Biochem. Cell. Arch. Vol. 22, No. 1, pp. 1785-1791, 2022	www.connectjournals.com/bca	*ISSN 0972-5075*
DocID: https://connectjournals.com/03896.2022.22.1785		*eISSN 0976-1772*

PHARMACODYNAMICS ANALYSIS OF FOSFOMYCIN AGAINST MULTI-DRUGS RESISTANT *E. COLI* O157: H7 ISOLATED FROM URINARY TRACT INFECTION

Samir Abed Mohammed[1] and Orooba M.S. Ibrahim[2]

[1]Department of Biology, Al-Farabi University College, Iraq.
[2]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq.
*e-mail: samir@alfarabiuc.edu.iq, auroba.m@covm.uobaghdad.edu.iq
Orcid.ID1 : 0000-0003-4573-051x, Orcid.ID2 : 0000-0002-0682-8621

(Received 15 January 2021, Revised 8 March 2022, Accepted 15 March 2022)

ABSTRACT : The rates of urinary tract infection with multidrug-resistant (MDR) *Escherichia coli* have dramatically increased and the treatment of these infections with antimicrobial agents (β-lactams, fluoroquinolones, trimethoprim–sulfamethoxazole, nitrofurantoin, etc.) are becoming limited or ineffective due to the increasing frequency of antibiotic-resistant bacteria. The present study aimed to evaluate Fosfomycin pharmacodynamics; the elected *E. coli* O157:H7 isolate had been subjected to antibiotic susceptibility test, minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) by utilizing of Time kill curve, Post antibiotic effect (PAE) and Mutant prevention concentration (MPC), the results showed that the *E. coli* O157:H7 isolate was found to be multidrug-resistant (MDR), the MIC value of Fosfomycin against *E. coli* O157:H7 was 2000 µg/ml, the values of MBC and MPC were 4000 µg/ml for each of them. Furthermore, time-kill analysis demonstrated that Fosfomycin exhibited the highest bactericidal effects of fosfomycin against *E. coli* O157:H7 was achieved after 4 hrs of post-treatment and lasted up to 24 hrs, while the post-antibiotic effect of Fosfomycin against *E. coli* O157: H7 was remaining for 72 min. Therefore, we report here that the Fosfomycin was strong enough to overcome of bacterial resistance to these antibiotics and has been suggested as an effective alternative and a promising antimicrobial agent for restoring the efficacy of many antibiotics against MDR *E. coli* O157:H7.

Key words : *E. coli* O157:H7, fosfomycin, minimum inhibitory concentration, time killing curve, urinary tract infection.

How to cite : Samir Abed Mohammed and Orooba M.S. Ibrahim (2022) Pharmacodynamics analysis of Fosfomycin against multi-drugs resistant *E. coli* O157: H7 isolated from urinary tract infection. *Biochem. Cell. Arch.* **22**, 1785-1791. DocID: https://connectjournals.com/03896.2022.22.1785

INTRODUCTION

Urinary tract infections (UTIs) are represent one of the most common clinical infections in the world that affecting the human and animal (Flores-Mireles *et al*, 2015). Pathogenic urinary tract infections occur in about 14% of dogs throughout their life. *E. coli* is the most common bacterium causing 80–90% of community-acquired UTIs and 30–50% of nosocomially acquired UTIs. However, the recurrent UTIs were decreased to 25% of women within 6 months of an acute UTI episode and pose a major problem (Ejrnaes, 2011).

In recent years, the rates of UTIs with multidrug-resistant *E. coli* have dramatically increased and the treatment of these infections with antimicrobial agents (β-lactams, fluoroquinolones, trimethoprim–sulfamethoxazole, nitrofurantoin, etc.) are becoming limited or ineffective due to the increasing frequency of antibiotic-resistant bacteria (Zhanel *et al*, 2006). As a result, there is need to search for alternative to these drugs. Fosfomycin has been suggested as a promising antimicrobial agent for restoring the efficacy of many antibiotics against MDR Gram-negative bacteria (Bilal *et al*, 2018). Thus, the present study aimed to test the pharmacodynamics activity of fosfomycin against MDR *E. coli* O157:H7 isolated from urinary tract infections.

MATERIALS AND METHODS

Studied isolates

The uropathogenic *E. coli* O157:H7 isolate was obtained from microbiology laboratory at Alyarmook Hospital, Baghdad, Iraq, during the period of 2020. This isolate was cultured by streaking on Cefixime Tellurit Sorbitol MacConkey agar (CT-SMAC) for isolation and incubated at 37°C for 24 hrs as same as the isolation and

identification protocol that used by Yousif, (2019) to *E. coli* O157:H7 confirmation.

Antimicrobial agent

Fosfomycin (Sigma-Aldrich, St. Louis, MO, USA) was purchased from LabCompany (Londrina, Paraná, Brasil). Fosfomycin was dissolved in water to form 10 ìg/mL stock solution, which was stored at -20°C (stock solution).

Susceptibility testing

The following antibiotic discs (Oxoid) were used for antibiotic susceptibility testing of *E. coli* O157:H7 isolate by The agar disc diffusion method was adapted according to performance standards of CLSI (Clinical and Laboratory Standards Institute, 2012): Cefotaxime (20µg/disc), gentamicin (10µg/disc), Meropenem (10µg/disc), Methicillin (10µg/disc), Novobiocin (15µg/disc), Oxacillin (10µg/disc), Tetracycline (30µg/disc), Vancomycin (10 µg/disc) and Penicillin G (10 µg/disc) were used, for assessing the antibacterial activity.

Minimum Inhibitory Concentration (MIC)

The overnight cultures of *E. coli* O157:H7 were adjusted to 10^8 CFU/ml and diluted 1:100 with broth to obtain a 10^6 CFU/ml suspension, by checkerboard assay, each well of a standard microwell plate 100 ìL of the 10^6 CFU/mL bacterial suspensions were transferred and mixed with an equal volume of fosfomycin at ranged (15.6-8000) µg/ml and incubated on 37ºC for 24 hrs.(CLSI., 2018), For colorimetric identification of bacterial growth, 20 µL of TTC solution 0.125% (w/v) was added to each well of the test and re-incubated for 2hrs. (vaga *et al*., 2019).

***In-vitro* time kill curve**

The time-kill curve assay of fosfomycin against *E. coli* O157:H7 isolate was based on the National Committee for Clinical Laboratory Standards. Briefly, bacterial suspension equivalent to 0.5 Mcfarland (1.5 x 10^8 CFU/ml) was prepared from overnight bacterial culture. 0.1 ml of the prepared bacterial suspension was diluted in 14.9 ml of Mueller-Hinton broth and incubated at 37ºC for 1 hr. to obtain 10^6 CFU/ml bacterial suspensions. Fosfomycin concentrations from 4x MIC to 0.25x MIC had been prepared in 6 McCartney's bottles in addition to controlling positive tube and each bottle was inoculated with 0.1 ml of bacterial suspension and incubated on 37ºC for 24 hrs. Bacterial colonies were calculated at 0, 2, 4, 6 and 24 hrs. through the incubation time (NCCLS, 1999).

Post antibiotic effect

Freshly prepared 10^6 CFU/ml bacterial suspension and incubated on 37ºC for 2hrs. to bring *E.coli* to the logarithmic phase of bacterial growth. Fosfomycin had been prepare 10x, 1x and 0.1xMICs was inoculated with 0.1 of bacterial suspension and incubated on 37ºC for 2 hrs; after incubation, the antibacterial effect of antibiotic was removed by diluting then tubes re-incubated on 37ºC for 24 hrs (Aeschlimann and Rybak, 1998; Ozbek Celik *et al*, 2014). Bacterial colonies were calculated at 0, 2, 4 and 6 hrs. by agar plate and colonies were calculated as mentioned previously (Miles *et al*, 1938). PAE was calculated as (Ozbek Celik *et al*, 2014): PAE = T – C

Where, T and C are the time required to increase 1-log10 CFU following 1:1000 dilution for the bacteria treated with (T) and without (C) the agents, respectively.

Mutant Prevention concentration

freshly prepared 10^6 CFU/ml bacterial suspension then incubated on 37ºC for 2 hrs. to bring *E. coli* O157:H7 to the log phase of bacterial growth. Plates of Mueller-Hinton agar and Fosfomycin had been prepared and a calculated aliquot was diluted in previously prepared Mueller-Hinton agar (45-50ºC) to produce 10x, 1x and 0.1x MICs concentrations. Each concentration was poured into a petri dish (triplicate) and 0.1 ml of bacterial suspension was spread on each plate then incubated at 37ºC for 72 hrs. The lowest antibiotic concentration that recorded no visible growth of bacteria is considered the concentration that prevents bacterial mutations (Dahdouh *et al*, 2014).

RESULTS

The positive result of our isolate showed a smooth, circular and colorless on SMA-CT agar at - 24hrs post-culturing and incubation at 37°C. In Sorbitol MacConkey agar, lactose is replaced by Sorbitol. Most strains of *E. coli* ferment Sorbitol to produce acid but *E. coli* O157:H7 could not ferment Sorbitol. This method explains that *E. coli* O157:H7 unlike 90% of *E. coli* isolates does not ferment Sorbitol rapidly.

E. coli O157:H7 strain was isolated from UTI. High resistance to various antibiotics. Including Cefotaxim, Gentamycin, methicillin, Oxacillin, Vancomycin and Penicillin G and sensitive to Meropenem, Novobiocin and Tetracycline. According to results of microdilution method; the MIC results showed that the concentration of 2000 µg/ml of fosfomycin was the minimal amount that inhibits the growth of *E. coli* O157:H7 that used in the test while other lower concentrations failed to inhibit the growth of the bacteria.

Time-Kill Curve Kinetics of Fosfomycin

Results of time kill curve kinetics are based on the

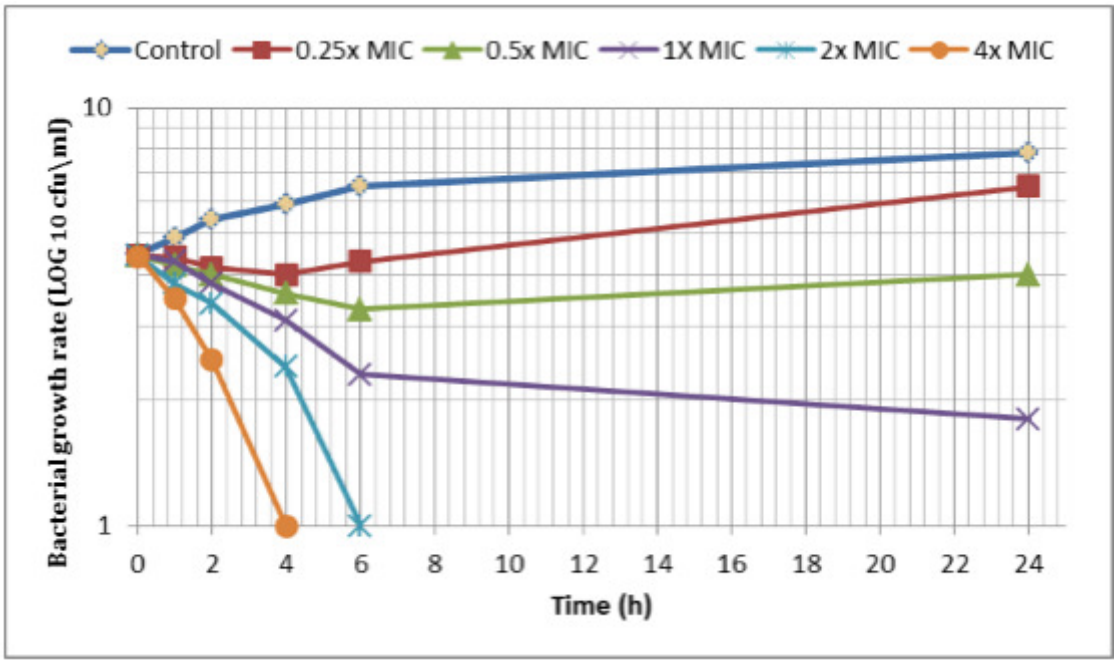

Fig. 1 : Time kill curve kinetics of Fosfomycin against *E. coli* O157:H7.

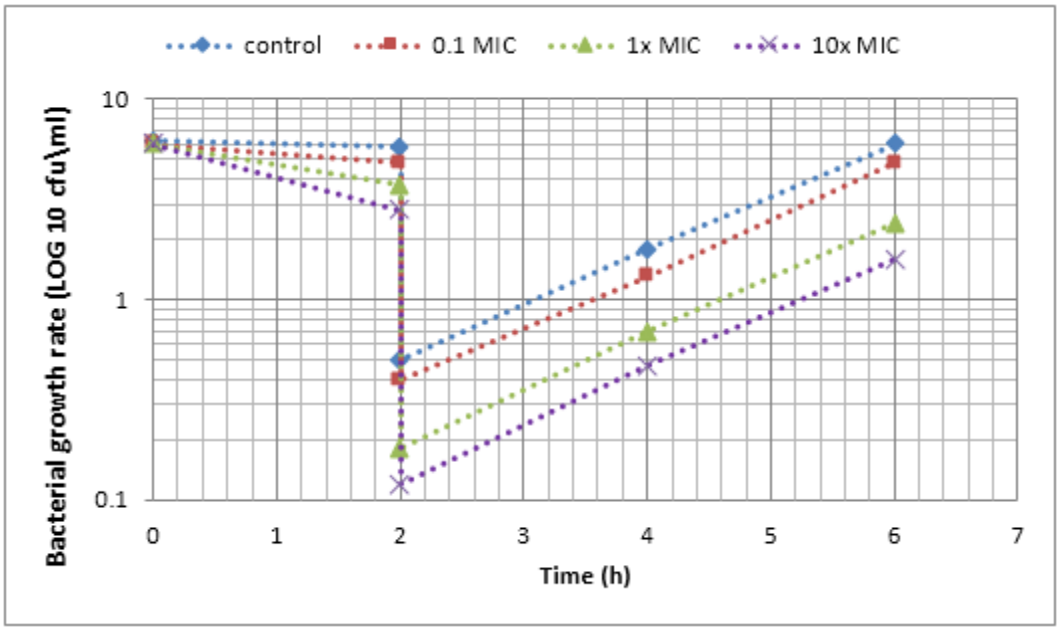

Fig. 2 : Post antibiotic effect of Fosfomycin against *E. coli* O157:H7 (h.).

highest MIC recorded from the microdilution MIC assay which was 2000 µg/ml for the *E. coli* O157:H7, the *in-vitro* concentrations that involved in the study were 0.25x MIC, 0.5x MIC, 1x MIC, 2x MICs and 4x MICs. All of concentrations from 4x MIC through 2x MICs achieved obvious bactericidal effect by reducing of ≥3 $\log_{10}$ of the total number of cfu/ml of *E. coli* O157:H7 in comparison to control, 0.25x MIC and 0.5x MIC; while 1x MIC showed a drop in growth curve at 1st six hours, then after, there was continued inhibition of growth as reported at the 24th hour (Fig. 1).

The area under the time of killing curve was calculated and compared to control inoculum growth rate, the difference in the area under the curve values among different treatments were set as an endpoint whereas the lowest area under the curve refers to the highest bactericidal effect as reported in the Table 1. The results showed that all of 2x (MICs) and 4x (MICs) achieved the highest significant bactericidal effect (P≥ 0.05) in comparison to other treatments. The 1x MIC concentration achieved purely bacteriostatic effect (Pe" 0.05) in comparison to all concentrations and control groups; Both of 0.5x MIC and 0.25x MIC failed to achieve a significant bacteriostatic or bactericidal effect (P≤ 0.05)

Table 1 : Area under the time-kill curve of Fosfomycin against *E. coli* O157:H7 (h*log CFU/ml).

Control	0.25x MIC	0.5x MIC	1x MIC	2x MICs	4x MICs
162.23 ±0.30A	**123.24±0.4A**	**88.62±0.12A**	**58.05±0.28B**	**27.95±0.38C**	**12.46±0.75C**

Values represent mean ± S.E, Different letters denoted a significant difference (p≤0.05) among the groups.

Table 2 : Post antibiotic effect of Fosfomycin against *E. coli* O157:H7 (h).

Concentrations	Control(C)	0.1xMIC	1xMIC	10xMIC
Growth after fosfomycin removal ($1 \log_{10}$/hrs.)	3.60 ± 0.01A	3.90 ± 0.06A	4.80 ± 0.07B	5.40 ± 0.01B
Post antibiotic effect (hrs.) (C-T)	N.A	0.30 ± 0.0025A	1.20± 0.0035B	1.90 ± 0.0014B

• Values represent mean ± S.E, Different letters denoted a significant difference (p<0.05) among the groups. N.A. (Not Applicable).

Table 3 : Mutant preventive concentration of Fosfomycin against *E. coli* O157:H7.

Concentration	Observation
Control	+
0.25 x MIC	+
0.5x MIC	+
1x MIC	+
2x MIC	-
4x MIC	-
MPC/MIC = 2	

(+) Visible bacterial growth, (-) No bacterial growth

in comparison to 1xMIC, 2xMIC, and 4xMIC.

Post antibiotic effect of Fosfomycin

The post antibacterial effect of Fosfomycin regarded the difference in the time that consumed by treatment and control culture for one $\log_{10}$ increment in colonies count of *E. coli* O157:H7 after removal of fosfomycin effect by dilution. Regarding the MIC value (2000µg/ml), three different concentrations of fosfomycin (0.1x MIC, 1x MIC and 10x MIC) had been tested against control culture.

These findings, as graphed in the Fig. 2 showed insignificant increment (P≤0.05) in the time required for *E. coli* O157:H7that exposed to 0.1x MIC concentration of fosfomycin to rise in growth by 1 Log_{10} after removal of fosfomycin from the medium by dilution in comparison to control culture.

The results also exhibited significant increment (P≥ 0.05) in the required time for bacterial regrowth by 1Log_{10} of *E. coli* O157:H7 that exposed to 1x MIC and 10x MIC concentrations of Fosfomycin in comparison to both control and 0.1x MIC cultures (Table 2). The time of post-antibiotic effect that recorded in 1x MIC and 10x MIC culture after removal of Fosfomycin effect were 1.2 hr. (72 min) and 1.9 hr. (114 min.) respectively which were significantly (P≥ 0.05) higher than the time of post-antibiotic effect that recorded by 0.1x MIC culture after removal of Fosfomycin who recorded 0.3 hr. (18 min.) as reported in the Table 2.

Mutant prevention concentration of Fosfomcin

Based on the recorded MICs of Fosfomycin against *E. coli* O157:H7 that was 2000 µg/ml, the obtained observations of our mutant prevention concentration (MPC) after 96 hrs. of incubation were reported in the Table 3. Our results revealed that there was no visible bacterial growth on the plates that contain 4x MIC, and 2x MIC of Fosfomycin in comparison to the control plate. The results of MPC for 1x MIC concentration of Fosfomycin showed a visible weak growth of *E. coli* O157:H7 in comparison to the control plate. Also, there was a heavier bacterial growth was appeared in the plate that contains 0.5x MIC concentration of Fosfomycin in comparison to the plate that contains 1x MIC concentration of Fosfomycin. The Mutation prevention index (MPC/MIC) was calculated depending on the recorded results of mutant prevention concentration to the minimum inhibitory concentration of the used isolate of *E. coli* O157:H7 in the test.

DISCUSSION

The infections with MDR uropathogens have become significantly challenging due to their high resistance to commonly used antibiotics. In the present study, the *E. coli* O157:H7 characteristics as same as that reported by Al-Rudha *et al* (2016). The examined uropathogenic *E. coli* O157:H7 isolate exhibited MDR phenotypes and this isolate was resistant to 66% of the antibiotics. The high prevalence of resistance to most common antibiotics could be due to the intensive and excessive use of these antibiotics in treating UTI (Laxminarayan *et al*, 2013). These results are consistent with those recently reported in Egypt mentioned that the rates of UTIs with multidrug-resistant *E. coli* O157:H7 have dramatically increased (El-Kashif and Eaid, 2018).

The minimum inhibitory concentration (MIC) plays a key role in determination of an antibacterial potency (Wiegand *et al*, 2008). Many methods were used to determine the MIC, but, the microdilution assay is the most adopted and accredited method by European committee on antimicrobial susceptibility testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) to determine the MIC (Elshikh *et al*, 2016). Our results of MIC study revealed that isolate of *E. coli* O157:H7 are highest MIC value was 2000 µg/ml. These results were consistent with time-kill curve is combined and extensive tool to assess both bacteriostatic and bactericidal effects of the antibiotics; it depends on the change in the logarithmic number of bacterial colonies through defined chronological pattern (Mouton *et al*, 2005).

According to the obtained curves (Fig. 1) and the calculated areas under each one of them (Table 1), both 0.25x MIC and 0.5x MIC concentrations showed no significant antibacterial effect in comparison to the control curve in contrast to 1x MIC that showed a significant bacteriostatic effect against *E. coli* O157:H7 throughout 24 hrs.; such bacteriostatic effect is expected since the 1x MIC of Fosfomycin has located within the determined range of *E. coli* O157:H7 sensitivity toward Fosfomycin which determined from ≥ 32 to 2048 µg/ml (El-Wafa and Ibrahim, 2019). Each of 2x MICs and 4x MICs concentrations showed a distinguished bactericidal effect at the 4th and 6th hr of the experiment respectively as same as what reported by Fransen *et al* (2017).

Fosfomycin as well as Fluoroquinolones which refer to the dependency of these antibacterial agents on the concentration that they spent in contact with the bacteria in site of infection to produce the bactericidal effect in contract to time-dependent killing antibiotics (Craig, 1993).

Estimation of Post antibiotic effect (PAE) is very important because it is the most *in-vitro* parameter mimicking the *in-vivo* situation that an antibiotic had been faced inside a living body. After all, the concentration of the tested antibiotics in other parameters like MIC and time-kill curve kinetics is constant in contrast to PAE how mimicking the drop in the concentration to sub-MIC values (Craig, 1991).

Despite the unclear mechanism of the PAE for most antibacterial agents, it has been proposed two mechanisms; induction of non-lethal damage and/or persistence of the antibacterial agent at the binding site with the bacterium (Zhanel *et al*, 1991).

The factors govern the duration of PAE; the type of the antibacterial, the bacterial isolate that is used in, the concentrations of the antibacterial in the medium and the variety of methodologies of assessment of that effect (Gottfredsson *et al*, 1996). Our achieved PAE values came in range with many previous studies (MacLeod *et al*, 2009 and Mazzei *et al*, 2006) that reported the PAE of different concentrations of fosfomycin against different *E. coli* isolates can last from 1-3.4 hrs; such wide range in PAE may be attributed to the difference among inoculum sizes and variability of *E. coli* isolates that used in the mentioned studies.

Mutant prevention concentration (MPC) is the concentration of the antibacterial agent that prevents heavy bacterial inoculum (> 10^9 CFU/ml) from visible growth on the plate (Dong *et al*, 1999).

The core difference between MIC and MPC is the used bacterial inoculum since the standard bacterial inoculum used in MIC (10^6 CFU/ml) is less dense than the inoculum that used in MPC (> 10^9 CFU/ml) because the 1st bacterial mutation might occur over 10^9 CFU/ml bacterial population; with other words, MPC will deprive the bacteria the chance to mutate or to develop resistance (Hesje *et al*, 2007).

In general, the mechanism that produces mutant prevention concentration of most antibiotic groups is not well evaluated as Fluoroquinolones (Smith *et al*, 2003), but, our speculations suppose that the rapid bactericidal mechanism of fosfomycin that achieved by the epoxy ring, which inhibits a cytoplasmic enzyme (phosphoenolpyruvate synthetase) active during the first step of bacterial cell wall (peptidoglycan) synthesis; will reduce the chances of bacterial mutation (Patel *et al*, 1997 and Gobernado, 2003).

In our observation, we record that presence of sub-bactericidal concentrations 1x MIC and 0.5x MIC (Table 3) in the inoculated plate didn't prevent bacterial mutation because such concentrations are not enough to produce the bactericidal effect; this result didn't come away from the typical foundation who states that the value of MPC is always lower than the MIC to prevent the growth of mutant subpopulations from density bacterial inoculum (Blondeau, 2009).

The ratio of the MPC to the MIC (MPC/MIC) is another calculated parameter and it is defined as "the concentration range over which the lower value is the MIC and the upper value is the MPC. Within this range, the growth of susceptible bacteria is suppressed but resistant mutant subpopulations can still be selectively amplified" (Zhao and Drlica, 2001).

CONCLUSION

The present study revealed that the Fosfomycin was strong enough to overcome of bacterial resistance to these antibiotics and has been suggested as a promising antimicrobial agent for restoring the efficacy of many antibiotics against MDR Gram-negative bacteria. Therefore, it could be a promising alternative to currently available first line antibiotics for the treatment of UTI, especially for a naive population where the consumption rate of fosfomycin is nil.

ACKNOWLEDGEMENTS

I would like to express my special appreciation and thanks my supervisor Professor Dr. Orooba M. S. Ibrahim for their great supervision, support, help, guidance and mentorship throughout this study, without her advice and support this research has never been done.

Conflict of interest

The authors declare that there are no conflicts of interest

REFERENCES

Aeschlimann J R and Rybak M J (1998) Pharmacodynamic analysis of the activity of quinupristin-dalfopristin against vancomycin-resistant Enterococcus faecium with differing MBCs via time-kill-curve and postantibiotic effect methods. *Antimicrobial Agents and Chemotherapy* **42**(9), 2188-2192.

Al-Rudha A M (2016) Distribution of *E. coli* O157: H7 in fecal and urine samples of cattle. *Iraqi J. Vet. Med.* **40**(1), 79-82.

Bilal H, Peleg A Y, McIntosh M P, Styles I K, Hirsch E B, Landersdorfer C B and Bergen P J (2018) Elucidation of the pharmacokinetic/pharmacodynamic determinants of fosfomycin activity against *Pseudomonas aeruginosa* using a dynamic *in vitro* model. *J. Antimicrob. Chemotherapy* **73**(6), 1570-1578.

Blondeau J M (2009) New concepts in antimicrobial susceptibility testing: the mutant prevention concentration and mutant selection window approach. *Vet. Dermatol.* **20**(5 6), 383-396.

Clinical and Laboratory Standards Institute (CLSI) (2018) Performance Standards for antimicrobial disk and dilution susceptibility tests for Bacteria isolated from animals; Approved standard. 5th ed. CSLI Supplement VET08. Wayne P A: Clinical and Laboratory Standards Institute USA, 33-53.

Craig W (1993) Pharmacodynamics of antimicrobial agents as a basis for determining dosage regimens. *Europ. J. Clin. Microbiol. Infect. Dis.* **12**(1), S6-S8.

Craig W A (1991) The postantibiotic effect. *Clin. Microbiol. Newslett.* **13**(16), 121-124.

Dahdouh E, Shoucair S H, Salem S E and Daoud Z (2014) Mutant prevention concentrations of imipenem and meropenem against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *The Scientific World Journal*, **2014**, Article ID 979648 , https://doi.org/10.1155/2014/979648

Dong Y, Zhao X, Domagala J and Drlica K (1999) Effect of fluoroquinolone concentration on selection of resistant mutants of *Mycobacterium bovis* BCG and *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **43**(7), 1756-1758.

Ejrnæs K (2011) Bacterial characteristics of importance for recurrent urinary tract infections caused by *Escherichia coli*. *Dan Med Bull*. **58**(4), B4187.

El-Kashif M M L and Elgazzar S E (2018) Maternal markers for detecting urinary tract infection among pregnant women in Port Said City, Egypt. *Am. J. Nurs.* **6**(5), 317-326.

Elshikh M, Ahmed S, Funston S, Dunlop P, McGaw M, Marchant R and Banat I M (2016) Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. *Biotech. Lett.* **38**(6), 1015-1019.

El-Wafa W M A and Ibrahim Y M (2020) *In vitro* activity of fosfomycin in double and triple combinations with imipenem, ciprofloxacin and tobramycin against multidrug-resistant *Escherichia coli*. *Curr. Microbiol.* 1-7.

Flores-Mireles A L, Walker J N, Caparon M and Hultgren S J (2015) Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat. Rev. Microbiol.* **13**(5), 269-284.

Fransen F, Hermans K, Melchers M J, Lagarde C C, Meletiadis J and Mouton J W (2017) Pharmacodynamics of fosfomycin against ESBL-and/or carbapenemase-producing Enterobacteriaceae. *J. Antimicrob. Chemotherapy* **72**(12), 3374-3381.

Gobernado M (2003) Fosfomycin. *Revista espanola de quimioterapia: publicacion oficial de la Sociedad Espanola de Quimioterapia* **16**(1), 15-40.

Hesje C K, Tillotson G S and Blondeau J M (2007) MICs, MPCs and PK/PDs: a match (sometimes) made in hosts. *Expert Rev. Resp. Med.* **1**(1), 7-16.

Laxminarayan R, Duse A, Wattal C, Zaidi A K, Wertheim H F, Sumpradit N and Cars O (2013) Antibiotic resistance—the need for global solutions. *The Lancet Infect. Dis.* **13**(12), 1057-1098.

Li Y, Feng B, Gu X, Yang D, Zeng Z, Zhang B and Ding H (2016) Correlation of PK/PD indices with resistance selection for cefquinome against *Staphylococcus aureus* in an *in vitro* model. *Front. Microbiol.* **7**, 466.

MacLeod D L, Barker L M, Sutherland J L, Moss S C, Gurgel J L, Kenney T F and Baker W R (2009) Antibacterial activities of a fosfomycin/tobramycin combination: a novel inhaled antibiotic for bronchiectasis. *J. Antimicrob. Chemotherapy* **64**(4), 829-836.

Mazzei T, Cassetta M I, Fallani S, Arrigucci S and Novelli A (2006) Pharmacokinetic and pharmacodynamic aspects of antimicrobial agents for the treatment of uncomplicated urinary tract infections. *Int. J. Antimicrobial Agents* **28**, 35-41.

National Committee for Clinical Laboratory Standards and Barry A L (1999) *Methods for determining bactericidal activity of antimicrobial agents: approved guideline* (Vol. **19**, No. 18). Wayne, PA: National Committee for Clinical Laboratory Standards.

Özbek Çelik B, Mataracý-Kara E and Yýlmaz M (2014) Effects of various antibiotics alone or in combination with doripenem against *Klebsiella pneumoniae* strains isolated in an intensive care unit. *Biomed. Res. Int*. **2014**, 397421.

Patel S S, Balfour J A and Bryson H M (1997) Fosfomycin tromethamine. *Drugs* **53**(4), 637-656.

Smith H J, Nichol K A, Hoban D J and Zhanel G G (2003) Stretching the mutant prevention concentration (MPC) beyond its limits. *J. Antimicrobial Chemotherapy* **51**(6), 1323-1325.

Veiga A, Maria da Graça T T, Rossa L S, Mengarda M, Stofella N C, Oliveira L J and Murakami F S (2019) Colorimetric microdilution assay: Validation of a standard method for determination of MIC, IC50%, and IC90% of antimicrobial compounds. *J. Microbiol. Methods* **162**, 50-61.

Wiegand I, Hilpert K and Hancock R E (2008) Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols* **3**(2), 163-175.

Yousif A A (2019) Effects of *E. coli* O157: H7 Experimental infections on rabbits. *Iraqi J. Vet. Med.* **43**(1), 34-42.

Zhanel G G, Zhanel M A and Karlowsky J A (2018) Intravenous fosfomycin: an assessment of its potential for use in the treatment of systemic infections in Canada. *Canad. J. Infect. Dis. Med. Microbiol. 2018*.

Zhao X and Drlica K (2001) Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. *Clin. Infect. Dis.* **33**(Supplement_3), S147-S156.