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In Vitro Activity of Fosfomycin Against Urinary Tract Isolates of *Escherichia coli* Isolated from Patients Across Canada from 2010 to 2013

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REVISED ABSTRACT

Background: *Escherichia coli* are responsible for 75-90% of uncomplicated urinary tract infections. Fosfomycin (FOS) tromethamine is indicated in the treatment of uncomplicated urinary tract infections and is usually administered as a single oral dose. FOS inactivates the enzyme UDP-*N*-acetylglucosamine-3-enolpyruvyltransferase (MurA) which ligates phosphoenolpyruvate (PEP) to the 3'-hydroxyl group of UDP-*N*-acetylglucosamine in peptidoglycan synthesis. A paucity of reference in vitro antimicrobial susceptibility testing (AST) MIC data exists for FOS because it must be tested by the agar dilution method (CLSI).

Methods: FOS AST was performed using CLSI agar dilution testing (MHA supplemented with 25 µg/ml of glucose-6-phosphate; M100-S23 [2013]); all other antibacterial agents were tested using CLSI broth microdilution panels. MICs were interpreted using M100-S23 (2013) criteria. FOS susceptible, intermediate, and resistant breakpoints are ≤64, 128, and ≥256 µg/ml, respectively. Isolates of *E. coli* tested were from the annual Canadian surveillance study, CANWARD. 868 *E. coli* isolated from urine from 2010 to 2013 were tested as were an additional 349 isolates of *E. coli* with known ESBL, AmpC, and carbapenemase resistance mechanisms.

Results: The table shows MIC_{90} (µg/ml) and % susceptible data for oral antimicrobial agents stratified by trimethoprim-sulfamethoxazole (SXT) and ciprofloxacin (CIP) susceptible (S) and resistant (R) phenotypes.

Conclusion: Frequently prescribed empiric agents for urinary tract infections, such as SXT and CIP, demonstrate compromised in vitro susceptibilities when tested against recent clinical isolates (SXT, 74.7% susceptible; CIP, 77.4% susceptible). >99% of *E. coli* tested were susceptible to FOS. Concurrent non-susceptibility to SXT and/or CIP did not affect the in vitro activity of FOS. FOS demonstrated potent in vitro activity against recent urine isolates of *E. coli* from Canadian patients.

	MIC$_{90}$ (µg/ml) / % Susceptible				
Isolate Phenotype (n)	FOS	SXT	NIT	CIP	AMC
All *E. coli* (868)	4 / 99.4	>8 / 74.7	32 / 96.1	>16 / 77.4	16 / 81.3
SXT-S *E. coli* (647)	4 / 99.5	0.25 / 100	32 / 97.5	>16 / 86.2	8 / 86.1
SXT-R *E. coli* (219)	4 / 99.1	>8 / 0	32 / 91.8	>16 / 51.6	16 / 67.1
CIP-S *E. coli* (672)	2 / 99.9	>8 / 83.2	32 / 97.5	≤0.06 / 100	16 / 85.7
CIP-R *E. coli* (195)	4 / 97.9	>8 / 45.9	32 / 91.3	>16 / 0	16 / 66.0
SXT-R & CIP-R *E. coli* (105)	4 / 98.1	>8 / 0	64 / 88.6	>16 / 0	16 / 56.2
ESBL-producing *E. coli* (33)	4 / 100	>8 / 30.3	64 / 84.8	>16 / 3.0	16 / 36.4
AmpC-producing *E. coli* (14)	4 / 100	>8 /78.6	32 / 100	>16 / 78.6	>32 / 7.1
E. coli R to ≥3 agents (15)	4 / 100	>8 / 6.7	128 / 60.0	>16 / 0	>32 / 13.3

Abbreviations: NIT, nitrofurantoin; AMC, amoxicillin-clavulanate.

MATERIALS & METHODS

Bacterial isolates. All urinary isolates of *E. coli* were collected as part of the ongoing, annual, Canadian national surveillance study, CANWARD (2). 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 (3) was not consistent with that provided by the submitting site, the isolate was removed from the study. From January 2010 to December 2013, 868 isolates of *E. coli* from urine were available for antimicrobial testing. To better delineate the activity of fosfomycin against β-lactam-resistant isolates of *E. coli*, an additional 236 ESBL-producing *E. coli*, 111 AmpC-producing *E. coli*, and 2 carbapenem-resistant *E. coli* collected by the CANWARD surveillance study from 2007 to 2012 were tested (urine specimens from 2010-2012 were excluded). These isolates were from blood, respiratory, and wound specimens from 2007 to 2012, and from urine specimens from 2007 to 2009; characterization of these isolates is described below.

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-S23 [2013]); all other antibacterial agents were tested using in-house-prepared 96-well broth microdilution panels according to CLSI guidelines (1,4). Fosfomycin susceptible, intermediate, and resistant MIC breakpoints are ≤64, 128, and ≥256 µg/ml, respectively (1). 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) (4). Quality control was performed following CLSI recommendations and minimum inhibitory concentrations (MICs) were interpreted using CLSI M100-S23 (2013) breakpoints (1). Fosfomycin-intermediate and -resistant isolates were each retested to confirm their phenotype.

Extended-spectrum β-lactamase (ESBL) Determination. CLSI criteria were used to screen for potential ESBL-producing isolates of *E. coli* (1). ESBL confirmatory testing followed the CLSI disk diffusion method using disks containing ceftazidime (30 µg), ceftazidime/clavulanic acid (30 µg/10 µg), cefotaxime (30 µg), and cefotaxime/clavulanic acid (30 µg/10 µg) (1) supplied by Mast Diagnostics (United Kingdom). Phenotypically confirmed ESBL-producing *E. coli* were screened by PCR and sequence analysis to identify bla_{SHV}, bla_{TEM}, bla_{CTX-M}, and bla_{OXA} as described previously (5).

AmpC Determination. Any putative ESBL-producing *E. coli* that was negative for the ESBL confirmatory test and resistant to cefoxitin (MICs ≥32 µg/mL) was identified as a putative AmpC producer. Putative AmpC-producers were screened for acquired *ampC* genes (bla_{ENT}, bla_{DHA}, bla_{FOX}, and bla_{CIT}) as previously described (6). All isolates producing a positive result for the presence of bla_{CIT}-related genes were further amplified by primers specific bla_{CMY} and sequenced appropriately. Sequencing for the detection of mutations within the chromosomal *ampC* promoter and/or attenuator region was performed on any isolate that was PCR negative for all acquired AmpC β-lactamases listed above, as previously described (7).

RESULTS

Table 1. In vitro activities of antimicrobial agents against 868 urine isolates of *E. coli* isolated by 15 laboratories across Canada from 2010 to 2013.

		(µg/ml)			MIC Interpretation		
Isolate Phenotype (n)	Antimicrobial agent	MIC$_{50}$	MIC$_{90}$	MIC range	% S	% I	% R
All *E. coli* (868)	Fosfomycin	≤1	4	≤1-512	99.4	0.5	0.1
	SXT	≤0.12	>8	≤0.12->8	74.7	-	25.3
	Nitrofurantoin	16	32	≤1-512	96.1	2.4	1.5
	Ciprofloxacin	≤0.06	>16	≤0.06->16	77.4	0.1	22.5
	Amoxicillin-clavulanate	4	16	0.5->32	81.3	13	5.7
Pan-susceptible (526)[a]	Fosfomycin	≤1	2	≤1-64	100	0	0
	SXT	≤0.12	0.25	≤0.12-2	100	-	0
	Nitrofurantoin	16	32	≤1-64	98.7	1.3	0
	Ciprofloxacin	≤0.06	≤0.06	≤0.06-1	100	0	0
	Amoxicillin-clavulanate	4	8	0.5-16	92.6	7.4	0
SXT-susceptible (647)	Fosfomycin	≤1	4	≤1-512	99.5	0.3	0.2
	SXT	≤0.12	0.25	≤0.12-2	100	-	0
	Nitrofurantoin	16	32	≤1-512	97.5	1.5	0.9
	Ciprofloxacin	≤0.06	>16	≤0.06->16	86.2	0	13.8
	Amoxicillin-clavulanate	4	16	0.5->32	86.1	8.5	5.4
SXT-resistant (219)	Fosfomycin	2	4	≤1-128	99.1	0.9	0
	SXT	>8	>8	4->8	0	-	100
	Nitrofurantoin	16	32	≤1-256	91.8	5	3.2
	Ciprofloxacin	1	>16	≤0.06->16	51.6	0.5	47.9
	Amoxicillin-clavulanate	8	16	1->32	67.1	26.5	6.4
Ciprofloxacin-susceptible (672)	Fosfomycin	≤1	2	≤1-512	99.9	0	0.1
	SXT	≤0.12	>8	≤0.12->8	83.2	-	16.8
	Nitrofurantoin	16	32	≤1-512	97.5	1.8	0.7
	Ciprofloxacin	≤0.06	≤0.06	≤0.06-1	100	0	0
	Amoxicillin-clavulanate	4	16	0.5->32	85.7	8.9	5.4
Ciprofloxacin-resistant (195)	Fosfomycin	2	4	≤1-128	97.9	2.1	0
	SXT	>8	>8	≤0.12->8	45.9	-	54.1
	Nitrofurantoin	16	32	≤1-256	91.3	4.6	4.1
	Ciprofloxacin	>16	>16	4->16	0	0	100
	Amoxicillin-clavulanate	8	16	1->32	66	27.3	6.7
Amoxicillin-clavulanate-susceptible (704)	Fosfomycin	≤1	2	≤1-128	99.6	0.4	0
	SXT	≤0.12	>8	≤0.12->8	79.1	-	20.9
	Nitrofurantoin	16	32	≤1-256	96.7	2.3	1
	Ciprofloxacin	≤0.06	>16	≤0.06->16	81.7	0.1	18.2
	Amoxicillin-clavulanate	4	8	0.5-8	100	0	0
Amoxicillin-clavulanate-resistant (49)	Fosfomycin	2	32	≤1-512	98	0	2
	SXT	≤0.12	>8	≤0.12->8	71.4	-	28.6
	Nitrofurantoin	16	32	8-512	91.9	2	6.1
	Ciprofloxacin	≤0.06	>16	≤0.06->16	73.5	0	26.5
	Amoxicillin-clavulanate	32	>32	32->32	0	0	100
SXT-resistant and ciprofloxacin-resistant (105)	Fosfomycin	2	4	≤1-128	98.1	1.9	0
	SXT	>8	>8	4->8	0	-	100
	Nitrofurantoin	16	64	4-256	88.6	6.7	4.8
	Ciprofloxacin	>16	>16	4->16	0	0	100
	Amoxicillin-clavulanate	8	16	2->32	56.2	35.2	8.6
ESBL-producing (33)[b]	Fosfomycin	2	4	≤1-4	100	0	0
	SXT	>8	>8	≤0.12->8	30.3	-	69.7
	Nitrofurantoin	16	64	≤1-256	84.8	12.1	3
	Ciprofloxacin	>16	>16	0.12->16	3	0	97
	Amoxicillin-clavulanate	16	16	1-32	36.4	54.5	9.1
AmpC-producing (14)[c]	Fosfomycin	2	4	≤1-4	100	0	0
	SXT	≤0.12	>8	≤0.12->8	78.6	-	21.4
	Nitrofurantoin	16	32	8-32	100	0	0
	Ciprofloxacin	≤0.06	>16	≤0.06->16	78.6	0	21.4
	Amoxicillin-clavulanate	>32	>32	8->32	7.1	7.1	85.8
Multidrug-resistant (15)[d]	Fosfomycin	2	4	≤1-4	100	0	0
	SXT	>8	>8	≤0.12->8	6.7	-	93.3
	Nitrofurantoin	16	128	16-256	60	0	40
	Ciprofloxacin	>16	>16	8->16	0	0	100
	Amoxicillin-clavulanate	32	>32	8->32	13.3	20	66.7

[a] Pan-susceptible is defined as susceptible to SXT, nitrofurantoin, ciprofloxacin, and amoxicillin-clavulanate.
[b] ESBL rate for *E. coli* was 4.6% (33/712; data from 2010-2012 isolates only).
[c] AmpC rate for *E. coli* was 2.0% (14/712; data from 2010-2012 isolates only).
[d] Multidrug-resistant was defined as isolates that were resistant to ≥3 agents from different antimicrobial classes.

Table 2. MIC distributions for fosfomycin against 868 urine isolates of *E. coli* isolated by 15 laboratories across Canada from 2010 to 2013 stratified by susceptible or resistant phenotype.

	Fosfomycin MIC (µg/ml)[a]									
	Cumulative % of isolates inhibited at MIC									
Isolate Phenotype (n)	≤1	2	4	8	16	32	64	128	256	512
All *E. coli* (868)	52.3	89.2	96.2	97.1	97.7	98.6	99.4	99.9	99.9	100
Pan-susceptible (526)[b]	56.3	91.1	97	98.1	98.5	99.2	100			
SXT-susceptible (647)	53.5	88.9	95.8	97.1	97.7	98.8	99.5	99.8	99.8	100
SXT-resistant (219)	48.9	90	97.3	97.3	97.7	98.2	99.1	100		
Ciprofloxacin-susceptible (672)	56.1	90.5	96.7	97.6	98.1	99	99.9	99.9	99.9	100
Ciprofloxacin-resistant (195)	39	84.6	94.4	95.4	96.4	97.4	97.9	100		
Amoxicillin-clavulanate-susceptible (704)	54.8	91.3	96.3	97.4	98.2	98.9	99.6	100		
Amoxicillin-clavulanate-resistant (49)	34.7	73.5	89.8	89.8	89.8	95.9	98	98	98	100
SXT-resistant and ciprofloxacin-resistant (105)	36.2	85.7	96.2	96.2	96.2	97.1	98.1	100		
ESBL-producing (33)[c]	33.3	81.8	100							
AmpC-producing (14)[d]	21.4	57.1	100							
Multidrug-resistant (15)[e]	26.7	86.7	100							

[a] Susceptible, intermediate, and resistant MIC breakpoints are ≤64, 128 (light orange), and ≥256 µg/ml (dark orange), respectively (1).
[b] Pan-susceptible is defined as susceptible to SXT, nitrofurantoin, ciprofloxacin, and amoxicillin-clavulanate.
[c] ESBL rate for *E. coli* was 4.6% (33/712; data from 2010-2012 isolates only).
[d] AmpC rate for *E. coli* was 2.0%(14/712; data from 2010-2012 isolates only).
[e] Multidrug-resistant was defined as isolates that were resistant to ≥3 agents from different antimicrobial classes.

Table 3. In vitro activity of fosfomycin against ESBL-producing and AmpC-producing *E. coli* collected by the CANWARD surveillance study from 2007 to 2012, excluding urine isolates from 2010 to 2012.

	MIC (µg/ml)			MIC Interpretation	
Isolate Phenotype (n)	MIC$_{50}$	MIC$_{90}$	Range	% S	% I	% R
ESBL-producing *E. coli* (236)[a]						
Fosfomycin	2	4	≤1->512	94.9	3.8	1.3
SXT	>8	>8	≤0.12->8	31.8	-	68.2
Nitrofurantoin	16	32	4-256	90.3	6.4	3.4
Ciprofloxacin	>16	>16	≤0.06->16	11.4	0.8	87.7
Amoxicillin-clavulanate	8	16	2->32	60.2	33.1	6.8
AmpC-producing *E. coli* (111)[b]						
Fosfomycin	2	16	≤1->512	96.4	1.8	1.8
SXT	0.5	>8	≤0.12->8	64	-	36
Nitrofurantoin	16	64	≤1-256	88.3	7.2	4.5
Ciprofloxacin	0.25	>16	≤0.06->16	59.5	0.9	39.6
Amoxicillin-clavulanate	16	>32	1->32	28.8	22.5	48.6

[a] Included 142 isolates from blood, 33 isolates from respiratory sources, 9 isolates from wound specimens (2007-2012); 52 isolates from urine (2007-2009).
[b] Included 62 isolates from blood, 15 isolates from respiratory sources, 7 isolates from wound specimens (2007-2012); 27 isolates from urine (2007-2009).

CONCLUSIONS

- 74.7 and 77.4% of urinary isolates of *E. coli* from across Canada in 2010-2013 were susceptible to SXT and ciprofloxacin, respectively, while susceptibility to the other agents tested was amoxicillin-clavulanate (81.3%) << nitrofurantoin (96.1%) < fosfomycin (99.4%) (Table 1); only 0.1% of isolates were resistant to fosfomycin. The current utility of SXT and fluoroquinolones, as well as amoxicillin-clavulanate, to provide reliable empiric therapy for urinary tract infections is in question.
- Fosfomycin activity remained unchanged (MIC_{90}, 2-4 µg/ml) across all important resistance phenotypes of *E. coli* compared with susceptible phenotypes (Table 1).
- Fosfomycin inhibited 100% of ESBL-producing, AmpC-producing, and multidrug-resistant isolates of *E. coli* from urine (Table 1).
- 5 isolates of fosfomycin non-susceptible *E. coli* from urine were identified from 2010 to 2013. The 5 isolates were each from a different medical centre in 4 different provinces; 4 isolates were collected in 2010 and 1 in 2011; patient locations were 2 from outpatient clinics, 2 from medical wards, and 1 from the ICU; patient ages ranged from 29 to 92 years; 3 isolates were from females; 4 isolates were resistant to ciprofloxacin, 2 were resistant to SXT, 1 was resistant to amoxicillin-clavulanate, 1 was intermediate to amoxicillin-clavulanate, and 1 was intermediate to nitrofurantoin; none of the isolates were multidrug-resistant (≥3 agents from different antimicrobial classes).
- Against a larger, more diverse set of ESBL-producing and AmpC-producing isolates of *E. coli*, fosfomycin inhibited >95% of isolates (Table 3). 16 isolates of fosfomycin non-susceptible *E. coli* from urine were identified from 2010 to 2013.
- **AmpC:** 4 fosfomycin non-susceptible AmpC-producing *E. coli* were identified at 4 different medical centres in 4 different provinces; isolates were collected in 2007, 2008, 2009, and 2010 (one isolate per year).
- **AmpC cont'd**: 3 isolates were collected from blood specimens of patients attending emergency rooms, while the remaining isolate was collected from the urine of a patient attending an outpatient clinic; patient ages ranged from 22 to 81 years old, and 2 of 4 isolates were from females; 2 were intermediate to fosfomycin; 2 were resistant to fosfomycin; 2 were resistant to SXT; 1 was intermediate to nitrofurantoin; 1 was resistant to nitrofurantoin; 3 were resistant to ciprofloxacin, and 2 were intermediate to amoxicillin-clavulanate; 1 isolate was MDR; 2 isolates produced CMY-2 and 2 isolates contained promoter/attenuator mutations within the chromosomal *ampC* gene; 1 isolate belonged to ST-131.
- **ESBL:** 12 fosfomycin non-susceptible ESBL-producing *E. coli* were identified at 9 different medical centres in 5 different provinces; 3 isolates were collected in 2007, 2 in 2008, 3 in 2009, 1 in 2010, 1 in 2011, and 2 in 2012; hospital locations included 2 from outpatient clinics, 2 from emergency rooms, 4 from the ICU, 3 from general medical wards, and 1 from a surgical ward; 5 isolates were collected from blood specimens, 5 from urine, 1 from a wound, and 1 from a respiratory specimen; patient ages ranged from 49 to 80 years old and 6 of 12 isolates were from females; 9 were intermediate to fosfomycin; 3 were resistant to fosfomycin; 6 were resistant to SXT; 1 was intermediate to nitrofurantoin; 11 were resistant to ciprofloxacin; 3 were intermediate to amoxicillin-clavulanate, and 2 were resistant to amoxicillin-clavulanate; 1 isolate was MDR; 15 of 16 isolates produced CTX-M-type ESBLs, of which 7 were CTX-M-15 and 3 were CTX-M-14; 8 isolates belonged to ST-131.
- Reasons for fosfomycin's low resistance rate may include its short contact time, high urine concentration (706 [± 466] ug/ml, 2-4 h after a single oral 3g dose), and potentially higher compliance compared with agents dosed for 3-5 days.
- Ongoing surveillance is required to monitor the development of resistance in *E. coli*. Fosfomycin represents an important therapeutic option for urinary tract infections as resistance to other antimicrobial agents increases.

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