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In Vitro Activity of Fosfomycin Against Bacterial Pathogens Isolated from Outpatient Urine Specimens in Canada from 2007 to 2013

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REVISED ABSTRACT

Background: In North America, fosfomycin (FOS) tromethamine is indicated for the treatment of uncomplicated urinary tract infections in women caused by *Escherichia coli* and *Enterococcus faecalis*. FOS has been shown to inactivate the enzyme UDP-*N*-acetylglucosamine-3-enolpyruvyltransferase (MurA) which ligates phosphoenolpyruvate (PEP) to the 3'-hydroxyl group of UDP-*N*-acetylglucosamine in peptidoglycan synthesis. FOS reference MIC antimicrobial susceptibility testing (AST) is rarely performed in clinical laboratories because the CLSI agar dilution method must be used. MIC data documenting the activity of FOS against outpatient urinary pathogens other than *E. coli* and *E. faecalis* are limited.

Methods: The isolates tested were cultured from urine specimens of outpatients attending emergency departments (EDs) and submitted to the annual CANWARD surveillance study from 2007 to 2013. FOS AST was performed using CLSI agar dilution testing (MHA supplemented with 25 µg/ml of glucose-6-phosphate; M100-S24 [2014]); all other antibacterial agents were tested using the CLSI broth microdilution method. MICs were interpreted using M100-S24 (2014) criteria. FOS susceptible (≤64 µg/ml), intermediate (128 µg/ml), and resistant (≥256 µg/ml) breakpoints were used to interpret MICs for all isolates tested.

Results: The table shows MIC_{90} (µg/ml) and % susceptible data for oral antimicrobial agents.

Organism (n)	MIC_{90} (µg/ml) / % Susceptible				
	FOS	SXT	NIT	CIP	AMC
E. coli (487)	4 / 99.6	>8 / 82.5	32 / 97.9	8 / 89.5	8 / 97.5
K. pneumoniae (98)	64 / 90.8	0.25 / 95.9	128 / 17.0	0.12 / 98.0	4 / 96.3
E. faecalis (50)	128 / 82.0	≤0.12 / NA	16 / 95.5	>16 / 62.5	1 / 100*
Pseudomonas aeruginosa (16)	256 / 37.5	>8 / NA	>512 / NA	>16 / 62.5	>32 / NA
Staphylococcus aureus (13)	32 / 100	≤0.12 / 100	16 / 100	>16 / 61.5	NA / 69.2**
Proteus mirabilis (11)	64 / 90.9	>8 / 81.8	128 / 0	2 / 81.8	16 / 90.9
Enterobacter cloacae (7)***	2->512 / 71.4	≤0.12-8 / 85.7	64-128 / 0	≤0.06-16 / 71.4	8->32 / 14.3
Klebsiella oxytoca (6)***	8-128 / 66.6	≤0.12 / 100	32 / 100	≤0.06 / 100	2 / 100
Citrobacter freundii (4)***	≤1-4 /100	≤0.12-4 / 75.0	16 / 100	≤0.06->16 / 75.0	1-32 / 50.0

Abbreviations: SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin; CIP, ciprofloxacin; AMC, amoxicillin-clavulanate; NA, not applicable. *AMC activity was predicted by testing ampicillin for *E. faecalis*. **AMC activity was predicted by testing cefoxitin for *S. aureus*. ***MIC range provided in place of MIC_{90} for organisms with <10 isolates.

Conclusion: The in vitro activities of SXT (82.5%) and CIP (89.5%), two frequently prescribed empiric agents for urinary tract infections, were compromised for outpatient urine isolates of *E. coli* compared to FOS (99.6% susceptible; 0% resistant). Only 2% of *E. faecalis* isolates were resistant to FOS (82.0% susceptible). FOS demonstrated broad spectrum activity against aerobic gram-negative (Enterobacteriaceae) and gram-positive (enterococci, staphylococci) pathogens frequently isolated from urinary tract infections of Canadian outpatients attending EDs.

MATERIALS & METHODS

Bacterial isolates. The isolates tested were cultured from urine specimens of outpatients attending emergency departments and submitted to the annual CANWARD surveillance study from 2007 to 2013 (4). Primary isolate identification was performed by the submitting site. If an isolate identification made by the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada) using morphological characteristics and spot tests (5) was not consistent with that provided by the submitting site, the isolate was removed from the study.

Antimicrobial susceptibility testing. Fosfomycin antimicrobial susceptibility testing was performed using CLSI agar dilution testing (MHA supplemented with 25 µg/ml of glucose-6-phosphate; M100-S24 [2014]); all other antibacterial agents were tested using in-house-prepared 96-well broth microdilution panels according to CLSI guidelines (3, 6). Fosfomycin was supplied by Triton Pharma Inc. (Concord, Ontario, Canada). Stock solutions and dilutions were prepared as described by the CLSI (M07-A9, 2012), in cation-adjusted Mueller-Hinton broth (MHB) (6). Quality control was performed following CLSI recommendations and minimum inhibitory concentrations (MICs) were interpreted using CLSI M100-S24 (2014) breakpoints (3). Fosfomycin-resistant isolates were each retested to confirm their phenotype. ESBLs were identified following CLSI guidelines (3) and multidrug-resistant (MDR) isolates were defined using a published guideline (7).

BACKGROUND

The currently recommended empiric antimicrobial regimen for treating acute uncomplicated bacterial cystitis in otherwise healthy adult non-pregnant females is a five-day course of nitrofurantoin, a three-day course of double-strength trimethoprim-sulfamethoxazole (SXT) in settings where the prevalence of SXT resistance is <10-20%, or a 3g single dose of fosfomycin tromethamine; fluoroquinolones and β-lactams, such as amoxicillin-clavulanate, are second-line therapies (1). High urine concentrations (706 [± 466] µg/ml, 2-4 hours following a single oral 3g dose) of fosfomycin and its potentially higher rate of patient compliance compared with agents dosed for 3-5 days, likely underlie its reported low rate of resistance development among *Escherichia coli* (2). Currently, CLSI-approved susceptibility breakpoints for fosfomycin exist only for *Escherichia coli* and *Enterococcus faecalis* with a MIC ≤64 ug/ml considered susceptible (resistance, ≥256 ug/ml) and approved only for testing of isolates from urinary tract infections (3). EUCAST also publishes MIC breakpoints for fosfomycin for staphylococci and Enterobacteriaceae with a MIC ≤32 ug/ml considered susceptible for both parenteral (systemic infections) and oral (uncomplicated urinary tract infection only) (resistance, >32 ug/ml).

Fosfomycin, an agent known for >40 years, has received renewed interest recently because of resistance to traditionally used agents. However, there is a paucity of published in vitro MIC testing data for fosfomycin because it must be tested by the agar dilution method (3). Recent North American MIC data documenting the activity of fosfomycin against outpatient urinary pathogens other than *E. coli* and *E. faecalis* are very limited. Observed and potential increases in antimicrobial resistance among urinary tract pathogens suggest fosfomycin may be given consideration in the treatment of uncomplicated urinary tract infections caused by pathogens other than *E. coli* and *E. faecalis*.

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RESULTS

Table 1. In vitro activities of fosfomycin and comparative antimicrobial agents against outpatient urine isolates collected by 15 laboratories in Canada from 2007 to 2013.

Organism (n)	Antimicrobial agent	(µg/ml) MIC_{50}	(µg/ml) MIC_{90}	(µg/ml) MIC range	CLSI MIC Interpretation[a] % Susceptible	CLSI % Intermediate	CLSI % Resistant	EUCAST MIC Interpretation[b] % Susceptible	EUCAST % Intermediate	EUCAST % Resistant
E. coli (487)[c]	Fosfomycin	≤1	4	≤1-128	99.6	0.4	0	98.2	-	1.8
	SXT	≤0.12	>8	≤0.12->8	82.5	-	17.5	82.5	0.2	17.3
	Nitrofurantoin	16	32	4-128	97.9	1.7	0.4	99.6	-	0.4
	Ciprofloxacin	≤0.06	8	≤0.06->16	89.5	0.2	10.3	88.7	0.8	10.5
	Amoxicillin-clavulanate	4	8	≤0.06->32	97.5	1.6	0.9	99.7	-	0.3
K. pneumoniae (98)[c]	Fosfomycin	32	64	2->512	90.8	3.1	6.1	66.3	-	33.7
	SXT	≤0.12	0.25	≤0.12->8	95.9	-	4.1	95.9	0	4.1
	Nitrofurantoin	128	128	8->512	17.0	24.5	58.5	41.5	-	58.5
	Ciprofloxacin	≤0.06	0.12	≤0.06->16	98.0	1.0	1.0	98.0	0	2.0
	Amoxicillin-clavulanate	2	4	1->32	96.3	2.4	1.3	98.8	-	1.7
E. faecalis (50)	Fosfomycin	64	128	32->512	82.0	16.0	2.0	-	-	-
	SXT	≤0.12	≤0.12	≤0.12-2	NA	NA	NA	UD[d]	UD	UD
	Nitrofurantoin	8	16	8-64	95.5	4.5	0	100	-	0
	Ciprofloxacin	1	>16	0.25->16	62.5	10.4	27.1	72.9	-	27.1
	Amoxicillin-clavulanate	0.5	1	0.25-2	NA	NA	NA	100	0	0
Pseudomonas aeruginosa (16)	Fosfomycin	128	256	4->512	37.5	25.0	37.5	-	-	-
	SXT	8	>8	2->8	NA	NA	NA	-	-	-
	Nitrofurantoin	>512	>512	>512	NA	NA	NA	-	-	-
	Ciprofloxacin	0.25	>16	0.12->16	62.5	0	37.5	56.3	6.2	37.5
	Amoxicillin-clavulanate	>32	>32	>32	NA	NA	NA	-	-	-
Staphylococcus aureus (13)	Fosfomycin	16	32	≤1-32	100	0	0	100	-	0
	SXT	≤0.12	≤0.12	≤0.12	100	-	0	100	0	0
	Nitrofurantoin	16	16	8-16	100	0	0	100	-	0
	Ciprofloxacin	0.5	>16	0.25->16	61.5	0	38.5	61.5	-	38.5
	Amoxicillin-clavulanate	NA	NA	NA	69.2	-	30.8	-	-	-
Proteus mirabilis (11)	Fosfomycin	8	64	2-256	90.9	0	9.1	81.8	-	18.2
	SXT	≤0.12	>8	≤0.12->8	81.8	-	18.2	81.8	0	18.2
	Nitrofurantoin	128	128	128	0	0	100	0	-	100
	Ciprofloxacin	≤0.06	2	≤0.06->16	81.8	9.1	9.1	81.8	0	18.2
	Amoxicillin-clavulanate	1	16	0.5-16	90.9	9.1	0	100	0	0
Enterobacter cloacae (7)	Fosfomycin	UD	UD	2->512	71.4	14.3	14.3	71.4	-	28.6
	SXT	UD	UD	≤0.12-8	85.7	-	14.3	85.7	0	14.3
	Nitrofurantoin	UD	UD	64-128	0	66.6	33.4	66.6	-	33.4
	Ciprofloxacin	UD	UD	≤0.06-16	71.4	14.3	14.3	71.4	0	28.6
	Amoxicillin-clavulanate	UD	UD	8->32	14.3	14.3	71.4	28.6	-	71.4
Klebsiella oxytoca (6)	Fosfomycin	UD	UD	8-128	66.6	33.4	0	50.0	-	50.0
	SXT	UD	UD	≤0.12	100	-	0	100	0	0
	Nitrofurantoin	UD	UD	32	100	0	0	100	-	0
	Ciprofloxacin	UD	UD	≤0.06	100	0	0	100	0	0
	Amoxicillin-clavulanate	UD	UD	2	100	0	0	100	-	0
Citrobacter freundii (4)	Fosfomycin	UD	UD	≤1-4	100	0	0	100	-	0
	SXT	UD	UD	≤0.12-4	75.0	-	25.0	75.0	25.0	0
	Nitrofurantoin	UD	UD	16	100	0	0	100	-	0
	Ciprofloxacin	UD	UD	≤0.06->16	75.0	0	25.0	75.0	0	25.0
	Amoxicillin-clavulanate	UD	UD	1-32	50.0	25.0	25.0	100	-	0

[a] CLSI breakpoints for fosfomycin are only available for *E. coli* (UTI only) and *E. faecalis* (UTI only): MIC ≤64 ug/ml = susceptible, MIC 128 ug/ml = intermediate, and MIC ≥256 µg/ml = resistant.
[b] EUCAST breakpoints for fosfomycin for Enterobacteriaceae (uncomplicated UTI only) and *Staphylococcus* (intravenous): MIC ≤32 ug/ml = susceptible and >32 ug/ml = resistant. EUCAST does not publish MIC breakpoints for *Enterococcus* or *Pseudomonas aeruginosa*.
[c] 0/487 *E. coli* were ESBL-positive; 6/487 *E. coli* were multidrug-resistant. 2/98 (2.0%) *K. pneumoniae* were ESBL-positive; MIC range for ESBL-positive isolates was 64 ug/ml; 1/98 *K. pneumoniae* were multidrug-resistant.
[d] UD, Unable to determine.

Table 2. MIC distributions for fosfomycin against outpatient urine isolates collected by 15 laboratories in Canada from 2007 to 2013.

Genus/species (n)	Fosfomycin MIC (µg/ml)[a] Cumulative % of isolates inhibited at MIC ≤1	2	4	8	16	32	64	128	256	512
E. coli (487)	65.3	87.3	92.6	94.9	96.9	98.2	99.6	100		
K. pneumoniae (98)		1.0	2.0	10.2	29.6	66.3	90.8	93.9	96.9	100[b]
E. faecalis (50)						4.0	82.0	98.0		100[b]
P. aeruginosa (16)			6.2		12.5	25.0	37.5	62.5	93.7	100[b]
S. aureus (13)	7.7		23.1	30.8	61.5	100				
Proteus mirabilis (11)		18.1	45.4	54.5	72.7	81.8	90.9	100		
Enterobacter cloacae (7)		14.3	28.6	42.8		71.4		85.7		100[b]
Klebsiella oxytoca (6)				16.6	33.3	50.0	66.6	100		
Citrobacter freundii (4)	75.0		100							

[a] CLSI fosfomycin susceptible, intermediate, and resistant MIC breakpoints are ≤64, 128 (light orange), and ≥256 µg/ml (dark orange), respectively.
[b] MIC >512 µg/mL.

CONCLUSIONS

- Fosfomycin demonstrated potent in vitro activity against *E. coli* with 99.6% of isolates susceptible (no resistant isolates were identified).
- >90% of *K. pneumoniae* and *P. mirabilis* were inhibited by fosfomycin when MICs were interpreted using *E. coli* breakpoints; too few isolates of other species of Enterobacteriaceae were tested to fairly assess fosfomycin's activity against those species.
- >60% of fosfomycin MICs for isolates of *P. aeruginosa* were intermediate or resistant when MICs were interpreted using *E. coli* breakpoints
- Only 2% of *E. faecalis* were resistant to fosfomycin; 82.0% of isolates were susceptible to fosfomycin.
- 100% of fosfomycin MICs for isolates of *S. aureus* were susceptible when MICs were interpreted using *E. coli* breakpoints.
- A literature review suggested that the antibacterial spectrum of fosfomycin includes the majority of enteric Gram-negative bacteria and *Haemophilus* spp. and that fosfomycin demonstrates considerably higher MICs for *Klebsiella*, *Enterobacter*, and *Serratia* than for *E. coli*, *Citrobacter*, and *Proteus* (although the activity of fosfomycin against *Klebsiella* and *Enterobacter* appears variable) (8, 9); we did not generate enough data for non-*E. coli* isolates of Enterobacteriaecae to support or refute previously published data. Fosfomycin has been previously reported to be moderately active against *P. aeruginosa* with variable MICs ranging from 4 to >512 µg/ml; our data confirms previous reports. *Acinetobacter* spp. and Gram-negative anaerobic bacteria are not susceptible to fosfomycin.
- A literature review suggests that fosfomycin appears more active against *S. aureus*, including MRSA, enterococci, and some streptococci, including *S. pneumoniae*, than other Gram-positive bacteria (8, 9); our data, although limited, appears to support these previous findings. Some streptococci, *Staphylococcus saprophyticus*, corynebacteria, *Chlamydia*, and mycoplasmas have been reported to be resistant to fosfomycin, likely due to the absence or low abundance of the MurA target.
- The difference in CLSI and EUCAST MIC breakpoints impacts the percentage of isolates of Enterobacteriaceae reported as susceptible.