

Determination of fosfomycin resistant *Enterobacteriaceae* in isolates and frequency of *fos* genes in Tabriz Hospitals during 2018

Aidin Lalehzadeh, Mohammad Hossein Soroush, Javid Sadeghi, Mohammad Ahangarzadeh Rezaee, Tahereh Pirzadeh, Fatemeh Yeganeh Sefidan, Mohammad Yousef Memar, Elham Sheykhsharan, Reza Ghotaslou*

Received: 22 September 2018 / Received in revised form: 15 March 2019, Accepted: 24 March 2019, Published online: 25 April 2019
© Biochemical Technology Society 2014-2019
© Sevas Educational Society 2008

Abstract

Background: The members of *Enterobacteriaceae* are considered significant agents in human infections. The emergence of drug resistance is important for physicians. The aims of this study were investigating of fosfomycin resistance and the distribution rate of resistance genes among *Enterobacteriaceae* in Tabriz Hospitals. **Materials and Methods:** To determine the susceptibility patterns in 250 isolates, the disk diffusion method was performed. The minimum inhibitory concentration (MIC) of fosfomycin was determined by the agar dilution. The distribution rate of fosfomycin resistance genes (*fosA*, *fosA3*, *fosB*, *fosB2*, *fosC*, *fosC2*, and *fosX*) was done by the PCR. **Results:** The resistance rate to fosfomycin was in a value of 15 (6%) and 24 (9.6%) isolates by the disk diffusion and MIC methods, respectively. The MIC₅₀ and MIC₉₀ were 8 µg/mL and 16 µg/mL, respectively. The frequency of fosfomycin resistance genes was 1.6%, 1.6%, 1.6% and 0.8% for *fosA*, *fosC*, *fosX* and *fosA3*. The *fosB*, *fosB2*, and *fosC2* was not detected. **Conclusion:** Fosfomycin is a dramatically useful antibiotic against *Enterobacteriaceae*-caused infections. A trivial resistance rate is observed to fosfomycin in some isolates, and all detected resistance-genes are plasmid mediate agents. Resistance to other therapeutic options is much more than fosfomycin, which is an alarming point.

Key words: Resistance, Fosfomycin, *fos* genes, *Enterobacteriaceae*

Introduction

Enterobacteriaceae as a diverse bacterial family cause a various number of infections particularly urinary tract infection (UTI), septicemia and gastroenteritis in human (Sheykhsharan, Bannazadeh Baghi et al. 2018). Some members of this family are always pathogen and others serve as opportunistic infections (Ghotaslou, Yeganeh Sefidan et al. 2017). The increased resistance rate to antimicrobial agents has led to a reduction in the reliable and effective use of them in the treatment of *Enterobacteriaceae* infections (Falagas, Maraki et al. 2010). During the past few years, the introduction of new antibiotics to clinical purposes has been facing restrictions, some old drugs have also come out of the clinical routine. However, fosfomycin remains an effective antibiotic against the infections caused by *Enterobacteriaceae* (Livermore, Warner et al. 2011). Fosfomycin was firstly discovered in Spain in 1969, it is an acid fosfonic derivative compound with low molecular weight. It has not shown nearly any combination of proteins, also is not chemically related to other antibacterial factors and is unique in this respect (Beuk, Hill et al. 2013). Fosfomycin as a bactericide drug interferes in the synthesis of peptidoglycan. It occurs through the inhibition of UDP-*N*-acetylglucosamine enolpyruvyl transferase

Aidin Lalehzadeh, Fatemeh Yeganeh Sefidan, Mohammad Yousef Memar

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

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

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

Mohammad Hossein Soroush, Javid Sadeghi, Mohammad Ahangarzadeh Rezaee, Tahereh Pirzadeh, Reza Ghotaslou*

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

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

Elham Sheykhsharan

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

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

*Email: rzgottaslo@yahoo.com

(Falagas, Giannopoulou et al. 2008). For a number of decades, it has a unique mechanism to deal with resistant microorganisms (Lee, Park et al. 2012). It has a dramatic effect against on the Gram-negative/positive bacteria (Suárez and Mendoza 1991). Three forms of fosfomycin are included fosfomycin tromethamine as a soluble salt, fosfomycin calcium for oral use (UTI treatment), and fosfomycin disodium for intravenous use (Michalopoulos, Livaditis et al. 2011). Earlier study expressed a significant susceptibility to fosfomycin in MDR (Multi Drug-Resistant) and XDR (Extensively Drug-Resistant) *Enterobacteriaceae* (Falagas, Maraki et al. 2010). Recently, some studies reported fosfomycin-resistant *E. coli* isolates (Oteo, Bautista et al. 2010). Overall resistance to fosfomycin has been induced by chromosomal and plasmid agents including *fos* determinants (Cottell and Webber 2017). In the current research, the resistance rate of *Enterobacteriaceae* to fosfomycin and the distribution rate of seven *fos* genes were investigated.

Materials and Methods

Bacterial isolates

Two-hundred and fifty *Enterobacteriaceae* isolates belong to various clinical specimens were collected from Emam Reza, Children, and Asad Abadi Hospitals. The identification of *Enterobacteriaceae* was conducted through the common differential biochemical tests (Hansen, Aucken et al. 2004). The isolates were stored in TSB (Tryptic soy broth) medium including glycerol and preserved in -70°C (Rohman, Ijong et al. 2013). This study was approved by the Ethics Committee of Tabriz University of Medical Sciences (Ir.Tbzmed.Rec.1397.579).

The susceptibility testing

The susceptibility testing was conducted through the disk diffusion agar method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2012). The antimicrobial disks were including fosfomycin, imipenem, trimethoprim/sulfamethoxazole, amikacin, gentamicin, ciprofloxacin, ceftazidime, and cefotaxime (Mast, UK). The quality control strain was *E. coli* ATCC (American type culture collection) 25922.

MIC Determination

The MIC (minimum inhibitory concentration) of fosfomycin in *Enterobacteriaceae* isolates was determined by the agar dilution method. The interpretation of results was done according to the CLSI guidelines (CLSI 2012). The quality control strain was *E. coli* ATCC 25922.

The PCR

The DNA extraction of fosfomycin-resistant isolates was done by the boiling method (Peng, Yu et al. 2013). To detect fosfomycin resistance genes (*fosA*, *fosA3*, *fosB*, *fosB2*, *fosC*, *fosC2*, and *fosX*), the PCR was used as previously described (Bi, Li et al. 2017). Finally, PCR amplicons were investigated via electrophoresis on a 1% agarose gel and detected bands were visualized under UV light (LaMontagne, Michel Jr et al. 2002).

Statistical analysis

The obtained data were analyzed by descriptive analytic assays in SPSS software (Washington, the USA), version 22.

Results

In the current study, 250 nonduplicate *Enterobacteriaceae* isolates was collected from 91 males (36.4%) and 159 females (63.6%) with the mean age of 42±34 years. The isolates were collected from internal wards (29.2%) followed by outpatients, emergency, surgery, ICU (Intensive Care Unit), neurology, urology, respiratory, infectious, transplantation, trauma, ENT (Ear, Nose and Throat), NICU (Neonatal Intensive Care Unit), oncology and rheumatology wards in a value of 29.2%, 20.8%, 12%, 5.6%, 3.6%, 3.2%, 2.8%, 2.8%, 2.4%, 0.8%, 0.8%, 0.8%, 0.8% and 0.4%, respectively. Differential tests revealed that 169 (68%) of isolates were *Escherichia coli*, followed by 64 (26%) *Klebsiella pneumoniae*, 5 (2%) *Enterobacter cloacae*, 4 (1.6%) *Enterobacter agglomerance*, 2 (0.8%) *Klebsiella oxytoca*, 2 (0.8%) *Proteus vulgaris*, 2 (0.8%) *Morganella morganii*, and 2 (0.8%) *Proteus mirabilis*. According to the disk agar diffusion results, the most resistant rate was observed to cotrimoxazole followed by cefotaxime, ciprofloxacin, gentamicin, ceftazidime, imipenem, amikacin, and fosfomycin (Figure-1).

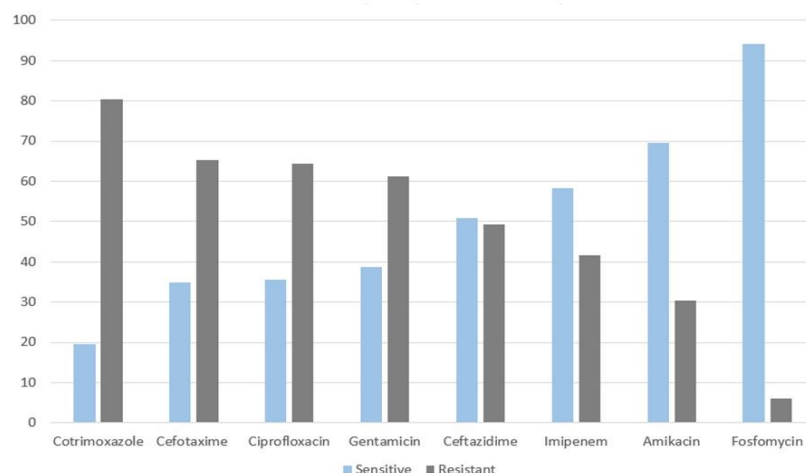


Figure 1. Antimicrobial susceptibility patterns of Enterobacteriaceae isolates.

Resistance to fosfomycin in *E. coli* isolates was 2.95% followed by 10.92% in *K. pneumoniae*, 20% in *E. cloacea* and 100% in *M. morgani* isolates. The rate of resistance to fosfomycin was 9.8% by the MIC method, and the MIC₅₀ and MIC₉₀ were 8 µg/mL and 16 µg/mL, respectively. Also, the MIC range was 1-256 µg/mL. The distribution rate of *fos* tested genes in fosfomycin-resistant isolates were as follow, *fosA* 4 (1.6%), *fosC* 4 (1.6%), *fosX* 4 (1.6%) and *fosA3* 2 (0.8%). However, there was no trace of *fosB*, *fosB2* and *fosC2* genes in the resistant isolates (Table-1 and Figure-2)

Table 1. The distribution rate of *fos* tested genes in fosfomycin-resistant isolates

Bacteria/n	<i>fosA</i> n (%)	<i>fosA3</i> n (%)	<i>fosC</i> n (%)	<i>fosX</i> n (%)
<i>E. coli</i> / 169	1 (0.59)	2 (1.18)	3 (1.77)	2 (1.18)
<i>K. pneumoniae</i> / 64	2 (3.12)	0 (0)	1 (1.56)	2 (3.12)
<i>E. cloacea</i> / 5	1 (20)	0 (0)	0 (0)	0 (0)
<i>M. morgani</i> / 2	0 (0)	0 (0)	0 (0)	0 (0)

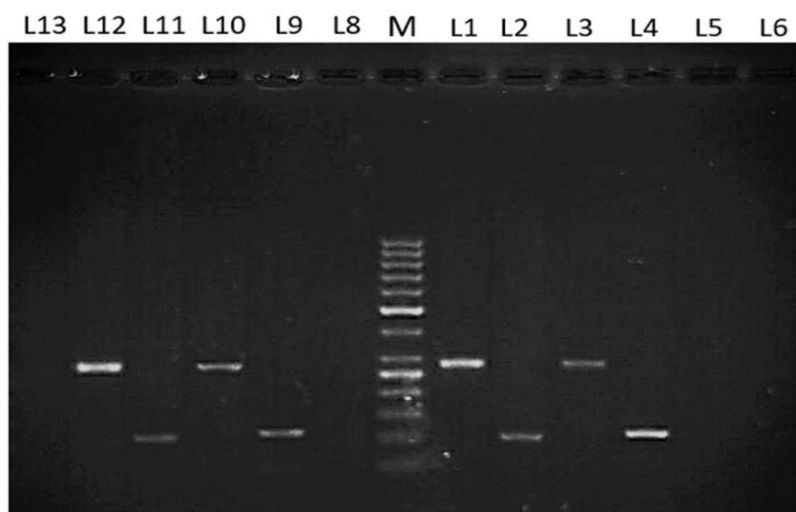


Figure 2. The gel electrophoresis patterns of fosfomycin resistance genes in 1% agarose gel. Lane M, DNA size marker (50 bp); *fosA* gene (271 bp), L1: positive isolate, L3: positive control, L5: negative control and L6: negative isolate; *fosX* gene (131 bp), L2: positive isolate, L4: positive control, L5: negative control and L6: negative isolate; *fosC* gene (112 bp), L9: positive isolate, L11: positive control, L8: negative control, L10 and L13: negative isolates; *fosA3* gene in (282 bp), L10: positive isolate, L12: positive control, L8: negative control, L9, L11 and L13: negative isolates

Discussion

Enterobacteriaceae as significant causative agents contribute to a number of important clinical infections. The emergence of antibiotic resistance has restricted the prescribing of some available options (Paterson 2006). In the current study, the high resistance rate is observed to trimethoprim/sulfamethoxazole followed by cefotaxime. The rate of resistance to tested antibiotics such as aminoglycosides and beta-lactams in the treatment of infections due to *Enterobacteriaceae* has reported in earlier studies from the different regions (Akram, Shahid et al. 2007; Sadeghi, Ghotaslou et al. 2016). The most significant finding in most of the researches suggests increasing the resistance rate. In the current research, the significant observed resistance to most antibiotics in particular trimethoprim/sulfamethoxazole can be considered as a big medical concern.

The previous studies indicated that fosfomycin has a broad spectrum antibiotic to be prescribed against the Gram-negative and Gram-positive bacteria (Aghamali, Sedighi et al. 2018). It is an established antibiotic in the treatment of UTI infections and has a high distribution in the various organs (Roussos, Karageorgopoulos et al. 2009). In the current research, the resistance rate to fosfomycin was 15 (6%). Matthew et al in Greece indicated that approximately 91.8% of MDR *Enterobacteriaceae* isolates were sensitive to fosfomycin (Falagas, Maraki et al. 2010). Also, fosfomycin was an effective antibiotic against the *E. coli* and *K. pneumoniae* isolates in a value of 92.9% and 95.2%, respectively, according to So-Young Lee and colleagues investigation in South Korea (Lee, Park et al. 2012). These findings are similar to more investigations from different countries (Beuk, Hill et al. 2013, Seroy, Grim et al. 2016, Keepers, Gomez et al. 2017). In our research, the most fosfomycin-resistant isolates have belonged to *E. cloacea*, *M. morgani*, *K. pneumoniae*, and *E. coli*. In 2013 in Iran, the resistance rate to fosfomycin in *K. pneumoniae* isolates was reported at 3.6% (Taherpour and Hashemi 2013). The other notable case in the present investigation is overwhelming resistance to fosfomycin in the *M. morgani* isolates, although the number of isolates is low, these two isolates have shown resistance to this antibiotic.

The fosfomycin resistance patterns also were conducted through the MIC method in China, which had a resistance rate in a value of 10.2%, the *fosA3* gene was detected in 9% of isolates, while, *fosC2* and *fosA* genes were found in none of the isolates (Hou, Huang et al. 2012). The *fosA3* gene had found in a third of resistant *E. coli* isolates in Hong Kong (Ho, Chan et al. 2013). The *fosA3* is a plasmid-mediated gene (Hou, Huang et al. 2012), which is frequent in China but rare in European countries based on sequencing data (Villa, Guerra et al. 2015). The resistance rate to fosfomycin has been detected 9.6% by the MIC method, which is consistent with mentioned studies, except that, the *fosA3* gene had the lowest distribution rate in our resistant isolates. Also, the obtained results of resistance to fosfomycin in both the disk diffusion and agar dilution methods in the current research are in accordance, only the very slight and neglectable difference was observed between them. The dominant founded genes in the present study were *fosA*, *fosC*, and *fosX* with equal proportions. None of resistant *M. morgani* isolates displayed any 4 detected genes in the current study, however, in the *E. cloacea* isolates only *fosA* gene was traced. These 4 genes were present in the resistant-*E. coli* isolates. *K. pneumonia* isolates were included 3 genes except for the *fosA3* gene.

The most notable point is the effectiveness of fosfomycin in treating infections in most of the cases. The percentage of resistance differs slightly in the world, fortunately, has not grown. The trivial difference in the obtained statistics can be explained according to the patterns of prescribed-antibiotics in the various regions. On the other hand, low prevalence resistance rate to fosfomycin, the identification of *fosX* gene is in the utmost of importance in the current study because of the scarcity of this gene in previous investigations.

As might be expected, the high level of resistant isolates was observed in blood or urine sources, because of abundantly *Enterobacteriaceae* members in mentioned sources. Nowadays it is recommended to combine the fosfomycin with trometamol, an oral formula in the treatment of UTI, especially in women with severe UTI (Raz 2012). According to the previous investigation, fosfomycin is a dramatically effective antibiotic against the UTI caused by uropathogenic *Enterobacteriaceae* (Yeganeh-Sefidan, Ghotaslou et al. 2016). Due to the dispersion of *Enterobacteriaceae* isolates in different places and food supplies and fosfomycin usage in veterinary, bacteria are readily transferred to new places (Hou, Huang et al. 2012).

Although fosfomycin has dramatically antibacterial activity, the trivial identified resistance cases is an alarm because of the determinant locations on mobile genetic elements like the plasmids. Due to a high probability to spread of resistance agents, prevention of these interactions is utmost of importance. One of the most important options would be combination therapy. This study surveys the resistance rate to fosfomycin comprehensively in Northwest of Iran. Since the rigorous researches have not been done in Iran, firstly, the results obtained from our investigation can be a proper pattern to physicians in order to optimal prescription. Secondly, it is suggested to use by other advanced molecular techniques to reach exact statistics in emerging resistance genes in future studies.

Conclusion

It is concluded that fosfomycin is an effective antibiotic against infections caused by *Enterobacteriaceae*. A trivial resistance rate is observed to fosfomycin in a number of isolates, all detected resistance-determinants are plasmid mediate. Resistance to other therapeutic options is much more than fosfomycin, which is an alarming point.

Acknowledgment

This research was financially supported by Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Conflict of interest

Any conflict of interest to declare.

References

- Aghamali, M., M. Sedighi, N. Mohammadzadeh, S. Abbasian, Z. Ghafouri and E. Kouhsari (2018). "Fosfomycin: mechanisms and the increasing prevalence of resistance." *Journal of medical microbiology*.
- Akram, M., M. Shahid and A. U. Khan (2007). "Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India." *Annals of clinical microbiology and antimicrobials* 6(1): 4.
- Beuk, C., C. Hill, S. Whitehead, E. Blondel-Hill, K. Wagner and N. Cheeptham (2013). "Determination of susceptibility to fosfomycin and tigecycline of Enterobacteriaceae, particularly Escherichia coli isolates, producing extended-spectrum β -lactamases from multiple regional Canadian hospitals." *Canadian Journal of Infectious Diseases and Medical Microbiology* 24(3): e80-e82.
- Bi, W., B. Li, J. Song, Y. Hong, X. Zhang, H. Liu, H. Lu, T. Zhou and J. Cao (2017). "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* 50(1): 29-34.
- CLSI, C. (2012). "Performance standards for antimicrobial susceptibility testing." *Clinical and Laboratory Standards Institute (M100eS22) (s22nd Informational Supplement)*.
- Cottell, J. L. and M. A. Webber (2017). "Prevalence, mechanisms and comparison of detection methods of fosfomycin resistance in E. coli from urinary tract infections." *bioRxiv*: 234435.
- Falagas, M. E., K. P. Giannopoulou, G. N. Kokolakis and P. I. Rafailidis (2008). "Fosfomycin: use beyond urinary tract and gastrointestinal infections." *Clinical infectious diseases* 46(7): 1069-1077.
- Falagas, M. E., S. Maraki, D. E. Karageorgopoulos, A. C. Kastoris, E. Mavromanolakis and G. Samonis (2010). "Antimicrobial susceptibility of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Enterobacteriaceae isolates to fosfomycin." *International journal of antimicrobial agents* 35(3): 240-243.
- Ghotaslou, R., F. Yeganeh Sefidan, M. T. Akhi, M. Asgharzadeh and Y. Mohammadzadeh Asl (2017). "Dissemination of Genes Encoding Aminoglycoside-Modifying Enzymes and armA Among Enterobacteriaceae Isolates in Northwest Iran." *Microbial Drug Resistance* 23(7): 826-832.
- Hansen, D. S., H. M. Aucken, T. Abiola and R. Podschun (2004). "Recommended test panel for differentiation of Klebsiella species on the basis of a trilateral interlaboratory evaluation of 18 biochemical tests." *Journal of clinical microbiology* 42(8): 3665-3669.
- Ho, P.-L., J. Chan, W.-U. Lo, E. L. Lai, Y.-Y. Cheung, T. C. Lau and K.-H. Chow (2013). "Prevalence and molecular epidemiology of plasmid-mediated fosfomycin resistance genes among blood and urinary Escherichia coli isolates." *Journal of medical microbiology* 62(11): 1707-1713.
- Hou, J., X. Huang, Y. Deng, L. He, T. Yang, Z. Zeng, Z. Chen and J.-H. Liu (2012). "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*: AAC. 05104-05111.
- Keepers, T. R., M. Gomez, C. Celeri, K. M. Krause, D. Biek and I. Critchley (2017). "Fosfomycin and comparator activity against select Enterobacteriaceae, Pseudomonas, and Enterococcus urinary tract infection isolates from the United States in 2012." *Infectious diseases and therapy* 6(2): 233-243.
- LaMontagne, M., F. C. Michel Jr, P. Holden and C. Reddy (2002). "Evaluation of extraction and purification methods for obtaining PCR-amplifiable DNA from compost for microbial community analysis." *Journal of Microbiological Methods* 49(3): 255-264.
- Lee, S.-Y., Y.-J. Park, J. K. Yu, S. Jung, Y. Kim, S. H. Jeong and Y. Arakawa (2012). "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* 67(12): 2843-2847.

- Livermore, D. M., M. Warner, S. Mushtaq, M. Doumith, J. Zhang and N. Woodford (2011). "What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline." *International journal of antimicrobial agents* 37(5): 415-419.
- Michalopoulos, A. S., I. G. Livaditis and V. Gougoutas (2011). "The revival of fosfomycin." *International journal of infectious diseases* 15(11): e732-e739.
- Oteo, J., V. Bautista, N. Lara, O. Cuevas, M. Arroyo, S. Fernández, E. Lázaro, F. J. De Abajo, J. Campos and S. E.-E.-N. S. Group (2010). "Parallel increase in community use of fosfomycin and resistance to fosfomycin in extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli*." *Journal of Antimicrobial Chemotherapy* 65(11): 2459-2463.
- Paterson, D. L. (2006). "Resistance in gram-negative bacteria: Enterobacteriaceae." *American journal of infection control* 34(5): S20-S28.
- Peng, X., K.-Q. Yu, G.-H. Deng, Y.-X. Jiang, Y. Wang, G.-X. Zhang and H.-W. Zhou (2013). "Comparison of direct boiling method with commercial kits for extracting fecal microbiome DNA by Illumina sequencing of 16S rRNA tags." *Journal of microbiological methods* 95(3): 455-462.
- Raz, R. (2012). "Fosfomycin: an old—new antibiotic." *Clinical Microbiology and Infection* 18(1): 4-7.
- Rohman, A., F. Ijong and I. Suwetja (2013). "Viability of *Edwardsiella tarda* and *Escherichia coli* preserved with glycerol-tryptone soy broth (TSB) kept at freezing temperature." *AQUATIC SCIENCE & MANAGEMENT (Jurnal Ilmu dan Manajemen Perairan)* 1(2): 154-159.
- Roussos, N., D. E. Karageorgopoulos, G. Samonis and M. E. Falagas (2009). "Clinical significance of the pharmacokinetic and pharmacodynamic characteristics of fosfomycin for the treatment of patients with systemic infections." *International journal of antimicrobial agents* 34(6): 506-515.
- Sadeghi, M. R., R. Ghotaslou, M. T. Akhi, M. Asgharzadeh and A. Hasani (2016). "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* 65(11): 1322-1331.
- Seroy, J. T., S. A. Grim, G. E. Reid, T. Wellington and N. M. Clark (2016). "Treatment of MDR urinary tract infections with oral fosfomycin: a retrospective analysis." *Journal of Antimicrobial Chemotherapy* 71(9): 2563-2568.
- Sheykhsaran, E., H. Bannazadeh Baghi, M. H. Soroush Barhaghi, N. Alizadeh, M. Y. Memar, S. Etemadi and R. Ghotaslou (2018). "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* 22(3): 205-212.
- Suárez, J. E. and M. C. Mendoza (1991). "Plasmid-encoded fosfomycin resistance." *Antimicrobial agents and chemotherapy* 35(5): 791.
- Taherpour, A. and A. Hashemi (2013). "Detection of OqxAB efflux pumps, OmpK35 and OmpK36 porins in extended-spectrum- β -lactamase-producing *Klebsiella pneumoniae* isolates from Iran." *Hippokratia* 17(4): 355.
- Villa, L., B. Guerra, S. Schmoger, J. Fischer, R. Helmuth, Z. Zong, A. Garcia-Fernandez and A. Carattoli (2015). "IncA/C plasmid carrying blaNDM-1, blaCMY-16, and fosA3 in *Salmonella enterica* Corvallis from a migratory wild bird in Germany." *Antimicrobial agents and chemotherapy: AAC*. 00944-00915.
- Yeganeh-Sefidan, F., R. Ghotaslou, M. T. Akhi, M. R. Sadeghi, Y. Mohammadzadeh-Asl and H. B. Baghi (2016). "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* 8(2): 125.