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Pharmacodynamic Activity of Fosfomycin versus Multidrug-Resistant (MDR) Genotypically Characterized Extended Spectrum ß-lactamase (ESBL) - and/or Carbapenemase-Producing *Escherichia coli* using an *In vitro* Model

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ABSTRACT

Background: *E. coli* is the most common cause of urinary tract infections. *E. coli* are now frequently ESBL-producing and some isolates may also be resistant to carbapenems; both of these resistance phenotypes are commonly associated with isolates with MDR profiles. Fosfomycin (FOS) inhibits peptidoglycan synthesis by a mechanism distinct from β-lactams and is available orally for the treatment of urinary tract infections caused by *E. coli*. The current study assessed the pharmacodynamic (PD) activity of FOS against molecularly characterized MDR ESBL- and/or carbapenemase-producing *E. coli* using an in vitro PD model (IVPM).

Methods: 8 ESBL-producing and 3 carbapenemase-producing *E. coli* were studied. ESBL-producing strains were CTX-M-15 or CTX-M-14 genotypes and demonstrated a MDR phenotype with resistance to ceftriaxone, ciprofloxacin, TMP-SMX, gentamicin and doxycycline. The carbapenemase-producing *E. coli* studied were KPC-3 (n=2) or NDM-1 (n=1) producing strains with a MDR phenotype and ertapenem MICs ≥2 mg/L. The IVPM was inoculated with an inoculum of (1x10^6 CFU/mL). FOS was dosed once daily at 0 hours to simulate free (*f*) urine (U) maximum concentrations and a $t_{1/2}$ obtained after a standard 3 gram oral dose in healthy volunteers (*f*Umax 4000 mg/L; $t_{½}$ 6 hrs). Sampling was performed over 24 h to assess viable growth.

Results: FOS MICs ranged from 1-4 mg/L for ESBL producers, while all 3 carbapenemase-producing *E. coli* demonstrated FOS MICs of 2 mg/L. FOS PD parameters $T_{>MIC}$ 100% resulted in bacterial killing (log_{10} killing assessed relative to the starting inoculum at 1, 2, 4, 6, 12 and 24 hours) of ≥4.0, ≥4.0, ≥4.0, ≥4.0, ≥4.0 and ≥4.0, respectively, versus all ESBL-producing and carbapenemase-producing *E. coli*. No significant regrowth occurred over the 24 h study period.

Conclusion: Simulated FOS urine concentrations obtained after a 3 gram single dose were bactericidal as early as 1 hour with complete bacterial eradication at all time points over the 24 hour testing period against MDR ESBL - and/or carbapenemase-producing *E. coli.*

INTRODUCTION

E. coli is the most common cause of urinary tract infections. Extended-spectrum ß-lactamase (ESBL)-producing as well as carbapenem-resistant *E. coli* have rapidly spread in the community, extended-care facilities and hospital settings.[1-3] ESBL-producing *E. coli* are frequently multi-drug resistant (MDR, defined as resistant to 3rd generation cephalosporins and ≥ 2 other chemically unrelated antimicrobial classes).[1-3] Fosfomycin inhibits peptidoglycan synthesis by a mechanism distinct from β-lactams and is available orally for the treatment of urinary tract infections caused by *E. coli*. *In vitro,* fosfomycin is very active versus ESBL and MDR *E. coli* with MIC_{50}s and MIC_{90}s of 2 and 4 mg/L, respectively.[4] Little data are available regarding the pharmacodynamics of fosfomycin against MDR ESBL-producing or carbapenem-resistant *E. coli.*

PURPOSE

This study assessed the pharmacodynamic activity of fosfomycin against molecularly characterized MDR ESBL - and/or carbapenemase-producing *E. coli* using an *in vitro* pharmacodynamic model.

MATERIALS & METHODS

Bacterial strains and culture conditions

The *E. coli* isolates were obtained from the CANWARD study (www.can-r.ca), a national, ongoing Health Canada endorsed surveillance study assessing antimicrobial resistance in Canadian hospitals.[2,3] In the CANWARD study, any *E. coli* with a ceftriaxone MIC ≥ 1 mg/L was identified as a putative ESBL.[3] Putative ESBL phenotypes were confirmed by the disk diffusion method as described by Clinical and Laboratory Standards Institute (CLSI). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were the control strains. Genotypic characterization of ESBLs was performed by PCR and sequencing of bla_{SHV}, bla_{TEM}, bla_{CTX}, bla_{OXA} and bla_{VEB} genes as previously described.[3] A BLAST search of the DNA sequence was conducted to determine the specific ESBL genotype. All putative carbapenemase-producing *E. coli* 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 *et al.*[3] We selected a wild-type strain (non-ESBL and non carbapenem-resistant) and eight MDR ESBL producing *E. coli.* ESBL-producing strains were CTX-M-15 or CTX-M-14 genotypes and demonstrated a MDR phenotype with resistance to ceftriaxone, ciprofloxacin, TMP-SMX, gentamicin and/or doxycycline. The carbapenemase-producing *E. coli* studied were KPC-3 (n=2) or NDM-1 (n=1) producing strains with a MDR phenotype and ertapenem MICs ≥2 mg/L.

For pharmacodynamic studies, logarithmic phase cultures at 0.5 McFarland (1 x 10^8 CFU/mL) in cation-supplemented Mueller Hinton broth were prepared as previously described.[5] Viable bacterial counts consistently yielded a starting inoculum of approximately 1x10^6 CFU/mL. A growth control was included in every experiment. Growth controls peaked at ~ 1-5 x 10^9 CFU/mL and were maintained over the 24 h experiment.

Antibiotic preparations and susceptibility testing

Antibiotic agents were obtained as laboratory-grade powders from their respective manufacturers (Fosfomycin, Paladin labs, Montreal, Quebec). Stock solutions were made according to the Clinical and Laboratory Standards Institute-CLSI M7-A6 method. MICs were determined by the CLSI-approved broth microdilution method. All MICs were performed in triplicate on separate days.[2]

Pharmacokinetics of fosfomycin in the *in vitro* pharmacodynamic model

Experiments were performed simulating peak urine concentrations (U_{max}) of fosfomycin, achieved in human urine after a standard 3 gram single dose (Table 1).[6] Peak fosfomycin urinary concentrations of ~4,000 mg/L and clearance simulated using a reported serum half-life of 6 h.[6] The pharmacokinetics of fosfomycin were evaluated after administration of a single 3 gram dose administered into the central compartment and sampling from this compartment at 0, 1, 2, 4, 6, 12 and 24 h. Fosfomycin concentrations were determined using a modification of the bioassay procedure described by Shimizu.[7]

***In vitro* pharmacodynamic model/pharmacodynamic experiments**

The *in vitro* pharmacodynamic model used in this study has been previously described.[5] Logarithmic phase cultures were diluted into fresh cation-supplemented Mueller Hinton broth to achieve a final inoculum of approximately 1x10^6 CFU/mL. Clinically achievable urinary concentrations were simulated.[6] Pharmacodynamic experiments were performed in duplicate (on separate days) in ambient air at 37˚ C at 0, 1, 2, 4, 6, 12 and 24 h as previously described.[5] 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). Antibiotic carryover was minimized by diluting samples withdrawn from the model or by repeated washing and centrifugation. No difference in antibiotic carryover was observed between dilution and washing. Measurement of antibacterial effects was assessed as log_{10} changes in bacterial counts at 1, 2, 4, 6, 12 and 24 h with respect to time 0.

RESULTS

Figure 1. Fosfomycin Killing of *E. coli* Strain 80960

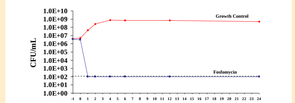

Figure 2. Fosfomycin Killing of *E. coli* Strain 85332

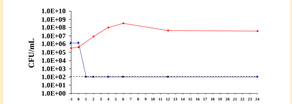

Figure 3. Fosfomycin Killing of *E. coli* Strain 89439

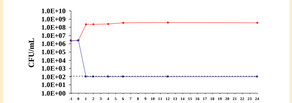

Figure 4. Fosfomycin Killing of *E. coli* Strain 90789

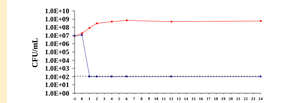

Figure 5. Fosfomycin Killing of *E. coli* Strain ECMH01

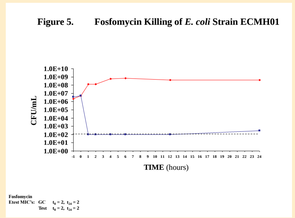

Table 1. Fosfomycin and comparator MICs (mg/L) of ESBL-and carbapenemase-producing *E. coli* inocula

Strain	Fosfomycin	Ciprofloxacin	TMP/SMX	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 simulated

Strain	*E. coli* Genotype	Fosfomycin MIC (mg/L)	Fosfomycin $T_{>MIC}$ h [%]	Fosfomycin Umax /MIC
79768	wild type	1	24 [100]	3800
80083	CTX-M-15,OXA-1	2	24 [100]	1900
80960	CTX-M-15,TEM-1	4	24 [100]	950
85332	CTX-M-14,TEM-1	2	24 [100]	1900
88273	CTX-M-15,TEM-1,OXA-1	2	24 [100]	1900
89439	CTX-M-15,OXA-1	1	24 [100]	3800
90087	CTX-M-15,OXA-1	2	24 [100]	1900
90789	KPC-3,TEM-1	2	24 [100]	1900
92969	CTX-M-15,OXA-1	4	24 [100]	950
95882	KPC-3,TEM-1	2	24 [100]	1900
N-10-1631	CTX-M-15,OXA-1	4	24 [100]	950
ECMH01	NDM-1	1	24 [100]	3800

Table 3. Fosfomycin killing of *E. coli* simulating urinary concentration Log_{10} killing at 1, 2, 6, 12 and 24 h, respectively[a]

Strain (Fosfomycin MIC, mg/L)	1h	2h	6h	12h	24h
79768 (1)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
80083 (2)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
80960 (4)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
85332 (2)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
89439 (2)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
90087 (2)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
90789 (2)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
92969 (4)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
95882 (2)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
N-10-1631 (4)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0
ECMH01 (1)	≥4.0	≥4.0	≥4.0	≥4.0	≥4.0

[a] = growth reduction relative to initial inoculum

CONCLUSIONS

1. Fosfomycin MICs vs MDR ESBL - and carbapenemase-producing *E. coli* were 1-4 mg/L.
2. Fosfomycin 1 gram daily was bactericidal at all timepoints over the 24 hour testing period.
3. Simulated fosfomycin urine concentrations obtained after a 3 gram single dose were bactericidal as early as 1 hour with complete bacterial eradication at all time points over the 24 hour testing period against MDR ESB L- and/or carbapenemase-producing *E. coli.*

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