05111, 2012.
- [31] P. L. Ho, J. Chan, W. U. Lo et al., "Prevalence and molecular epidemiology of plasmid-mediated fosfomycin resistance genes among blood and urinary *Escherichia coli* isolates," *Journal of Medical Microbiology*, vol. 62, no. 11, pp. 1707–1713, 2013.
- [32] L. Villa, B. Guerra, S. Schmogger et al., "IncA/C plasmid carrying bla_{NDM-1}, bla_{CMY-16}, and fosA3 in *Salmonella enterica* Corvallis from a migratory wild bird in Germany," *Antimicrobial Agents and Chemotherapy*, pp. 00944–00915, 2015.
- [33] F. R. Zaniani, M. Savari, E. A. Montazeri, R. M. Nejad, and S. Khoshnood, "Distribution of fosfomycin and AmpC β -lactamase resistance genes in urinary *Escherichia coli* isolates obtained from patients admitted to an educational hospital in Ahvaz, southwest Iran," *Gene Reports*, vol. 17, Article ID 100533, 2019.
- [34] S. H. Jahromy, M. Rahbar, A. Z. Bialvaei et al., "Prevalence of fosfomycin resistance genes and antimicrobial susceptibility of clinical urinary extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates," *Reviews in Medical Microbiology*, vol. 31, no. 2, pp. 86–91, 2020.
- [35] A. Ghasemian, A. M. Mobarez, S. N. Peerayeh, A. T. B. Abadi, S. Khodaparast, and F. Nojoomi, "Report of plasmid-mediated colistin resistance in *Klebsiella oxytoca* from Iran," *Reviews in Medical Microbiology*, vol. 29, no. 2, pp. 59–63, 2018.
- [36] M. Kashefieh, H. Hosainzadegan, S. Baghbanijavid, and R. Ghotaslou, "The molecular epidemiology of resistance to antibiotics among *Klebsiella pneumoniae* isolates in Azerbaijan, Iran," *The Journal of Tropical Medicine*, vol. 2021, Article ID 9195184, 9 pages, 2021.
- [37] J. Y. Sharahi, A. Hashemi, A. Ardebili, and S. Davoudabadi, "Molecular characteristics of antibiotic-resistant *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from hospitalized patients in Tehran, Iran," *Annals of Clinical Microbiology and Antimicrobials*, vol. 20, no. 1, pp. 32–34, 2021.
- [38] R. Raz, "Fosfomycin: an old—new antibiotic," *Clinical Microbiology and Infection*, vol. 18, no. 1, pp. 4–7, 2012.
- [39] X. Zhang, M. Ma, Y. Cheng et al., "Spread and molecular characteristics of Enterobacteriaceae carrying fosA-like genes from farms in China," *Microbiology Spectrum*, vol. 10, no. 4, Article ID e0054522, 2022.
- [40] F. Yeganeh-Sefidan, R. Ghotaslou, M. T. Akhi, M. R. Sadeghi, Y. Mohammadzadeh-Asl, and H. Bannazadeh Baghi, "Fosfomycin, interesting alternative drug for treatment of urinary tract infections created by multiple drug resistant and extended spectrum β -lactamase producing strains," *Iranian Journal of Microbiology*, vol. 8, no. 2, pp. 125–131, 2016.
- [41] A. Marino, S. Stracquadanio, M. Ceccarelli, A. Zagami, G. Nunnari, and B. Cacopardo, "Oral fosfomycin formulation for acute bacterial prostatitis; a new role for an old molecule: a case report and brief literature review," *World Academy of Sciences Journal*, vol. 4, no. 4, pp. 26–6, 2022.
- [42] E. F. Meijer, A. van Renssen, I. Maat, L. van der Graaf-van Bloois, B. Duim, and E. M. Broens, "Canine *Staphylococcus argenteus*: case report from The Netherlands," *Pathogens*, vol. 11, no. 2, p. 153, 2022.