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In Vitro Activity of Fosfomycin Against Bacterial Pathogens Isolated from Urine Specimens in Canada from 2007 to 2020: CANWARD Surveillance Study

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Introduction

The current IDSA/ESCMID guidelines recommend a 5-7 day course of nitrofurantoin, a 3-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).

Fosfomycin is a broad-spectrum, bactericidal, epoxide antibacterial agent that inhibits an initial step in peptidoglycan synthesis. Oral fosfomycin is associated with high urine concentrations (~4,000 μg/ml) 2-4 hours following a single oral 3g dose, potent *in vitro* bactericidal activity, and a low rate of resistance development in *Escherichia coli* (2-4).

CLSI (M100, 2022) publishes fosfomycin MIC breakpoints that only apply to urine isolates of *E. coli* and *Enterococcus faecalis*: susceptible, ≤64 ug/ml; intermediate, 128 ug/ml; and resistant, ≥256 ug/ml (5). EUCAST publishes MIC breakpoints for parenteral fosfomycin for *Enterobacterales* and staphylococci (susceptible, ≤32 ug/ml; resistant, >32 ug/ml) and for oral fosfomycin for *E. coli* (uncomplicated urinary tract infection only) (susceptible, ≤8 ug/ml; resistant, >8 ug/ml) (6).

Parenteral fosfomycin was approved for patient use in Canada in 2019 and is used in many European countries and Japan. It is indicated, in combination with other antimicrobials, for the treatment of a variety of infections including complicated UTI (7,8).

There is a paucity of published *in vitro* MIC testing data for fosfomycin because reference MIC testing must use the agar dilution method (4). North American MIC data documenting the activity of fosfomycin against outpatient urinary pathogens other than *E. coli* and *E. faecalis* are also limited.

The current study assessed the *in vitro* activity of fosfomycin against pathogens causing UTIs in Canadian patients.

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 2020 (9). 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 (9) 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 (5); all other antibacterial agents were tested using in-house-prepared 96-well broth microdilution panels according to CLSI standards (10). Fosfomycin was supplied by Paladin Labs (Montreal, Quebec, Canada). Stock solutions and dilutions were prepared as described by the CLSI in cation-adjusted Mueller-Hinton broth (MHB) (5). Quality control was performed following CLSI recommendations and MICs were interpreted using CLSI M100 2022 breakpoints and EUCAST 2022 criteria (5,6). Fosfomycin-resistant isolates were each retested to confirm their phenotype. ESBLs were identified following CLSI guidelines (5).

Results

Table 1. MIC distributions for fosfomycin against urine isolates collected by 15 laboratories in Canada from 2007 to 2020

Species/phenotype (n)	Fosfomycin MIC (μg/ml) Cumulative % of isolates inhibited at MIC									
	≤1	2	4	8	16	32	64	128	256	512
Escherichia coli (2871)	60.2	88.3	94.3	95.8	97.2	98.4	99.2	99.8	99.9	100 [a]
E. coli - ESBL (209)	51.2	88.5	94.3	95.2	96.2	96.7		98.6	99.0	100 [a]
Klebsiella pneumoniae (393)	0.3	1.8	4.8	12.5	37.4	71.5	90.1	94.1	96.9	100 [a]
K. pneumoniae - ESBL (25)		4.0	8.0	16.0	48.0	72.0	80.0	88.0	96.0	100 [a]
Enterococcus faecalis (346)			0.3	0.6	1.7	33.5	88.4	98.6	99.4	100 [a]
Proteus mirabilis (164)	10.4	34.1	62.2	70.7	75.6	81.7	87.2	95.7	97.0	100 [a]
Pseudomonas aeruginosa (140)	0.7		4.3		9.3	16.4	52.1	86.4	95.0	100 [a]
Staphylococcus aureus (94)	7.4	16.0	48.9	74.5	90.4	97.9	98.9		100	
Enterobacter cloacae (88)	8.0	17.0	19.3	26.1	42.0	60.2	69.3	80.7	87.5	100 [a]
Klebsiella oxytoca (79)	1.3	3.8	5.1	21.5	55.7	78.5	87.3	96.2	98.7	100 [a]
Klebsiella aerogenes (31)		3.2		9.7	35.5	80.6	87.1	90.3	96.8	100
Citrobac*ter freundii* (30)	83.3	96.7			100					

[a] MIC >512 ug/mL.

Table 2. *In vitro* activities of fosfomycin against urine isolates collected by 15 laboratories in Canada from 2007 to 2020

Species/phenotype (n)	MIC_{50}/MIC_{90} (μg/ml)	% Susceptible (MIC breakpoint concentration, μg/ml)		
	FOS	FOS (≤64)[a]	FOS (≤32)[b]	FOS (≤8)[c]
Escherichia coli (2871)	≤1 / 4	99.2	98.4	95.8
E. coli - ESBL (209)	≤1 / 4	96.7	96.7	95.2
Klebsiella pneumoniae (393)	32 / 64	90.1	71.5	12.5
K. pneumoniae - ESBL (25)	32 / 256	80.0	72.0	16.0
Enterococcus faecalis (346)	64 / 128	88.4	33.5	0.6
Proteus mirabilis (164)	4 / 128	87.2	81.7	70.7
Pseudomonas aeruginosa (140)	64 / 256	52.1	16.4	4.3
Staphylococcus aureus (94)	8 / 16	98.9	97.9	74.5
Enterobacter cloacae (88)	32 / 512	69.3	60.2	26.1
Klebsiella oxytoca (79)	16 / 128	87.3	78.5	21.5
Klebsiella aerogenes (31)	32 / 128	87.1	80.6	9.7
Citrobacter freundii (30)	≤1 / 2	100	100	96.7

[a] CLSI MIC susceptible breakpoint for oral fosfomycin tested against urine isolates of *E. coli* and *E. faecalis*.
[b] EUCAST MIC susceptible breakpoint for parenteral fosfomycin against systemic and urine isolates of staphylococci and Enterobacterales.
[c]EUCAST MIC susceptible breakpoint for oral fosfomycin against urine isolates of *E. coli*.

Table 3. *In vitro* activities of comparative antimicrobial agents against outpatient urine isolates collected by 15 laboratories in Canada from 2007 to 2020

Species/ phenotype (n)	Antimicrobial agent	(μg/ml)			CLSI MIC Interpretation			EUCAST MIC Interpretation		
		MIC_{50}	MIC_{90}	MIC range	% S	% I	% R	% S	% I	% R
Escherichia coli (2871)	SXT	≤0.12	>8	≤0.12->8	75.3	-	24.7	75.3	0.3	24.4
	Nitrofurantoin	16	32	≤1->512	97.1	1.8	1.1	98.9	-	1.1
	Ciprofloxacin	≤0.06	>16	≤0.06->16	76.5	1.1	22.4	76.5	1.1	22.4
	Amoxicillin-clavulanate	4	16	≤0.06->32	82.1	13.1	4.8	98.5	-	1.5
E. coli - ESBL (209)	SXT	>8	>8	≤0.12->8	34.4	-	65.6	34.4	1.0	64.6
	Nitrofurantoin	16	64	≤1-512	88.5	7.2	4.3	95.7	-	4.3
	Ciprofloxacin	>16	>16	≤0.06->16	15.8	0.9	83.3	15.8	0.9	83.3
	Amoxicillin-clavulanate	8	32	1->32	51.6	36.9	11.5	95.8	-	4.2
Klebsiella pneumoniae (393)	SXT	≤0.12	>8	≤0.12->8	87.5	-	12.5	87.5	1.3	11.2
	Nitrofurantoin	64	128	2->512	37.5	36.7	25.8	74.2	-	25.8
	Ciprofloxacin	≤0.06	0.5	≤0.06->16	88.3	3.0	8.7	88.3	3.0	8.7
	Amoxicillin-clavulanate	2	8	1->32	91.1	5.0	3.9	98.6	-	1.4
K. pneumoniae - ESBL (25)	SXT	>8	>8	≤0.12->8	8.0	-	92.0	8.0	0	92.0
	Nitrofurantoin	64	256	32-512	23.8	42.9	33.3	66.7	-	33.3
	Ciprofloxacin	4	>16	≤0.06->16	24.0	4.0	72.0	24.0	4.0	72.0
	Amoxicillin-clavulanate	16	32	4->32	30.4	39.2	30.4	95.7	-	4.3
Enterococcus faecalis (346)	SXT	≤0.12	0.5	≤0.12->8	NA[a]	NA	NA	UD[b]	UD	UD
	Nitrofurantoin	8	16	2-128	99.6	0	0.4	99.6	-	0.4
	Ciprofloxacin	1	>16	0.12->16	68.0	8.7	23.3	77.3	-	22.7
	Amoxicillin-clavulanate	0.5	1	0.12-2	100[c]	-	0	100	0	0
Proteus mirabilis (164)	SXT	≤0.12	>8	≤0.12->8	76.8	-	23.2	76.8	0.6	22.6
	Nitrofurantoin	128	128	64-512	0	20.4	79.6	20.4	-	79.6
	Ciprofloxacin	≤0.06	4	≤0.06->16	82.3	0.6	17.1	82.3	0.6	17.1
	Amoxicillin-clavulanate	1	8	0.5->32	93.0	3.2	3.8	97.5	-	2.5
Pseudomonas aeruginosa (140)	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	16	≤0.06->16	75.7	5.7	18.6	75.7[d]	-	24.3
	Amoxicillin-clavulanate	>32	>32	32->32	NA	NA	NA	NA	NA	NA
Staphylococcus aureus (94)	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	58.1	1.0	40.9	58.1[d]	-	41.9
	Amoxicillin-clavulanate	4	>32	0.5->32	74.7[e]	-	25.3	NA	NA	NA
Enterobacter cloacae (88)	SXT	≤0.12	8	≤0.12->8	89.8	-	10.2	89.8	0	10.2
	Nitrofurantoin	64	128	2->512	33.3	39.2	27.5	72.5	-	27.5
	Ciprofloxacin	≤0.06	2	≤0.06->16	88.6	1.2	10.2	88.6	1.2	10.2
	Amoxicillin-clavulanate	>32	>32	2->32	8.2	4.7	87.1	25.9	-	74.1
Klebsiella oxytoca (79)	SXT	≤0.12	≤0.12	≤0.12->8	96.2	-	3.8	96.2	0	3.8
	Nitrofurantoin	32	64	4-256	89.2	6.2	4.6	95.4	-	4.6
	Ciprofloxacin	≤0.06	≤0.06	≤0.06-2	98.7	0	1.3	98.7	0	1.3
	Amoxicillin-clavulanate	4	16	1->32	89.0	6.9	4.1	98.6	-	1.4
Klebsiella aerogenes (31)	SXT	≤0.12	0.5	≤0.12-1	100	-	0	100	0	0
	Nitrofurantoin	64	128	32-128	7.1	71.5	21.4	78.6	-	21.4
	Ciprofloxacin	≤0.06	≤0.06	≤0.06-8	93.3	0	6.7	93.3	0	6.7
	Amoxicillin-clavulanate	>32	>32	2->32	3.6	3.5	92.9	7.1	-	92.9
Citrobacter freundii (30)	SXT	≤0.12	>8	≤0.12->8	76.7	-	23.3	76.7	3.3	20.0
	Nitrofurantoin	16	32	8-256	91.7	4.1	4.2	95.8	-	4.2
	Ciprofloxacin	≤0.06	0.25	≤0.06->16	90.0	3.3	6.7	90.0	3.3	6.7
	Amoxicillin-clavulanate	>32	>32	1->32	7.1	14.3	78.6	35.7	-	64.3

[a] NA, No CLSI breakpoints available.
[b] UD, Unable to determine.
[c] AMC activity predicted by testing ampicillin for *E. faecalis*.
[d] % Susceptible included isolates categorized as "Susceptible, increased exposure".
[e] AMC activity predicted by testing cefoxitin for *S. aureus*.
SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin; CIP, ciprofloxacin; AMC, amoxicillin-clavulanate.

Discussion and Conclusions

- 99.2% of all *E. coli* and 96.7% of ESBL-producing *E. coli* isolates had fosfomycin MICs ≤64 μg/ml (CLSI susceptible breakpoint).
- 95.8% of all *E. coli* and 95.2% of ESBL-producing *E. coli* isolates had fosfomycin MICs ≤8 μg/ml (EUCAST susceptible breakpoint for oral fosfomycin).
- 71.5% of all *K. pneumoniae* and 72.5% of ESBL-producing *K. pneumoniae* were fosfomycin-susceptible when MICs were interpreted using EUCAST Enterobacterales/staphylococci breakpoint for parenteral fosfomycin (MIC ≤32 μg/ml).
- For other *Enterobacterales*, fosfomycin susceptibilities ranged from 60.2-100% (MIC ≤32 μg/ml).
- 52.1% (MIC ≤64 μg/ml), 16.4% (≤32 μg/ml), and 4.3% (≤8 μg/ml) of fosfomycin MICs for isolates of *P. aeruginosa* were susceptible using the three different MIC breakpoints.
- 88.4% of *E. faecalis* were fosfomycin-susceptible (MIC ≤64 μg/ml).
- 97.9% of *S. aureus* were fosfomycin-susceptible (MIC ≤32 μg/ml).
- Our data are consistent with published 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* (7).
- Fosfomycin demonstrated limited activity against *P. aeruginosa* in our study, with variable MICs ranging from ≤1 to >512 μg/ml, which is consistent with the literature (7).
- *Acinetobacter* spp. and Gram-negative anaerobic bacteria are not susceptible to fosfomycin (7).
- 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. (7). The majority of isolates of *S. aureus*, and enterococci (including VRE) have fosfomycin MICs ≤32 ug/ml. 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 (7).
- 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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