

Introduction

- In North America, oral fosfomycin tromethamine is approved for treatment of uncomplicated urinary tract infection (UTI).
- In North America, intravenous (IV) fosfomycin sodium is indicated alone for complicated UTI and in combination with other antimicrobials, for treatment of serious infections in adults and children.
- Fosfomycin inhibits susceptible bacteria by irreversibly inactivating the enzyme UDP-N-acetylglucosamine-3-enolpyruvyltransferase (MurA) which ligates phosphoenolpyruvate (PEP) to the 3'-hydroxyl group of UDP-N-acetylglucosamine, an essential building block of peptidoglycan.
- Clinical laboratories do not routinely determine fosfomycin MICs using the CLSI reference method (agar dilution) because it is labor-intensive and impairs workflow.
- Susceptibility to fosfomycin may also be determined by Etest® (gradient strip) or by disk diffusion; however, the appearance of colonies within the zone of inhibition can complicate zone size reading.
- Broth dilution testing is not an acceptable method for fosfomycin (CLSI, EUCAST).
- Current CLSI, EUCAST, and FDA MIC/zone size breakpoints for fosfomycin are restricted to only a few bacterial species.

Methods

- 4,008 urine isolates of *E. coli*, *K. pneumoniae*, and *E. faecalis* collected by the annual CANWARD surveillance study (12–15 Canadian tertiary-care hospital clinical laboratories/year) from 2007 to 2022 were included in the main study (Table 2).
- 458 other *Enterobacterales* species, 158 *P. aeruginosa*, 100 *S. aureus*, and 50 *E. faecium* urine isolates were also tested against fosfomycin (Table 3).
- Fosfomycin susceptibility testing was performed using the CLSI M07 reference agar dilution method (Mueller-Hinton agar supplemented with 25 µg/ml of glucose-6-phosphate); all other antibacterial agents were tested by the CLSI M07 reference broth microdilution method.
- ESBL-positive isolates of *E. coli* and *K. pneumoniae* were identified following CLSI guidelines.

Results

Table 2. MIC₅₀, MIC₉₀, and percent susceptible data at MIC breakpoint concentrations for fosfomycin and percent susceptible data for comparators tested against *E. coli*, *K. pneumoniae*, and *E. faecalis* isolated from urine specimens in Canada from 2007 to 2022

Isolate Phenotype (n)	MIC ₅₀ /MIC ₉₀ µg/ml	% Susceptible (MIC breakpoint concentration)			% Susceptible			
	Fosfomycin	Fosfomycin (≤64 µg/ml) ^a	Fosfomycin (≤32 µg/ml) ^b	Fosfomycin (≤8 µg/ml) ^c	SXT	NIT	CIP	AMC
<i>Escherichia coli</i> (3,115)	≤1/4	99.2	NA	95.8	75.3	97.2	76.5	80.9
<i>Escherichia coli</i> , ESBL-positive (237)	≤1/4	96.2	NA	94.9	33.3	88.6	15.6	48.2
<i>Klebsiella pneumoniae</i> (446)	32/128	NA	71.5	NA	87.9	37.0	88.1	91.0
<i>Klebsiella pneumoniae</i> , ESBL-positive (28)	16/256	NA	75.0	NA	10.7	20.8	25.0	26.9
<i>Enterococcus faecalis</i> (447)	64/128	88.8	NA	NA	NA	99.5	66.2	99.7 ^d

Abbreviations: SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin; CIP, ciprofloxacin; AMC, amoxicillin-clavulanate; NA, not applicable.
^a MICs interpreted using CLSI M100 (36th Edition, 2026) breakpoints (urine) for *E. coli* and *E. faecalis*.
^b MICs interpreted using FDA breakpoint for IV fosfomycin for *K. pneumoniae*.
^c MICs interpreted using EUCAST (Version 16.0, 2026) oral (uncomplicated UTI) and IV (infections originating from the urinary tract) breakpoints for *E. coli*, and the FDA breakpoint for IV fosfomycin for *E. coli*.
^d AMC activity was predicted using MIC breakpoints for ampicillin for *E. faecalis*.

Table 3. Cumulative MIC distributions for fosfomycin against urine isolates collected across Canada from 2007 to 2022

Genus/species (n)	Fosfomycin MIC (µg/ml)									
	Cumulative % of isolates inhibited at MIC									
	≤1	2	4	8 ^a	16	32 ^b	64 ^c	128	256	≥512
Gram-Negative Urine Isolates										
<i>E. coli</i> (3,115)	61.9	88.5	94.3	95.8	97.2	98.4	99.2	99.7	99.8	100
<i>E. coli</i> , ESBL-positive (237)	53.6	89.0	94.1	94.9	95.8	96.2	96.2	97.9	98.3	100
<i>K. pneumoniae</i> (446)	0.4	1.8	5.2	13.2	37.7	71.5	89.5	93.7	96.9	100
<i>K. pneumoniae</i> , ESBL-positive (28)	0	3.6	7.1	17.9	50.0	75.0	82.1	89.3	96.4	100
<i>Proteus mirabilis</i> (196)	10.7	32.7	60.7	69.9	74.5	80.6	86.2	94.4	96.4	100
<i>Pseudomonas aeruginosa</i> (158)	0.6	1.3	4.4	4.4	10.1	17.1	51.3	85.4	94.9	100
<i>Enterobacter cloacae</i> (100)	9.0	18.0	22.0	28.0	44.0	62.0	72.0	82.0	88.0	100
<i>Klebsiella oxytoca</i> (90)	1.1	3.3	5.6	23.3	55.6	77.8	85.6	93.3	97.8	100
<i>Citrobacter freundii</i> (37)	81.1	97.3	97.3	97.3	100					
<i>Klebsiella aerogenes</i> (35)	0	2.9	2.9	11.4	40.0	80.0	88.6	91.4	97.1	100
Gram-Positive Urine Isolates										
<i>E. faecalis</i> (447)	0.2	0.2	0.4	0.7	1.6	30.9	88.8	98.7	99.6	100
<i>Staphylococcus aureus</i> (100)	7.0	17.0	51.0	76.0	91.0	98.0	99.0	99.0	100	
<i>E. faecium</i> (50)						2.0	48.0	98.0	98.0	100

^a EUCAST (Version 16.0, 2026) oral (uncomplicated UTI) and IV (infections originating from the urinary tract) fosfomycin-susceptible breakpoints for *E. coli*, and the FDA fosfomycin-susceptible breakpoint for IV fosfomycin for *E. coli*.
^b FDA fosfomycin-susceptible breakpoint for IV fosfomycin for *K. pneumoniae*.
^c CLSI M100 (36th Edition, 2026) fosfomycin-susceptible breakpoints (urine) for *E. coli* and *E. faecalis*.

Conclusions

- Fosfomycin demonstrated *in vitro* activity against pathogens frequently causing UTIs in Canadian patients.
 - >99% of all *E. coli*, including 96.2% of ESBL-positive isolates, were fosfomycin-susceptible using the CLSI MIC breakpoint for urine (≤64 µg/ml).
 - 95.8% of all *E. coli*, including 94.9% of ESBL-positive isolates, were fosfomycin-susceptible using applicable EUCAST and FDA MIC breakpoints (≤8 µg/ml).
 - 71.5% of all *K. pneumoniae*, including 75.0% of ESBL-positive isolates, were fosfomycin-susceptible using the FDA MIC breakpoint (≤32 µg/ml).
 - 88.8% of *E. faecalis* were susceptible to fosfomycin using the CLSI MIC breakpoint for urine (≤64 µg/ml).
- Clinicians should be aware of differences in fosfomycin formulations and their associated MIC breakpoints.
- The differences in CLSI, EUCAST, and FDA MIC breakpoints impacts the percentage of *Enterobacterales* isolates reported as susceptible.
- The *in vitro* susceptibilities to trimethoprim-sulfamethoxazole (75%) and ciprofloxacin (77%), two frequently prescribed empiric agents for UTIs, were ~20% lower than to fosfomycin (96–99%) for *E. coli*.
- ~89% of *E. faecalis* isolates were susceptible to fosfomycin compared to 99.5% for nitrofurantoin and 99.7% for amoxicillin-clavulanate.
- Fosfomycin demonstrated broad-spectrum *in vitro* activity against *Enterobacterales*, enterococci, and staphylococci frequently isolated from Canadian patients with UTIs.

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References

- Monurol® package insert. 2019. Allergan, Inc.
- Contepo package insert. 2025. Meitheal Pharmaceuticals Inc.
- M07, 12th edition. 2024. CLSI.
- M100, 36th edition. 2026. CLSI.
- Clinical breakpoints – bacteria (v 16.0). 2026. EUCAST.
- <https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria>