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In Vitro Activity of Fosfomycin Against Bacterial Pathogens Isolated from Urine Specimens in Canada from 2007 to 2019: CANWARD Surveillance Study

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Introduction

The current IDSA/ESCMID guidelines recommend a five-seven 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 oral fosfomycin tromethamine as empirical regimens for treating acute uncomplicated bacterial cystitis in otherwise healthy adult nonpregnant females (1). Fluoroquinolones and β-lactams, such as amoxicillin-clavulanate, are second-line therapies (1). High urine concentrations (~4,000 µg/ml, 2-4 hours following a single oral 3g dose) of fosfomycin, potent in-vitro bactericidal activity and high rate of patient compliance due to single dose therapy compared with agents dosed for 3-5 days, likely underlie its reported low rate of resistance development among *Escherichia coli* (2-4). 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 it is only approved for testing isolates from urinary tract infections (CLSI M100, 29th Edition (2019). EUCAST also publishes MIC breakpoints for fosfomycin for staphylococci and *Enterobacterales* with a MIC ≤32 ug/ml considered susceptible (resistance, >32 ug/ml) for both parenteral (systemic infections) and oral (uncomplicated urinary tract infection only) fosfomycin therapy.

Oral fosfomycin, an agent known for >40 years, has received renewed interest recently because of resistance to traditionally used agents (2,4). In addition, IV fosfomycin (which is available in many countries and recently became available in Canada) is indicated for the treatment of a variety of infections including complicated UTI (5,6). However, there is a paucity of published in vitro MIC testing data for fosfomycin because reference MIC testing must use the agar dilution method (4). 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 oral and intravenous fosfomycin may be given consideration in the treatment of uncomplicated and complicated urinary tract infections caused by pathogens other than *E. coli* and *E. faecalis.*

Materials and 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 2019 (7). 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 (7) 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 30th edition [2020]); all other antibacterial agents were tested using in-house-prepared 96-well broth microdilution panels according to CLSI standards (8). Fosfomycin was supplied by Paladin Labs (Montreal, Quebec, Canada). Stock solutions and dilutions were prepared as described by the CLSI (M100 30th edition-2020), in cation-adjusted Mueller-Hinton broth (MHB) (9). Quality control was performed following CLSI recommendations and minimum inhibitory concentrations (MICs) were interpreted using CLSI M100 30th edition [2020] breakpoints and EUCAST criteria (9, 10). Fosfomycin-resistant isolates were each retested to confirm their phenotype. ESBLs were identified following CLSI guidelines (9).

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 2019

Organism (n)	Antimicrobial agent	(µg/ml)			CLSI MIC Interpretation[a]			EUCAST MIC Interpretation[b]		
		MIC_{50}	MIC_{90}	MIC range	% S	% I	% R	% S	% I	% R
Escherichia coli	Fosfomycin	≤1	4	≤1->512	99.2	0.6	0.2	98.3	-	1.7
(2785)	SXT[c]	≤0.12	>8	≤0.12->8	75.4	-	24.6	75.4	0.3	24.3
	Nitrofurantoin	16	32	≤1->512	97.1	1.8	1.1	98.9	-	1.1
	Ciprofloxacin	≤0.06	>16	≤0.06->16	76.3	1.1	22.6	76.3	1.1	22.6
	Amoxicillin-clavulanate	4	16	≤0.06->32	82.0	13.2	4.8	98.6	-	1.4
Escherichia coli	Fosfomycin	2	4	≤1->512	96.4	2.1	1.5	96.4	-	3.6
ESBL	SXT	>8	>8	≤0.12->8	35.2	-	64.8	35.2	1.0	63.8
(196)	Nitrofurantoin	16	64	≤1-512	88.3	7.6	4.1	95.9	-	4.1
	Ciprofloxacin	>16	>16	≤0.06->16	14.9	0.9	84.2	14.8	1.0	84.2
	Amoxicillin-clavulanate	8	32	1->32	51.4	37.7	10.9	96.2	-	3.8
Klebsiella pneumoniae	Fosfomycin	32	128	≤1->512	89.7	4.5	5.8	70.2	-	29.8
(359)	SXT	≤0.12	>8	≤0.12->8	87.7	-	12.3	87.7	1.4	10.9
	Nitrofurantoin	64	128	4->512	36.5	37.1	26.4	73.6	-	26.4
	Ciprofloxacin	≤0.06	0.5	≤0.06->16	88.6	3.3	8.1	88.6	3.3	8.1
	Amoxicillin-clavulanate	2	8	1->32	91.6	5.4	3	99.4	-	0.6
Klebsiella pneumoniae	Fosfomycin	32	256	2->512	78.3	8.7	13	69.6	-	30.4
ESBL	SXT	>8	>8	≤0.12->8	8.7	-	91.3	8.7	0	91.3
(23)	Nitrofurantoin	64	512	32-512	26.3	36.9	36.8	63.2		36.8
	Ciprofloxacin	4	>16	≤0.06->16	26.1	4.3	69.6	26.1	4.3	69.6
	Amoxicillin-clavulanate	16	32	4->32	28.6	42.8	28.6	95.2	-	4.8
Enterococcus faecalis	Fosfomycin	64	128	4->512	88.3	10.2	1.5	NA	NA	NA
(333)	SXT	≤0.12	0.5	≤0.12->8	NA	NA	NA	UD[c]	UD	UD
	Nitrofurantoin	8	16	2-128	99.6	0	0.4	99.6	-	0.4
	Ciprofloxacin	1	>16	0.12->16	67.1	9.0	23.9	76.7	-	23.3
	Amoxicillin-clavulanate	0.5	1	0.12-2	100[e]	-	0	100	0	0
Proteus mirabilis	Fosfomycin	4	128	≤1->512	86.9	8.3	4.8	81.4	-	18.6
(145)	SXT	≤0.12	>8	≤0.12->8	77.2	-	22.8	77.2	0.7	22.1
	Nitrofurantoin	128	128	64-512	0	16.9	83.1	16.9	-	83.1
	Ciprofloxacin	≤0.06	4	≤0.06->16	83.4	0.7	15.9	83.4	0.7	15.9
	Amoxicillin-clavulanate	1	8	0.5->32	93.0	2.8	4.2	97.2	-	2.8
Pseudomonas aeruginosa	Fosfomycin	128	256	≤1->512	49.2	37.1	13.7	NA	NA	NA
(124)	SXT	8	>8	1->8	NA	NA	NA	NA	NA	NA
	Nitrofurantoin	>512	>512	512->512	NA	NA	NA	NA	NA	NA
	Ciprofloxacin	0.25	8	≤0.06->16	75.8	5.7	18.5	75.8[d]	-	24.2
	Amoxicillin-clavulanate	>32	>32	32->32	NA	NA	NA	NA	NA	NA
Staphylococcus aureus	Fosfomycin	8	32	≤1-256	98.9	0	1.1	97.8	-	2.2
(89)	SXT	≤0.12	≤0.12	≤0.12-0.5	100	-	0	100	0	0
	Nitrofurantoin	16	16	4-32	100	0	0	NA	NA	NA
	Ciprofloxacin	0.5	>16	0.12->16	55.7	1.1	43.2	55.7[d]	-	44.3
	Amoxicillin-clavulanate	4	>32	0.5->32	73.3[f]	-	26.7	NA	NA	NA
Enterobacter cloacae	Fosfomycin	32	512	≤1->512	67.1	12.6	20.3	57.0	-	43.0
(79)	SXT	≤0.12	1	≤0.12->8	92.4	-	7.6	92.4	0	7.6
	Nitrofurantoin	64	128	2->512	33.3	38.4	28.3	71.7	-	28.3
	Ciprofloxacin	≤0.06	2	≤0.06->16	88.6	1.3	10.1	88.6	1.3	10.1
	Amoxicillin-clavulanate	>32	>32	2->32	9.2	5.3	85.5	25.0	-	75.0

Table 1. In vitro activities of fosfomycin and comparative antimicrobial agents against outpatient urine isolates collected by 15 laboratories in Canada from 2007 to 2019 (Continued)

Organism (n)	Antimicrobial agent	(µg/ml)			CLSI MIC Interpretation[a]			EUCAST MIC Interpretation[b]		
		MIC_{50}	MIC_{90}	MIC range	% S	% I	% R	% S	% I	% R
Klebsiella	Fosfomycin	16	128	≤1->512	86.1	9.7	4.2	77.8	-	22.2
oxytoca	SXT	≤0.12	≤0.12	≤0.12->8	95.8	-	4.2	95.8	0	4.2
(72)	Nitrofurantoin	32	32	4-256	91.4	5.2	3.4	96.6	-	3.4
	Ciprofloxacin	≤0.06	≤0.06	≤0.06-2	98.6	0	1.4	98.6	0	1.4
	Amoxicillin-clavulanate	4	16	1->32	88.2	7.4	4.4	98.5	-	1.5
Klebsiella aerogenes	Fosfomycin	32	256	2-512	86.2	3.5	10.3	79.3	-	20.7
(29)	SXT	≤0.12	0.5	≤0.12-1	100	-	0	100	0	0
	Nitrofurantoin	64	128	64-128	0	75.0	25.0	75.0	-	25.0
	Ciprofloxacin	≤0.06	0.12	≤0.06-8	92.9	0	7.1	92.9	0	7.1
	Amoxicillin-clavulanate	>32	>32	2->32	3.7	3.7	92.6	7.4	-	92.6
Citrobacter freundii	Fosfomycin	≤1	2	≤1-16	100	0	0	100	-	0
(26)	SXT	≤0.12	>8	≤0.12->8	76.4	-	23.6	76.9	3.9	19.2
	Nitrofurantoin	16	32	8-32	100	0	0	100	-	0
	Ciprofloxacin	≤0.06	0.25	≤0.06->16	92.3	3.9	3.8	92.3	3.9	3.8
	Amoxicillin-clavulanate	>32	>32	1->32	8.3	12.5	79.2	33.3	-	66.7

[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. However, these same breakpoints were used for all pathogens in CLSI column.
[b] EUCAST breakpoints for fosfomycin for *Enterobacterales* (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] UD, Unable to determine.
[d] % Susceptible included isolates categorized as "Susceptible, increased exposure'.
[e] AMC activity predicted by testing ampicillin for *E. faecalis*.
[f] AMC activity predicted by testing cefoxitin for *S. aureus*.
SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin; CIP, ciprofloxacin; AMC, amoxicillin-clavulanate; NA, not applicable.

Table 2. MIC distributions for fosfomycin against urine isolates collected by 15 laboratories in Canada from 2007 to 2019.

Genus/species (n)	Fosfomycin MIC (µg/ml)[a] Cumulative % of isolates inhibited at MIC									
	≤1	2	4	8	16	32	64	128	256	512
Escherichia coli (2785)	59.5	88.0	94.2	95.7	97.2	98.3	99.2	99.8	99.9	100[b]
Escherichia coli-ESBL (196)	49.5	87.8	93.9	94.9	95.9	96.4		98.5	99.0	100[b]
Klebsiella pneumoniae (359)	0.3	1.9	4.5	10.9	34.8	70.2	89.7	94.2	96.9	100[b]
Klebsiella pneumoniae-ESBL (23)		4.3		8.7	43.5	69.6	78.3	87.0	95.7	100[b]
Enterococcus faecalis (333)			0.3	0.6	1.8	34.2	88.3	98.5	99.4	100[b]
Proteus mirabilis (145)	9.0	30.3	60.0	69.7	75.2	81.4	86.9	95.2	96.6	100[b]
Pseudomonas aeruginosa (124)	0.8		4.8		10.5	16.9	49.2	86.3	95.2	100[b]
Staphylococcus aureus (89)	6.7	15.7	47.2	73.0	89.9	97.8	98.9		100	
Enterobacter cloacae (79)	8.9	19.0	21.5	27.8	40.5	57.0	67.1	79.7	86.1	100[b]
Klebsiella oxytoca (72)	1.4	4.2		18.1	52.8	77.8	86.1	95.8	98.6	100[b]
Klebsiella aerogenes (29)		3.4		10.3	34.5	79.3	86.2	89.7	96.6	100
Citrobacter freundii (26)	88.5	96.2			100					

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

Conclusions

- Fosfomycin demonstrated potent in vitro activity against *E. coli* with 99.2% of isolates susceptible and 96.4% of ESBL producing *E. coli*.
- 89.7% of *K. pneumoniae* were inhibited by fosfomycin when MICs were interpreted using *E. coli* breakpoints and 78.3 of ESBL producing *K. pneumoniae*.
- For other *Enterobacterales*, fosfomycin susceptibilities ranged from 67.1-100%.
- 49.2% of fosfomycin MICs for isolates of *P. aeruginosa* were susceptible when interpreted using *E. coli* breakpoints.
- 88.3% of *E. faecalis* were susceptible to fosfomycin.
- 98.9% of fosfomycin MICs for isolates of *S. aureus* were susceptible when MICs were interpreted using *E. coli* breakpoints.
- Our data are consistent with the literature which reports the antibacterial spectrum of fosfomycin includes the majority of enteric Gram-negative bacteria and that fosfomycin demonstrates higher MICs for *Klebsiella*, *Enterobacter*, and *Serratia* than for *E. coli*, *Citrobacter*, and *Proteus* (5).
- Fosfomycin demonstrated activity against *P. aeruginosa* in our study, with variable MICs ranging from ≤ 1 to >512 µg/ml, which is consistent with the literature (5).
- *Acinetobacter* spp. and Gram-negative anaerobic bacteria are not susceptible to fosfomycin (5).
- Our data are consistent with the literature which reports that fosfomycin is active versus Gram-positive cocci including *S. aureus*, *S. pneumoniae* and *Enterococcus* spp. (5). The majority of isolates of *S. aureus*, and enterococci (including VRE) have fosfomycin MICs ≤32 ug/ml. Some streptococci, *Staphylococcus saprophyticus*, corynebacteria, *Chlamydia*, and mycoplasmsas have been reported to be resistant to fosfomycin, likely due to the absence or low abundance of the MurA target (5).
- The difference in CLSI and EUCAST MIC breakpoints appears to impact the % of isolates of *Enterobacterales* from urine reported as susceptible.
- In general, fosfomycin possesses broad-spectrum activity against most Gram-negative and Gram-positive bacterial pathogens causing urinary tract infections.

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