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In Vitro Activity of Fosfomycin Against Bacterial Pathogens Isolated from Urine Specimens of Outpatients Attending Emergency Departments in Canada from 2007 to 2014

J.A. KARLOWSKY[1,2], H.J. ADAM[1,2], M.R. BAXTER[2], N. LAING[2], B. WESHNOWESKI[1], R. VASHISHT[2], and G.G. ZHANEL[2]
Diagnostic Services Manitoba[1], and Dept. of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba[2], Winnipeg, Manitoba, Canada

Dr. James A. Karlowsky
Dept of Clinical Microbiology
St. Boniface Hospital
409 Tache Avenue, L4025-10
Winnipeg, MB R2H 2A6 Canada
Email: jkarlowsky@dsmanitoba.ca

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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: 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 panels. MICs were interpreted using M100-S24 (2014) criteria. FOS susceptible, intermediate, and resistant breakpoints are ≤64, 128, and ≥256 µg/ml, respectively. 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 2014.
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
Escherichia coli (877)	4 / 99.7	>8 / 78.2	32 / 97.8	>16 / 85.4	16 / 88.8
Klebsiella pneumoniae (106)	128 / 89.6	0.25 / 96.2	128 / 34.9	≤0.06 / 98.1	8 / 96.7
Enterococcus faecalis (50)	128 / 83.8	NA	8 / 100	>16 / 72.1	1 / 100*
Proteus mirabilis (40)	128 / 85.0	>8 / 87.5	256 / 0	2 / 87.5	16 / 90.0
Pseudomonos aeruginosa (27)	256 / 48.1	NA	NA	>16 / 66.7	NA
Staphylococcus aureus (26)	32 / 100	≤0.12 / 100	16 / 100	>16 / 50.0	NA / 66.7**
Klebsiella oxytoca (22)	64 / 90.9	≤0.12 / 100	32 / 95.5	≤0.06 / 100	16 / 89.5
Enterobacter cloacae (14)	128 / 85.7	≤0.12 / 92.9	128 / 35.7	2 / 85.7	>32 / 21.4

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*.

Conclusion: The in vitro activities of SXT (78%) and CIP (85%), two frequently prescribed empiric agents for urinary tract infections, were compromised against recent urinary isolates of *E. coli* compared to FOS (>99%). 84% of *E. faecalis* isolates were susceptible to FOS. FOS demonstrated broad spectrum activity against facultative 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 2014 (5). 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 (6) 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 standards (4,7). Fosfomycin was supplied by Paladin Labs Inc. (Saint-Laurent, Quebec, Canada). Stock solutions and dilutions were prepared as described by the CLSI (M07-A9, 2012), in cation-adjusted Mueller-Hinton broth (MHB) (7). Quality control was performed following CLSI recommendations and minimum inhibitory concentrations (MICs) were interpreted using CLSI M100-S24 (2014) breakpoints (4). Fosfomycin-resistant isolates were each retested to confirm their phenotype. ESBLs were identified following CLSI guidelines (4).

BACKGROUND

The currently recommended empiric antimicrobial regimen for treating acute uncomplicated bacterial cystitis in otherwise healthy adult non-pregnant females is a five to 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 fosfomycin tromethamine; fluoroquinolones and β-lactams, such as amoxicillin-clavulanate, are second-line therapies (1). High urine concentrations (~4000 µg/ml, 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* (2,3). 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 (4). EUCAST also publishes MIC breakpoints for fosfomycin for staphylococci and Enterobacteriaceae with a MIC ≤32 ug/ml considered susceptible (resistance, >32 ug/ml) for both parenteral (systemic infections) and oral (uncomplicated urinary tract infection only) therapy.

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 (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 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 2014.

Organism (n)	Antimicrobial agent	(µg/ml) MIC_{50}	MIC_{90}	MIC range	CLSI MIC Interpretation[a] % S	% I	% R	EUCAST MIC Interpretation[b] % S	% I	% R
Escherichia coli (877)[c]	Fosfomycin	≤1	4	≤1-128	99.7	0.3	0	98.6	-	1.8
	SXT	≤0.12	>8	≤0.12->8	78.2	-	21.8	78.2	0.6	21.2
	Nitrofurantoin	16	32	≤1-256	97.8	1.5	0.7	99.3	-	0.7
	Ciprofloxacin	≤0.06	>16	≤0.06->16	85.4	0	14.6	84.3	1.1	14.6
	Amox-clav	4	16	≤0.06->32	88.8	8.9	2.3	99.4	-	0.6
Klebsiella pneumoniae (106)[c]	Fosfomycin	32	128	2->512	89.6	3.8	6.6	67.0	-	33.0
	SXT	≤0.12	0.25	≤0.12->8	96.2	-	3.8	96.2	0	3.8
	Nitrofurantoin	64	128	4->512	34.9	28.3	36.8	63.2	-	36.8
	Ciprofloxacin	≤0.06	≤0.06	≤0.06->16	98.1	1.0	0.9	98.1	0	1.9
	Amox-clav	2	8	1->32	96.7	2.2	1.1	98.9	-	1.1
Enterococcus faecalis (68)	Fosfomycin	64	128	32-256	83.8	13.3	2.9	-	-	-
	SXT	≤0.12	≤0.12	≤0.12->8	NA	NA	NA	UD[d]	UD	UD
	Nitrofurantoin	8	8	4-16	100	0	0	100	-	0
	Ciprofloxacin	1	>16	0.25->16	72.1	5.8	22.1	77.9	-	22.1
	Amox-clav	0.5	1	0.12-2	NA	NA	NA	100	0	0
Proteus mirabilis (40)	Fosfomycin	4	128	≤1->512	85.0	10.0	5.0	77.5	-	22.5
	SXT	≤0.12	>8	≤0.12->8	87.5	-	12.5	87.5	0	12.5
	Nitrofurantoin	128	256	64-256	0	17.5	82.5	17.5	-	82.5
	Ciprofloxacin	≤0.06	2	≤0.06->16	87.5	7.5	5.0	87.5	0	12.5
	Amox-clav	1	16	0.5->32	90.0	7.5	2.5	97.5	0	2.5
Pseudomonas aeruginosa (27)	Fosfomycin	128	256	4->512	48.1	22.3	29.6	-	-	-
	SXT	8	>8	2->8	NA	NA	NA	-	-	-
	Nitrofurantoin	>512	>512	512->512	NA	NA	NA	-	-	-
	Ciprofloxacin	0.5	>16	≤0.06->16	66.7	3.7	29.6	59.3	7.4	33.3
	Amox-clav	>32	>32	>32	NA	NA	NA	-	-	-
Staphylococcus aureus (26)	Fosfomycin	8	32	≤1-32	100	0	0	100	-	0
	SXT	≤0.12	≤0.12	≤0.12	100	-	0	100	0	0
	Nitrofurantoin	16	16	8-32	100	0	0	100	-	0
	Ciprofloxacin	16	>16	0.25->16	50.0	0	50.0	50.0	-	50.0
	Amox-clav	4	>32	4->32	66.7	-	33.3	50.0	-	50.0
Klebsiella oxytoca (22)	Fosfomycin	16	64	8-128	90.9	9.1	0	81.8	-	18.2
	SXT	≤0.12	≤0.12	≤0.12-0.25	100	-	0	100	0	0
	Nitrofurantoin	32	32	8-64	95.5	4.5	0	100	-	0
	Ciprofloxacin	≤0.06	≤0.06	≤0.06-0.25	100	0	0	100	0	0
	Amox-clav	2	16	1-16	89.5	10.5	0	100	-	0
Enterobacter cloacae (14)	Fosfomycin	16	128	≤1->512	85.7	7.2	7.1	85.7	-	14.3
	SXT	≤0.12	≤0.12	≤0.12-8	92.9	-	7.1	92.9	0	7.1
	Nitrofurantoin	64	128	8-128	35.7	28.6	35.7	64.3	-	35.7
	Ciprofloxacin	≤0.06	2	≤0.06-16	85.7	7.2	7.1	85.7	0	14.3
	Amox-clav	>32	>32	2->32	21.4	21.5	57.1	42.9	-	57.1
Citrobacter freundii (7)	Fosfomycin	≤1	≤1	≤1	100	0	0	100	-	0
	SXT	≤0.12	>8	≤0.12->8	71.4	-	28.6	71.4	14.3	14.3
	Nitrofurantoin	16	32	8-32	100	0	0	100	-	0
	Ciprofloxacin	≤0.06	>16	≤0.06->16	85.7	0	14.3	85.7	0	14.3
	Amox-clav	16	>32	1->32	28.6	28.5	42.9	71.4	-	28.6

[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 Enterobacteriacae (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] 28/877 (3.2%) *E. coli* were ESBL-positive; 2/106 (1.9%) *K. pneumoniae* were ESBL-positive; MIC range for ESBL-positive isolates was ≤1-128 ug/ml.
[d] UD, Unable to determine.

Table 2. MIC distributions for fosfomycin against outpatient urine isolates collected by 15 laboratories in Canada from 2007 to 2014.

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 (877)	61.7	88.7	94.4	96.2	97.6	98.6	99.7	100		
Klebsiella pneumoniae (106)		0.9	2.8	10.4	28.3	67.0	89.6	93.4	97.2	100[b]
Enterococcus faecalis (68)						23.5	83.8	97.1	100	
Proteus mirabilis (40)	2.5	17.5	55.0	62.5	72.5	77.5	85.0	95.0	97.5	100[b]
Pseudomonas aeruginosa (27)			7.4		11.1	22.2	48.1	70.4	92.6	100[b]
Staphylococcus aureus (26)	11.5	19.2	50.0	61.5	80.8	100				
Klebsiella oxytoca (22)				22.7	59.1	81.8	90.9	100		
Enterobacter cloacae (14)	28.6	35.7		50.0	64.3	85.7		92.9		100[b]
Citrobacter freundii (7)	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 99.7% of isolates susceptible.
- >85.0% 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.
- >50% of fosfomycin MICs for isolates of *P. aeruginosa* were intermediate or resistant when MICs were interpreted using *E. coli* breakpoints
- Only 84% of *E. faecalis* 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, and *S. pneumoniae*, than other Gram-positive bacteria; our data, although limited, appears to support these previous findings for *S. aureus*. The majority of isolates of *S. aureus*, and enterococci (including VRE) have fosfomycin MICs ≤32 µg/ml and would be potentially susceptible to urinary concentrations of fosfomycin. 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 appears to impact the % of isolates of Enterobacteriaceae reported as susceptible.
- In general, fosfomycin possesses broad-spectrum activity against most Gram-negative and Gram-positive bacterial pathogens causing urinary tract infections in outpatients.

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