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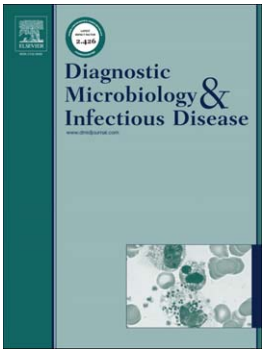

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Pharmacodynamic Activity of Fosfomycin Simulating Urinary Concentrations Achieved After a Single 3 Gram Oral Dose versus *Escherichia coli* Using an In Vitro Model

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Highlights

- We assessed the activity of fosfomycin simulating urinary concentrations achieved after a single 3 gram oral dose against *Escherichia coli* using an in vitro pharmacodynamic model. .
- Fosfomycin urinary concentrations obtained after a single 3 gram oral dose were bactericidal as early as 1 h after dosing with complete bacterial eradication at all time-points over the 48 h testing period against urinary isolates of *E. coli* (including MDR ESBL- and/or carbapenemase-producing strains).
- This study helps to explain the high (>90%) microbiological and clinical cure rates achieved with fosfomycin when used as a single 3 gram oral dose to treat patients with acute uncomplicated cystitis.

Abstract

We assessed the activity of fosfomycin simulating urinary concentrations achieved after a single 3 gram oral dose against *Escherichia coli* using an in vitro pharmacodynamic model. Eleven urinary isolates of *E. coli* were studied. Isolates were ESBL-producing or carbapenemase-producing. The in vitro pharmacodynamic model was inoculated with an inoculum of (~1 x 10^6 cfu/mL). Fosfomycin was administered to simulate maximum free (*f*) urine (U) concentrations and a $t_{1/2}$ obtained after a standard single 3 gram oral dose in healthy volunteers (fU_{max}, 4000 mg/L; $t_{1/2}$, 6 h). Sampling was performed over 48 h to assess the rate and extent of bacterial reduction as well as resistance selection. Complete bacterial eradication from the model was defined by no regrowth over the 48 h study period. Fosfomycin MICs ranged from 1-4 µg/mL for ESBL producers, while all three carbapenemase-producing *E. coli* demonstrated a fosfomycin MIC of 2 µg/mL. Fosfomycin $fT_{>MIC}$ of 100% ($fAUC_{0-24}$/MIC, ≥~7250) resulted in bacterial killing (reductions in $\log_{10}$ CFU assessed relative to the starting inoculum at 2, 4, 6, 12, 24, and 48 hours of ≥3.0) at each time-point versus all isolates of ESBL-producing and carbapenemase-producing *E. coli*. We conclude that fosfomycin urinary concentrations obtained after a single 3 gram oral dose were bactericidal as early as 1 h after dosing with complete bacterial eradication at all time-points over the 48 h testing period against urinary isolates of *E. coli* (including MDR ESBL- and/or carbapenemase-producing strains). Our data help to explain the high (>90%) microbiological and clinical cure rates achieved with fosfomycin when used as a single 3 gram oral dose to treat patients with acute uncomplicated cystitis.

1. Introduction

Escherichia coli is the most common etiologic agent of urinary tract infections. ESBL-producing and carbapenem-resistant *E. coli* have rapidly spread in the community, extended-care facilities, and hospital settings and are frequently multidrug-resistant (MDR) (concomitant resistance to ≥3 chemically different antimicrobial classes) (Zhanel et al, 2016; Zhanel et al, 2013; Denisuik et al, 2013). Fosfomycin, which inhibits peptidoglycan synthesis by a mechanism distinct from that of β-lactams, is recommended by a joint Infectious Diseases Society of America (IDSA) and European Society for Clinical Microbiology and Infectious Diseases (ESCMID) guideline as one of three first-line treatments for acute uncomplicated cystitis (Gupta et al, 2011). When fosfomycin is used to treat acute uncomplicated cystitis, it is administered as a single oral 3 gram dose (Gupta et al, 2011). In vitro, fosfomycin is very active against extended-spectrum β-lactamase (ESBL) and MDR *E. coli* with MIC_{50} and MIC_{90} values of 2 and 4 μg/mL, respectively (Karlowsky et al, 2014). However, there are currently no data that assess the pharmacodynamics of fosfomycin versus *E. coli* simulating its urinary concentration following a single 3 gram oral dose as used in the treatment of acute uncomplicated cystitis. The purpose of this study was to assess the activity of fosfomycin against molecularly characterized MDR ESBL- and/or carbapenemase-producing *E. coli* isolates using an in vitro pharmacodynamic model that simulated achievable urinary concentrations of fosfomycin following a single 3 gram oral dose.

2. Materials and methods:

2.1 Bacterial strains and culture conditions

The *E. coli* urinary isolates used in this study were obtained from the CANWARD study (www.can-r.ca), a national, ongoing Health Canada endorsed surveillance study assessing antimicrobial resistance in Canadian hospitals (Zhanel et al, 2013; Denisuik et al, 2013). In the CANWARD study, any *E. coli* with a ceftriaxone MIC ≥1 μg/mL is identified as a putative ESBL (Denisuik et al, 2013). Putative ESBL phenotypes are confirmed by the disk diffusion method described by CLSI using *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 as control strains (CLSI, 2016). Genotypic characterization of ESBLs was performed by PCR and DNA sequencing of bla_{SHV}, bla_{TEM}, bla_{CTX}, bla_{OXA} and bla_{VEB} genes, as previously described (Denisuik et al, 2013). A BLAST search of DNA sequences was conducted to determine specific ESBL genotypes. All potential carbapenemase-producing *E. coli* and *K. pneumoniae* (ertapenem MIC ≥1 μg/mL) were screened for the presence of bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{IMI}, bla_{NDM}, bla_{GES}, and bla_{OXA-48} by multiplex PCR as described by Denisuik and others (Denisuik et al, 2013). The NDM isolate was provided by Dr. Johann Pitout (Calgary Laboratory Services, Calgary, Canada), with all other isolates were obtained from the CANWARD study. MDR was defined as resistance to third-generation cephalosporins and ≥2 other chemically unrelated antimicrobial classes (Table 1).

For the pharmacodynamic studies, logarithmic phase cultures equivalent to a 0.5 McFarland (1×10^8 cfu/mL) in cation-supplemented Mueller Hinton broth supplemented with 25 μg/mL of glucose-6-phosphate were used (Zhanel et al, 2014). Viable bacterial counts consistently yielded a starting inoculum of approximately 1×10^6 cfu/mL in the model. A growth control was included in every experiment. Growth controls peaked at ~1×10^9 cfu/mL in the model and were maintained over the 48 hours of each experiment.

2.2 Antibiotic preparations and susceptibility testing

Antimicrobial agents were obtained as laboratory-grade powders from their respective manufacturers. Fosfomycin tromethamine was provided by Zambon (Milan, Italy). Stock solutions were prepared and broth microdilution MICs were determined for all antimicrobials except fosfomycin where MICs were performed using agar dilution with supplementation of 25 μg/mL of glucose-6-phosphate according to CLSI guidelines (CLSI, 2015). All MICs were performed in triplicate on separate days. Fosfomycin CLSI breakpoints are susceptible, intermediate, and resistant ≤64, 128, and ≥256 μg/mL, respectively (CLSI, 2016).

2.3 Pharmacokinetic and pharmacodynamic experiments with fosfomycin in the in vitro pharmacodynamic model

Experiments were performed simulating a single peak urine concentration (U_{max}) and urinary area under the curve over 24 hours (AUC_{0-24}) of fosfomycin achieved in human urine after a standard single 3 gram oral dose used when treating acute uncomplicated cystitis (Zhanel et al, 2016; Patel et al, 1997). As the protein binding of fosfomycin in human serum is very low, it was assumed that urinary concentrations of fosfomycin represented free (*f*, protein unbound) drug (Patel et al, 1997; Shimizu, 1997; Bergen T, 1990; Keating GM, 2013). Fosfomycin urinary clearance was simulated using a reported serum half-life of 6 h (i.e., half-life in urine was assumed to be 6 h) (Patel et al, 1997). The pharmacokinetics of fosfomycin were evaluated by simulating concentrations achieved after administration of a single 3 gram oral dose in the central compartment and sampling from this compartment at 0, 1, 2, 4, 6, 12, 18, 24, and 48 h. Thus, the simulated fosfomycin *f* drug U_{max} was ~4000 μg/mL, while at 24 hours and 48 hours it was ~250 μg/mL and ~16 μg/mL, respectively (Zhanel et al., 2016). Fosfomycin (*f* drug)

concentrations were determined in quadruplicate using *Bacillus subtilis* ATCC 6633 as the test organism and by modification of the agar diffusion method as previously described (Shimizu, 1997; Bergen, 1990). The correlation coefficient of this assay was 0.84. The intra-day and inter-day coefficients of variation were 3.5-6.9% and 2.5-6.1%, respectively. The $fAUC_{0\text{-}24}$ (μg.h/mL) for fosfomycin was calculated using the trapezoidal rule (Zhanel et al, 2014). The $fAUC_{0\text{-}24}$/MIC (free area under the curve over 24 hours relative to MIC) was calculated for fosfomycin against the specific isolates of *E. coli* studied. The in vitro pharmacodynamic model used in this study was a one-compartment model (Zhanel et al, 2014). Logarithmic phase cultures were diluted into fresh cation-supplemented Mueller Hinton broth supplemented with 25 μg/mL of glucose-6-phosphate to achieve a final inoculum of ~1 x 10^6 cfu/mL. Only *f* clinically achievable fosfomycin urinary concentrations were simulated. Pharmacodynamic experiments were performed in duplicate (on separate days) in ambient air at 37°C with sampling at 0, 1, 2, 4, 6, 12, 18, 24, and 48 h as previously described (Zhanel et al, 2014). The lowest dilution plated was 0.1 mL of undiluted sample and the lowest level of detection was 200 cfu/mL (20 colonies of 0.1 mL undiluted sample). Antimicrobial carryover was minimized by diluting samples withdrawn from the model or by repeated washing and centrifugation. No difference in antimicrobial carryover was observed between dilution and washing. Measurement of antibacterial effects was assessed as $\log_{10}$ changes in bacterial counts at 1, 2, 6, 12, 24, and 48 h with respect to time 0 (initial inoculum).

3. Results

Of the 12 isolates studied (one wild-type strain included), eight were ESBL CTX-M-producing urinary *E. coli* genotypes. All ESBL CTX-M-15 or CTX-M-14 genotypes demonstrated a MDR

phenotype with resistance to ceftriaxone and ciprofloxacin and frequently also trimethoprim-sulfamethoxazole, gentamicin, and/or doxycycline (Table 1). The carbapenemase-producing *E. coli* studied were KPC-3 (n=2) or NDM-1 (n=1) producing strains with MDR phenotypes and an ertapenem MIC ≥2 μg/mL (Table 1). All isolates were susceptible to fosfomycin with MICs ≤4 μg/mL (Table 1). All isolates were also susceptible to tigecycline and colistin (polymyxin E) (data not shown).

The achieved pharmacokinetic fosfomycin exposures in the central compartment of the pharmacodynamic model were within 6-12% of simulated pharmacokinetic values. The achieved fosfomycin mean (standard deviation; SD) pharmacokinetic parameters or exposures were fU_{max} 3900 (280) μg/mL, $t_{½}$ 5.8 (0.5) h and $fAUC_{0-24}$ ~29,000 (2800) μg.h/mL.

The pharmacodynamic parameters of fosfomycin achieved against MDR ESBL and carbapenamse-producing *E. coli* are displayed in Table 2. Fosfomycin $fT_{>MIC}$ of 100% was achieved versus all strains studied (Table 2). Fosfomycin $fAUC_{0-24}$ 7,250-29,000 (2800) μg.h/mL was achieved versus the isolates studied (Table 2). Fosfomycin $fAUC_{0-24}$ (μg.h/mL)/MIC (μg/mL) ≥7,250 and $fT_{>MIC}$ of 100% (fosfomycin MICs ≤4 μg/mL) resulted in bactericidal (reductions of ≥3 $\log_{10}$ CFU) activity at 1, 2, 6, 12, 24, and 48 h against all isolates tested (Table 3). In addition, achieved fosfomycin pharmacodynamics (when simulating urinary concentrations obtained after a single 3 gram oral dose) of $fAUC_{0-24}$ (μg.h/mL)/MIC (μg/mL) ≥7,250 and $fT_{>MIC}$ of 100% (fosfomycin MICs ≤4.0 μg/mL) resulted in complete eradication of all bacteria from the model at 2, 6, 12, 24, and 48 h against all isolates tested (Table 3). As all organisms were eradicated (and thus could not be grown to perform repeat MIC testing), it was assumed that no significant increase in fosfomycin MIC (≥4 fold) occurred with any isolate at any time point.

4. Discussion

It is well known that ESBL- and/or carbapenemase-producing *E. coli* possess a MDR phenotype yet are commonly susceptible to fosfomycin (Zhanel et al, 2016;Karlowsky et al, 2014;Falagas et al, 2010). In a previous study performed by our group we reported that fosfomycin demonstrated 99.4% susceptibility (fosfomycin MIC_{50} and MIC_{90} of 2 μg/mL and 4 μg/mL, respectively) versus 868 *E. coli* obtained from patients with urinary infection and 100% susceptibility versus 42 MDR ESBL-producing *E. coli* (Karlowsky et al, 2014). Fosfomycin as a single dose of 3 grams is recommended as first line treatment for acute uncomplicated cystitis, an infection primarily caused by *E. coli* (Gupta et al, 2011; Grigoryan et al, 2014). Despite the fact that fosfomycin is recommended as a potential first-line therapy for acute uncomplicated cystitis, little is known about the pharmacodynamics (rate and extent of bacterial inhibition/killing) of a single 3 gram oral dose of fosfomycin in urine. Our study is the first study that demonstrates that urinary concentrations achieved after a single 3 gram oral dose of fosfomycin results in rapid, bactericidal activity as early as 1 h after dosing. Complete eradication of all bacteria from the model was observed at 2, 6, 12, 24, and 48 h for all urinary isolates of *E. coli* studied (including MDR ESBL- and/or carbapenemase-producing strains). Our data help to explain the high (>90%) microbiological and clinical cure rates achieved with fosfomycin when used to treat patients with acute uncomplicated cystitis (Grigoryan et al, 2014).

Other than our current study, the available data regarding the rate and extent of bacterial inhibition/killing of *E. coli* and other organisms using fosfomycin as well as the pharmacodynamic parameters correlating with fosfomycin microbiological activity and clinical cure are limited (Mazzei et al, 2006;Walsh et al, 2015; Docobo-Perez et al, 2015; VanScoy et al,

2015; VanScoy et al, 2016). Mazzei *et al*. evaluated the in vitro pharmacodynamics of fosfomycin versus both *E. coli* and *Proteus mirabilis* (Mazzei et al, 2006). Using kill curves they reported that fosfomycin demonstrated concentration-dependent antibacterial activity with a concentration of ≥4-times the MIC resulting in bactericidal activity with no regrowth over 24 h. Similar experiments performed with *P. mirabilis* showed fosfomycin demonstrated concentration-dependent activity with a concentration of ≥8-times MIC resulting in bactericidal activity with no regrowth over the 24 h period (Mazzei et al, 2006). In addition, the postantibiotic effect (PAE) of fosfomycin was found to be dose-dependent with 4x MIC resulting in a PAE of 3.66 and 3.53 h versus *E. coli* and *P. mirabilis*, respectively. Walsh and colleagues assessed the in-vitro pharmacodynamics of fosfomycin versus *Pseudomonas aeruginosa* using time kill studies, population analysis profiles (PAPs), and PAE (Walsh et al, 2015). Time kill studies demonstrated moderate time-dependent killing at low inoculum (~1 x 10^6 cfu/mL) with virtually no killing at the high inoculum (~1 x 10^8 cfu/mL). Assessment of PAPs after fosfomycin exposure demonstrated the development of fosfomycin-resistant colonies versus fosfomycin-susceptible colonies. Of interest, baseline analysis (before fosfomycin exposure) identified fosfomycin heteroresistance in all *P. aeruginosa* isolates studied (Walsh et al, 2015). PAEs were concentration dependent ranging from 0.3 to 5.5 h. Docobo-Perez and coworkers assessed the in vitro pharmacokinetic-pharmacodynamic (PK-PD) relationships of fosfomycin versus ESBL-producing *E. coli* using a hollow fiber infection model (Docobo-Perez et al, 2015). The researchers simulated clinically achievable serum concentration after intravenous fosfomycin dosing at doses of 12, 24, and 36 grams per day. With the 24 gram per day dose ($fAUC_{0\text{-}24}$/MIC, ≥2,404.61) whether administered once a day or in divided doses resulted in complete eradication of the inoculum at 24 h with no subsequent regrowth with repeated dosing

(Docobo-Perez et al, 2015). Lower doses (12-18 grams per day) resulted in the overgrowth of high-level fosfomycin resistant mutants. $fAUC_{0-24}$/MIC was found to be the PK-PD linked index of suppression of bacterial resistance (Docobo-Perez et al, 2015). VanScoy *et al.* assessed the in vitro PK-PD relationships of fosfomycin versus *E. coli* using a one compartment in vitro infection model (VanScoy et al, 2015; VanScoy et al, 2016). The primary objective of their study was to identify the PK-PD index that best predicted efficacy of fosfomycin versus *E. coli.* It was observed in the mutational studies that even when using a standard inoculum for MIC testing, that an inherently fosfomycin resistant subpopulation of *E. coli* was present with MICs of 32-64 μg/mL versus the wild-type MIC of 1 μg/mL (VanScoy et al, 2015). The researchers reported that $fAUC_{0-24}$/MIC was the PK-PD index most predictive of efficacy, however, given that the majority of $fT_{>MIC}$ values were 100%, this PK-PD parameter could not be adequately explored (VanScoy et al, 2015). The researchers then found that free fosfomycin concentration above the drug resistant subpopulation (i.e., %T>RIC) was the PK-PD index most closely associated with fosfomycin efficacy (VanScoy et al, 2015). These same researchers also reported that fosfomycin exposures simulating human serum concentrations obtained after administration of fosfomycin 4 grams IV every 8 hours (Cmax ~230 μg/mL) did not select *E. coli* subpopulations with reduced fosfomycin susceptibility, however, fosfomycin exposures of 2 grams every 8 hours did (VanScoy et al, 2015; VanScoy et al, 2016). They also demonstrated that $fAUC_{0-24}$/MIC of ~1000 lead to maximal bacterial reduction. As we obtained obtained fosfomycin exposures $fAUC_{0-24}$ (μg.h/mL)/MIC (μg/mL) ≥ 7,250, it is not surprising that we observed rapid and extensive bacterial killing with no regrowth.

Thus, there appears at this time to be some confusion in the literature as to whether fosfomycin displays time or concentration dependent bactericidal activity (Walsh et al, 2016).

What has consistently been reported is that fosfomycin does demonstrate bactericidal activity, which is consistent with agents that inhibit peptidoglycan synthesis. As in our study, we simulated fosfomycin urinary concentrations only after a single 3 gram dose (and did not vary the dose or administer multiple doses) our study does not contribute to the literature to address whether fosfomycin displays time or concentration dependent bactericidal activity, but we do clearly show that fosfomycin is a rapidly and extensively bactericidal agent versus *E. coli* when simulating the very high urinary concentrations (considerably higher concentrations than what VanScoy et al, 2015 simulated in serum) that occur clinically when fosfomycin is administered as a single 3 gram oral dose for the treatment of acute uncomplicated cystitis.

Our study suffers from several limitations including the fact that we simulated urinary pharmacokinetic parameters reported in healthy volunteers after a single 3 gram oral dose of fosfomycin as we could not find such data in patients with acute uncomplicated cystitis (Patel et al, 1997; Bergan, 1990; Keating, 2013). Fosfomycin pharmacokinetic parameters obtained from healthy individuals may not necessary reflect pharmacokinetics in patients with disease states. In addition, we used fosfomycin pharmacokinetic parameters such as $t/_{1/2}$ and clearance from serum which may provide surrogates of of fosfomycin exposures reflective of those in bladder and urine. Given the physiology at these sites, which is subject to assumptions for drug accumulation in the bladder and voiding times, fluctuating concentration-time profiles would be expected. Due to these differences in concentration-time profiles at these sites, it is unclear at this time whether fosfomycin pharmacokinetic parameters from serum are truly applicable to the bladder and urine. As well, we only studied urinary concentrations of fosfomycin obtained after a single 3 gram oral dose and not multiple dosing or higher dosages, as we wanted to assess the rate and extent of *E. coli* inhibition/killing by urinary concentrations obtained after a single 3 gram oral dose which is

the recommended fosfomycin treatment regimen for treating acute uncomplicated cystitis (Gupta et al, 2011; Grigoryan et al, 2014). In addition, we assumed that all fosfomycin was acting as free drug owing to its previously documented low protein binding, whether this is actually the case is not known (Patel et al, 1997; Bergan, 1990; Keating, 2013). Another limitation of our study was that we simulated a fU_{max} of ~4000 μg/mL at the start of the experiment whereas in human volunteers the peak urinary concentration is typically not achieved until ~4 hours after dosing (Patel et al, 1997). We believe that this minor difference in time to peak urinary concentration would have had limited effect of the extent of bacterial eradication but may have reduced the rate of killing from 2 hours to 4 or 6 hours after dosing. In addition, it should be stated that we added glucose-6-phosphate to the media in our model (as per CLSI guidelines), however, glucose-6-phosphate is absent in human urine, which may affect fosfomycin pharmacodynamics in patients with urinary tract infections. We also used only a single inoculum of ~1 x 10^6 cfu/mL as this is a widely used and accepted inoculum in in-vitro pharmacodynamics studies (7), but may not necessarily represent the inoculum in a patient with acute uncomplicated cystitis and may not be optimal if one is trying to select out *E. coli* heteroresistance to fosfomycin. Finally, we only studied urinary *E. coli* isolates that were susceptible to fosfomycin with MICs 1-4 μg/mL because they represent the most common *E. coli* urinary isolates obtained in Canada (MIC_{50}, 2 μg/mL; MIC_{90}, 4 μg/mL) (Karlowsky et al, 2014), rather than testing isolates that were fosfomycin susceptible but with fosfomycin MICs of 8, 16, 32, and 64 μg/mL (Karlowsky et al, 2014; CLSI, 2015). These studies are currently ongoing by our group.

We conclude that fosfomycin urinary concentrations obtained after a single 3 gram oral dose were bactericidal as early as 1 h after dosing with complete bacterial eradication at all time-points over the 48 h testing period against urinary isolates of *E. coli* (including MDR ESBL-

and/or carbapenemase-producing strains). Our data help to explain the high (>90%) microbiological and clinical cure rates achieved with fosfomycin when used as a single 3 gram oral dose to treat patients with acute uncomplicated cystitis (14).

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Transparency Declarations

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References

1. Bergan T. 1990. Degree of absorption pharmacokinetics of fosfomycin trometamol and duration of urinary antibacterial activity. Infection 18(Suppl 2): 65-69.
2. Clinical and Laboratory Standards Institute. 2016. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Sixth Informational Supplement. M100-S26. CLSI, Wayne, PA, USA.
3. Clinical and Laboratory Standards Institute. 2015. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically – Tenth Edition: Approved Standard M07-A10. CLSI, Wayne, PA, USA.
4. Denisuik AJ, Lagacé-Wiens P, Pitout JD, Mulvey MR, Simner PJ, Tailor F, et al. 2013. Molecular epidemiology of extended-spectrum β-lactamase-, AmpC β-lactamase-, and carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from Canadian hospitals over a 5 Year Period: CANWARD 2007-2011. J Antimicrob Chemother 68 (Suppl 1): 57-65.
5. Docobo-Perez F, Drusano GL, Johnson A, Goodwin J, Whalley S, Ramos-Martín V, et al. 2015. Pharmacodynamics of fosfomycin: insights into clinical use for antimicrobial resistance. Antimicrob Agents Chemother 59: 5602-5610.
6. Falagas ME, Kastoris A, Kapaskelis AM, Karageorgopoulos DE. 2010. Fosfomycin for the treatment of multi-drug resistant including extended-spectrum β-lactamase producing Enterobacteriaceae infections: a systemic review. Lancet Infect Dis 10: 43-50.
7. Grigoryan L, Trautner BW, Gupta K. 2014. Diagnosis and management of urinary tract infections in the outpatient setting. JAMA 312(16): 1677-1684.
8. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. 2011. International

clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 52: 103-120.

9. Karlowsky JA, Denisuik AJ, Lagacé-Wiens PR, Adam HJ, Baxter MR, Hoban DJ, Zhanel GG. 2014. In vitro activity of fosfomycin against *Escherichia coli* isolated from patients with urinary tract infections in Canada as part of the CANWARD surveillance study. Antimicrob Agents Chemother 58: 1252-1256.
10. Keating GM. 2013. Fosfomycin trometamol: A review of its use as a single dose oral treatment for patients with acute lower urinary tract infections and pregnant women with asymptomatic bacteriuria. Drugs 73: 1951-1966.
11. Mazzei T, Cassetta MI, Fallani S, Arrigucci S, Novelli A. 2006. Pharmacokinetic and pharmacodynamics aspects of antimicrobial agents for the treatment of uncomplicated urinary tract infections. Int J Antimicrob Agents 28: 35-41.
12. Patel SS, Balfour JA, Bryson HM. 1997. Fosfomycin tromethamine: a review of its antibacterial activity pharmacokinetic properties and therapeutic efficacy as a single-dose oral treatment for acute uncomplicated lower urinary tract infections. Drugs 53: 637-656.
13. Shimizu K. 1977. Fosfomycin: absorption and excretion. Chemother 23(Suppl 1): 153-158.
14. Walsh CC, McIntosh MP, Peleg AY, Kirkpatrick CM, Bergen PJ. 2015. In vitro pharmacodynamics of fosfomycin against clinical isolates of *Pseudomonas aeruginosa*. J Antimicrob Chemother 70: 3042-3050.
15. VanScoy BD, McCauley J, Ellis-Grosse EJ, Olanrewaju OO, Bhavnani SM, Forrest A, Ambrose PG. 2015. Exploration of the pharmacokinetic-pharmacodynamic relationships for

fosfomycin efficacy using an in vitro infection model. Antimicrob Agents Chemother 59: 7170-7177.

16. VanScoy BD, McCauley J, Bhavnani SM, Ellis-Grosse EJ, Ambrose PG. 2016. Relationship between fosfomycin exposure and amplification of *Escherichia coli* subpopulations with reduced susceptibility in a hollow-fiber infection model. Antimicrob Agents Chemother 60: 5141-5145.
17. Zhanel GG, Adam H, Baxter M, Fuller J, Nichol KA, Denisuik AJ, et al. 2013. Antimicrobial susceptibility of 22,746 pathogens from Canadian hospitals: Results of the CANWARD 2007-2011 study. J Antimicrob Chemother 68 (Suppl 1): 7-22.
18. Zhanel GG, Denisuik A, Vashisht S, Yachison C, Adam HJ, Hoban DJ. 2014. Pharmacodynamic activity of ertapenem versus genotypically characterized extended spectrum β-lactamase (ESBL) or KPC or NDM producing *Escherichia coli* with reduced susceptibility or resistance to ertapenem using an in-vitro model. J Antimicrob Chemother 69: 2448-2452.
19. Zhanel GG, Walkty AY, Karlowsky JA. 2016. Fosfomycin A first-line oral therapy for acute uncomplicated cystitis. Can J Infect Dis Med Microbiol 2016, Article ID 2082693.

Table 1
Fosfomycin and comparator agent MICs for ESBL- and carbapenemase-producing *E. coli*

	MIC, μg/mL				
Strain	Fosfomycin	Ciprofloxacin	Trimethoprim-Sulfamethoxazole	Gentamicin	Ertapenem
79768	1	0.06	0.12	1	0.03
80083	2	>16	>8	32	0.25
80960	4	>16	>8	0.5	1
85332	2	>16	0.12	>32	0.12
88273	2	>16	0.12	0.5	0.5
89439	1	>16	>8	>32	1
90087	2	>16	>8	0.5	0.5
90789	2	>16	>8	32	2
92969	4	>16	4	32	2
95882	2	>16	>8	2	4
N-10-1631	4	>16	>8	0.5	>32
ECMH01	1	>16	>8	>32	>32

Table 2
Fosfomycin pharmacodynamic parameters achieved

Strain	*E. coli* Genotype	Fosfomycin MIC (μg/mL)	Fosfomycin $fT_{>MIC}$ h [%]	Fosfomycin $fAUC_{0-24}$/MIC
79768	wild type	1	24 [100]	29,000
80083	CTX-M-15,OXA-1	2	24 [100]	14,500
80960	CTX-M-15,TEM-1	4	24 [100]	7,250
85332	CTX-M-14,TEM-1	2	24 [100]	14,500
88273	NDM-1	2	24 [100]	14,500
89439	CTX-M-15,TEM-1,OXA-1	1	24 [100]	29,000
90087	CTX-M-15,OXA-1	2	24 [100]	14,500
90789	CTX-M-15,OXA-1	2	24 [100]	14,500
92969	KPC-3,TEM-1	4	24 [100]	7,250
95882	CTX-M-15,OXA-1	2	24 [100]	14,500
N-10-1631	KPC-3,TEM-1	4	24 [100]	7,250
ECMH01	CTX-M-15,OXA-1	1	24 [100]	29,000

Table 3
Fosfomycin reductions in log_{10} CFU relative to baseline of *E. coli* when simulating urinary concentrations after a single 3 gram oral dose [a]

	Log_{10} killing at:					
Strain (Fosfomycin MIC, µg/mL)	1h	2 h	6 h	12 h	24 h	48h
79768 (1)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
80083 (2)	3.2	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
80960 (4)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
85332 (2)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
89439 (1)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
90087 (2)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
90789 (2)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
92969 (4)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
95882 (2)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
N-10-1631 (4)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
ECMH01 (1)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0

[a] = growth reduction relative to initial inoculum.