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ORIGINAL RESEARCH

Fosfomycin and Comparator Activity Against Select Enterobacteriaceae, *Pseudomonas*, and *Enterococcus* Urinary Tract Infection Isolates from the United States in 2012

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ABSTRACT

Introduction: Fosfomycin is a broad-spectrum cell wall active agent that inhibits the MurA enzyme involved in peptidoglycan synthesis and is FDA-approved for treatment of uncomplicated urinary tract infections (UTIs) caused by *Escherichia coli* and *Enterococcus faecalis* in women. Data regarding the susceptibility of recent UTI isolates to fosfomycin are limited.

Methods: This study compared the fosfomycin susceptibility of 658 US UTI isolates with susceptibility to ciprofloxacin, levofloxacin, nitrofurantoin, and trimethoprim/sulfamethoxazole (SXT). Isolates included *E. coli* ($n = 257$), *Klebsiella* spp. ($n = 156$), *Enterobacter* spp. ($n = 79$), *Pseudomonas aeruginosa* ($n = 60$), *E. faecalis* ($n = 54$), and *Proteus* spp. ($n = 52$). Extended-spectrum β-lactamase (ESBL)-producing *E. coli*, *Klebsiella* spp., and *Proteus mirabilis*, ceftazidime-nonsusceptible *P. aeruginosa* and *Enterobacter* spp., and vancomycin-nonsusceptible *E. faecalis* were included.

Results: Overall, the minimum concentration inhibiting 50% of isolates (MIC_{50}) and 90% of isolates (MIC_{90}) for fosfomycin were 4 and 64 μg/mL, respectively. Of the 257 *E. coli* isolates, 99.6% were susceptible to fosfomycin. Ciprofloxacin, levofloxacin, SXT, and nitrofurantoin susceptibility rates were 65.4%, 65.8%, 59.9%, and 90.3%, respectively. The fosfomycin-susceptibility rate for *E. faecalis* (94.4%) was comparable with the nitrofurantoin-susceptibility rate (98.1%). Among the 144 ESBL-producing isolates, the fosfomycin MIC_{50} and MIC_{90} values were 2 and 32 μg/mL, respectively. Fosfomycin MIC_{50} and MIC_{90} values were 16 and 128 μg/mL for the 38 ceftazidime-nonsusceptible *Enterobacter* isolates and 64 and 128 μg/mL for the 15 ceftazidime-nonsusceptible *P. aeruginosa* isolates, respectively.

Conclusion: These results demonstrate that fosfomycin has in vitro activity against many US UTI isolates, including drug-resistant isolates, and may provide another therapeutic option for treatment of UTIs caused by antibiotic-resistant pathogens.

Keywords: Antimicrobial; Fosfomycin; Urinary tract infection

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INTRODUCTION

Uncomplicated urinary tract infections (UTIs) are one of the most common infections for which antibiotics are prescribed, and there is a current trend of increasing resistance to antibiotics typically used to treat these infections [1]. Contributing to this increased resistance is the increased frequency of extended spectrum β-lactamase (ESBL) production in organisms causing UTIs, particularly *Escherichia coli* and *Klebsiella* spp [2, 3].

Fosfomycin, trimethoprim/sulfamethoxazole (SXT), and nitrofurantoin are the 3 treatment options recommended for uncomplicated UTIs by the Infectious Diseases Society of America (IDSA) [1]. To safeguard against development of resistance, fluoroquinolones, such as ciprofloxacin and levofloxacin, are not recommended for use in treatment of uncomplicated UTI, despite their effectiveness. The IDSA guidelines also recommend that ampicillin not be used because of the increased prevalence of ESBL-producing organisms in uncomplicated UTIs, and that β-lactam antibiotics are used only when other treatment options are unavailable [1].

Ciprofloxacin and SXT resistance rates among urine isolates from female outpatients increased dramatically from 2003 to 2012, especially among elderly outpatients, highlighting the need for other oral therapeutic alternatives [4]. In a study of 1518 extraintestinal *E. coli* isolates, including 1034 urinary isolates, recovered from patients in the United States and Germany between 2010 and 2011, investigators observed resistance rates to nitrofurantoin, ciprofloxacin, and SXT of 6.8%, 17.1%, and 26.9%, respectively [5].

Fosfomycin is a bactericidal, cell wall active agent that binds to the MurA enzyme, thereby inhibiting the first step in peptidoglycan formation [6, 7]. It is approved, as fosfomycin tromethamine for oral administration, by the US Food and Drug Administration (FDA) for the treatment of women with uncomplicated UTIs (acute cystitis) caused by *E. coli* and *Enterococcus faecalis* and has broad-spectrum activity in vitro against Gram-positive and Gram-negative bacteria [8]. Fosfomycin has recently received renewed interest because of its activity against multidrug-resistant bacteria [7, 9, 10] and the low frequency of cross-resistance to it [8], likely due to its unique mechanism of action. Furthermore, fosfomycin has low and stable resistance rates worldwide (1–3% of *E. coli*) despite more than 20 years of clinical use in some countries [11, 12]. In cases where fosfomycin resistance does emerge, common mechanisms include mutations affecting transport proteins (e.g., GlpT and UhpT) and mutation of its target, MurA [11].

This study sought to compare the sensitivity of UTI isolates to fosfomycin with sensitivity to antibiotics commonly used for treatment of UTIs, including ciprofloxacin, levofloxacin, nitrofurantoin, and SXT, among US isolates from the 2012 Assessing Worldwide Antimicrobial Resistance Evaluation (AWARE) surveillance program. AWARE is a global surveillance program established to monitor the susceptibility of prespecified pathogens to a number of antimicrobial agents [13]. Medical centers from the nine US census regions contributed to the database, with organisms collected being sent for susceptibility testing to a central laboratory (JMI Laboratories, North Liberty, IA, USA) [13]. Isolates for the present study were selected proportionally to reflect the most commonly isolated species from uncomplicated UTIs.

To test the hypothesis that fosfomycin has activity against typical urinary tract pathogens, the activity against isolates with a resistant phenotype, ESBL-positive *E. coli*, *Klebsiella* spp., and *Proteus* spp. was assessed, as was the activity of fosfomycin against ceftazidime-nonsusceptible *P. aeruginosa*, *Enterobacter* spp., and vancomycin-nonsusceptible *E. faecalis* isolates.

METHODS

Isolates and Reagents

Isolates collected during the 2012 AWARE surveillance program were retrospectively obtained from JMI Laboratories. Isolates were

selected to obtain similar numbers of each species from each of the nine US census regions, based on their regional prevalence in the AWARE program [14]. Isolates ($n = 658$) included *E. coli* ($n = 257$), *Klebsiella* spp. ($n = 156$), *Enterobacter* spp. ($n = 79$), *P. aeruginosa* ($n = 60$), *E. faecalis* ($n = 54$), and *Proteus* spp. ($n = 52$). Resistant isolates included *E. coli*, *Klebsiella* spp., and *P. mirabilis* with an ESBL phenotype; ceftazidime-nonsusceptible *P. aeruginosa* and *Enterobacter* spp.; and vancomycin-nonsusceptible *E. faecalis*. The ESBL-phenotype was previously determined by JMI Laboratories and defined according to the Clinical and Laboratory Standards Institute (CLSI) ESBL screening criteria (ceftazidime and/or ceftriaxone and/or aztreonam minimum inhibitory concentration [MIC] value >1 μg/mL) [15]. Ceftazidime non-susceptibility (MICs ≥8 μg/mL) was used to categorize *Enterobacter* spp. Ceftriaxone and vancomycin susceptibilities were determined by JMI Laboratories using CLSI breakpoints [15]. Genotype determinations were made by JMI Laboratories using Check-points microarray kit CT–101 (Check-Points, Wageningen, The Netherlands) [16, 17]. This kit has the capability to detect $bla_{\text{CTX-M}}$ Groups 1, 2, 8 + 25, and 9, bla_{TEM} wild-type (wt) and ESBL, bla_{SHV} wt and ESBL, bla_{ACC}, $bla_{\text{ACT/MIR}}$, bla_{CMYII}, bla_{DHA}, bla_{FOX}, bla_{KPC}, and $bla_{\text{NDM-1}}$ (but not $bla_{\text{OXA-48}}$, bla_{IMP} or bla_{VIM}).

Antibiotics included in this study were fosfomycin (P5396-5G, lot BCBJ9873V; Sigma-Aldrich, St. Louis, MO, USA), ciprofloxacin (1134335, lot J0H307; USP, Rockville, MD, USA), levofloxacin (28266-1G-F, lot 1425507; Sigma-Aldrich, St. Louis, MO, USA), nitrofurantoin (1464001, lot K01060; USP), trimethoprim (Sigma T7883-5G, lot BCBB6418; Sigma-Aldrich), and sulfamethoxazole (Sigma S7507-10G, lot 048K0124; Sigma-Aldrich). Reagents used in this study included Mueller–Hinton agar (MHA; BBL 211438, lot 3217394; BD Diagnostic Systems, Sparks, MD, USA), glucose-6-phosphate (G7879-1G, lot 031M7004V; Sigma-Aldrich), and cation-adjusted Mueller–Hinton broth (CAMHB; BBL 21322; BD Diagnostic Systems).

Agar Dilution

Minimum inhibitory concentration for fosfomycin was determined by agar dilution according to CLSI documents M07-A9 and M100-S24 [15, 18]. MHA was prepared according to manufacturer's instructions followed by the addition of glucose-6-phosphate to a final concentration of 25 μg/mL. Fosfomycin was added to MHA in 2-fold serial dilutions at concentrations ranging from 0.25 to 256 μg/mL. Approximately 10^4 colony-forming units (CFU)/spot were added to plates. After incubation at 35 ± 2 °C for 16–20 h, the MIC was recorded as the lowest concentration of antimicrobial agent that completely inhibited visible growth.

Broth Microdilution

Minimum inhibitory concentration values for ciprofloxacin, levofloxacin, nitrofurantoin, and SXT were determined by broth microdilution methods according to CLSI documents M07-A9 and M100-S24 [15, 18], with testing of appropriate quality control strains.

Antibiotic test plates were prepared in-house using a twofold dilution series in 96-well round-bottom plates containing antibiotic (10× solution). Bacterial suspensions were diluted in CAMHB and added to the antibiotic test plates to yield a final culture density of 5×10^5 CFU/mL. After incubation for 18 h at 35 ± 2 °C, the MIC was recorded as the lowest concentration where there was no visible bacterial growth.

Analysis

Minimum inhibitory concentration values were interpreted with respect to CLSI interpretative criteria for *E. coli* and *E. faecalis* [fosfomycin breakpoints were MIC ≤64 μg/mL (susceptible), MIC = 128 μg/mL (intermediate), and MIC ≥256 μg/mL (resistant)] [15].

Compliance with Ethics Guidelines

All procedures followed were in accordance with the ethical standards of the responsible

committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2008. Informed consent was not required because isolates used in this study were from a previously conducted surveillance program and no patient-identifiable data were held by the investigators undertaking the current research.

RESULTS

A total of 658 UTI isolates were tested from the nine US census regions representing defined numbers of isolates of selected species collected during the 2012 AWARE surveillance program. Overall, MIC values for fosfomycin ranged from ≤0.25 to >256 μg/mL, and the fosfomycin MIC_{50} and MIC_{90} values for all isolates were 4 and 64 μg/mL, respectively (Table 1). A total of 202 isolates had a resistant phenotype (Table 2). Overall, 21.9% (144/658) of isolates were of the ESBL-phenotype (*E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis* only), 53 (8.1%) were ceftazidime-nonsusceptible *E. cloacae* and *P. aeruginosa* isolates and 5 (0.8%) were vancomycin-nonsusceptible *E. faecalis* isolates.

Susceptibility results of the 257 *E. coli* isolates tested are shown in Table 1. The fosfomycin MIC_{50} and MIC_{90} values for *E. coli* were 1 and 4 μg/mL, respectively. Only one (0.4%) *E. coli* isolate (an ESBL-phenotype) was resistant to fosfomycin. Susceptibility results of the 76 *E. coli* isolates with an ESBL phenotype are shown in Table 2.

Summaries of pathogen susceptibilities are provided in Tables 1 and 2. Fosfomycin had MIC_{50} and MIC_{90} values of 8 and 32 μg/mL for *Klebsiella* spp., 4 and 32 μg/mL for *Proteus* spp., and 16 and 32 μg/mL for *Enterobacter* spp., respectively (Table 1). Most *E. faecalis* isolates (94.4%) were susceptible to fosfomycin (Table 1). Fosfomycin MIC_{50} and MIC_{90} values against *P. aeruginosa* were 64 and 128 μg/mL, respectively (Table 1). Among all 202 resistant organisms tested (including those with an ESBL phenotype, ceftazidime-nonsusceptible Gram-negative pathogens, and vancomycin-nonsusceptible *E. faecalis*), fosfomycin had MIC_{50} and MIC_{90} values of 4 and 64 μg/mL, respectively (Table 2). Twenty-four of these isolates (23 *K. pneumoniae* and 1 *E. coli*) were carbapenemase producers based on the detection of a KPC gene and on being nonsusceptible to meropenem (data not shown). All but two of these carbapenem resistant isolates had MICs indicative of susceptibility to fosfomycin (based on the CLSI interpretive guidelines for *E. coli and E. faecalis,* one was intermediate and one resistant). There were only 5 vancomycin-nonsusceptible *E. faecalis* tested, but all were susceptible to fosfomycin with MIC values ≤64 μg/mL (Table 2). Fosfomycin MIC_{50} and MIC_{90} values for *Klebsiella* spp. with an EBSL-phenotype were 8 and 128 μg/mL and for ceftazidime-nonsusceptible *Enterobacter* spp. were 16 and 128 μg/mL, respectively (Table 2). By comparison, the fosfomycin MIC_{50} for ceftazidime-nonsusceptible *P. aeruginosa* was numerically higher (64 μg/mL), whereas the MIC_{90} value was similar (128 μg/mL; Table 2).

In total, 17 (2.6%) isolates had MIC values suggesting resistance to fosfomycin (data not shown), including *E. coli* (ESBL-phenotype; $n = 1$), ceftazidime-nonsusceptible *E. cloacae* ($n = 3$), vancomycin-susceptible *E. faecalis* ($n = 1$), *K. pneumoniae* [$n = 10$; ESBL-phenotype ($n = 5$) and non-ESBL-phenotype ($n = 5$)], non-ESBL-phenotype *P. mirabilis* ($n = 1$), and ceftazidime-nonsusceptible *P. aeruginosa* ($n = 1$). Among those isolates with an ESBL-phenotype that were fosfomycin-"resistant" ($n = 6$), there was no common β-lactamase gene detected among all the isolates, and there was no clear correlation between β-lactamase genes present and fosfomycin resistance (data not shown). Three isolates with $bla_{CTX\text{-}M}$ genes were resistant to fosfomycin (2 *K. pneumoniae* and 1 *E. coli*).

DISCUSSION

Overall, fosfomycin exhibited antibacterial activity against the panel of 658 organisms. With the exception of 1 isolate, all *E. coli* isolates tested were susceptible to fosfomycin. By comparison, the *E. coli* susceptibility rates were markedly lower for the other antibiotics tested,

Table 1 Pathogen characteristics and antibiotic susceptibilities

	MIC_{50}	MIC_{90}	Range	*S* (%)	*I* (%)	*R* (%)
All organisms ($n = 658$)						
Fosfomycin	4	64	≤0.25 to >256	–	–	–
Ciprofloxacin	0.06	>32	0.004 to >32	68.4	3.0	28.6
Levofloxacin	0.25	32	0.016 to >32	70.4	1.7	28.0
Nitrofurantoin[a]	32	128	1 to >128	56.4	23.3	20.3
SXT[b]	0.5	>32	0.06 to >32	63.4	–	36.6
E. coli ($n = 257$)						
Fosfomycin	1	4	≤0.25 to >256	99.6	0.0	0.4
Ciprofloxacin	0.03	>32	0.004 to >32	65.4	0.0	34.6
Levofloxacin	0.06	32	0.016 to >32	65.8	0.4	33.9
Nitrofurantoin	16	32	2 to >128	90.3	5.8	3.9
SXT	0.5	>32	0.06 to >32	59.9	–	40.1
Klebsiella spp. ($n = 156$)[c]						
Fosfomycin	8	32	0.5 to >256	–	–	–
Ciprofloxacin	0.06	>32	0.008 to >32	66.0	1.9	32.1
Levofloxacin	0.125	>32	0.016 to >32	68.6	1.3	30.1
Nitrofurantoin	64	128	2 to 128	9.0	41.0	50.0
SXT	0.5	>32	0.06 to >32	58.3	–	41.7
Proteus spp. ($n = 52$)[d]						
Fosfomycin	4	32	≤0.25 to >256	–	–	–
Ciprofloxacin	0.03	4	0.016 to >32	86.5	1.9	11.5
Levofloxacin	0.06	4	≤0.03 to 32	88.5	3.8	7.7
Nitrofurantoin	–	–	–	–	–	–
SXT	0.25	>32	0.06 to >32	73.1	–	26.9
Enterobacter spp. ($n = 79$)[e]						
Fosfomycin	16	32	0.5 to >256	–	–	–
Ciprofloxacin	0.03	32	0.008 to >32	83.5	5.1	11.4
Levofloxacin	0.06	32	0.016 to >32	83.5	5.1	11.4
Nitrofurantoin	64	128	1 to 128	11.4	59.5	29.1
SXT	0.5	>32	0.06 to >32	78.5	–	21.5
CAZ S *Enterobacter* spp. ($n = 41$)[f]						
Fosfomycin	16	32	0.5 to 128	–	–	–
Ciprofloxacin	0.03	0.25	0.016 to 32	97.6	0.0	2.4
Levofloxacin	0.06	0.25	0.016 to 32	97.6	0.0	2.4
Nitrofurantoin	64	128	2 to 128	7.3	58.5	34.1
SXT	0.25	2	≤0.25 to >32	90.2	–	9.8
E. faecalis ($n = 54$)						
Fosfomycin	32	64	16 to >256	94.4	3.7	1.9
Ciprofloxacin	1	>32	0.5 to >32	51.9	16.7	31.5
Levofloxacin	1	>32	0.5 to >32	68.5	0.0	31.5
Nitrofurantoin	8	16	8 to 64	98.1	1.9	0.0
SXT	–	–	–	–	–	–
VAN S *E. faecalis* ($n = 49$)						
Fosfomycin	64	64	16 to 256	93.9	4.1	2.0
Ciprofloxacin	1	>32	0.5 to >32	57.1	18.4	24.5

Table 1 continued

	MIC_{50}	MIC_{90}	Range	*S* (%)	*I* (%)	*R* (%)
Levofloxacin	1	>32	0.5 to >32	75.5	0.0	24.5
Nitrofurantoin	8	16	8 to 64	98.0	2.0	0.0
SXT	–	–	–	–	–	–
P. aeruginosa ($n = 60$)						
Fosfomycin	64	128	2 to >256	–	–	–
Ciprofloxacin	0.25	32	0.008 to >32	66.7	5.0	28.3
Levofloxacin	1	>32	0.016 to >32	63.3	3.3	33.3
Nitrofurantoin	–	–	–	–	–	–
SXT	–	–	–	–	–	–
CAZ S *P. aeruginosa* ($n = 45$)						
Fosfomycin	64	128	4 to 128	–	–	–
Ciprofloxacin	0.125	32	0.008 to >32	84.4	0.0	15.6
Levofloxacin	0.5	>32	0.016 to >32	80.0	4.4	15.6
Nitrofurantoin	–	–	–	–	–	–
SXT	–	–	–	–	–	–

Percent susceptible not calculable due to organisms in the group without CLSI breakpoints indicated by "–"
CAZ S ceftazidime-susceptible, *I* intermediate, MIC_{50} minimum concentration that inhibits 50% of isolates, MIC_{90} minimum concentration that inhibits 90% of isolates, *R* resistant, *S* susceptible, *SXT* trimethoprim/sulfamethoxazole, *VAN S* vancomycin-susceptible
[a] *P. aeruginosa* and *Proteus* spp. ($n = 546$) are intrinsically resistant to nitrofurantoin and were not included in the calculations
[b] *P. aeruginosa* and *E. faecalis* ($n = 544$) are intrinsically resistant to SXT, so those organisms were not included in the calculations
[c] *K. pneumoniae* ($n = 36$); *K. oxytoca* ($n = 20$)
[d] *P. mirabilis* ($n = 34$); *P. vulgaris* ($n = 18$)
[e] *E. cloacae* ($n = 49$); *E. aerogenes* ($n = 30$)
[f] *E. cloacae* ($n = 26$); *E. aerogenes* ($n = 15$)

a significant finding given that *E. coli* is the pathogen most commonly found in UTI infections. The fosfomycin susceptibility rate for *E. coli* isolates with an ESBL-phenotype was markedly higher than any other antibiotic tested. Among the isolates with an ESBL phenotype, $bla_{\text{CTX-M}}$ group 1 and non-ESBL bla_{TEM} were the most common β-lactamase genes detected (data not shown).

A study of *E. coli* isolated from urine of US outpatients from 2000 to 2010 reported resistance rates for ciprofloxacin, nitrofurantoin, and SXT that were slightly lower than those observed in the present study; this likely reflects a continued increase in resistance over the period 2010 to 2012 similar to that observed for the period 2000 to 2010 [20]. A recent study of *E. coli* isolates from patients with UTI in Canada during the period from 2010 to 2013 demonstrated susceptibility rates similar to the present study for fosfomycin (99.4% vs. 99.6%, respectively) and nitrofurantoin (96.1% vs. 90.3%, respectively) and higher than observed for ciprofloxacin (77.4% vs. 65.4%, respectively), and SXT (74.7% vs. 59.9%, respectively) [21]. US researchers also found *E. coli* to commonly exhibit susceptibility to fosfomycin (100%, tested using agar dilution) and nitrofurantoin (98.0%) [19].

Compared with the present study, similar susceptibility rates were also reported among ESBL-producing *E. coli* isolates from patients with UTI in the Canadian study for fosfomycin (100%) and nitrofurantoin (83.3%), with SXT (35.7%), and ciprofloxacin (9.5%) rates being somewhat lower [21]. Similarly, in a small US study, ESBL-producing *E. coli* isolates were susceptible to fosfomycin (96.7%; MIC values ranged from 0.25 to 4 μg/mL), but less so to nitrofurantoin (76.7%; MIC values of 8–64 μg/mL; susceptibility breakpoint ≤32 μg/mL) [22]. Another study of *E. coli* isolated from patients with UTI in China from 2004 to 2012 showed that fosfomycin susceptibility rates were high

Table 2 Pathogen characteristics and antibiotic susceptibilities for resistant isolates

	MIC_{50}	MIC_{90}	Range	*S* (%)	*I* (%)	*R* (%)
Resistant organisms ($n = 202$)						
Fosfomycin	4	64	≤0.25 to >256	–	–	–
Ciprofloxacin	32	>32	0.016 to >32	32.2	5.0	62.9
Levofloxacin	16	>32	0.016 to >32	33.7	3.0	63.4
Nitrofurantoin[a]	64	128	1 to 128	41.8	25.5	32.6
SXT[b]	>32	>32	0.06 to >32	40.7	–	59.3
ESBL-phenotype ($n = 144$)[c]						
Fosfomycin	2	32	≤0.25 to >256	–	–	–
Ciprofloxacin	>32	>32	0.016 to >32	25.7	2.1	72.2
Levofloxacin	16	>32	0.016 to >32	27.8	1.4	70.8
Nitrofurantoin[d]	64	128	8 to 128	46.8	17.0	36.2
SXT	>32	>32	0.06 to >32	34.0	–	66.0
E. coli ESBL-phenotype ($n = 76$)						
Fosfomycin	1	4	≤0.25 to >256	98.7	0.0	1.3
Ciprofloxacin	>32	>32	0.016 to >32	28.9	0.0	71.1
Levofloxacin	16	32	0.03 to >32	28.9	1.3	69.7
Nitrofurantoin	16	64	8 to 128	82.9	9.2	7.9
SXT	>32	>32	0.06 to >32	43.4	–	56.6
Klebsiella spp.: ESBL-phenotype ($n = 65$)[e]						
Fosfomycin	8	128	1 to >256	–	–	–
Ciprofloxacin	32	>32	0.016 to >32	21.5	4.6	73.8
Levofloxacin	16	>32	0.016 to >32	26.2	1.5	72.3
Nitrofurantoin	128	128	16 to >256	4.6	26.2	69.2
SXT	>32	>32	0.06 to >32	23.1	–	76.9
P. mirabilis: ESBL-phenotype ($n = 3$)						
Fosfomycin	NC	NC	0.5 to 2	–	–	–
Ciprofloxacin	NC	NC	0.03 to >32	33.3	0.0	66.7
Levofloxacin	NC	NC	0.125 to 32	33.3	0.0	66.7
Nitrofurantoin	–	–	–	0.0	33.3	66.7
SXT	NC	NC	1 to >32	33.3	–	66.7
CAZ NS *Enterobacter* spp. ($n = 38$)[f]						
Fosfomycin	16	128	0.5 to >256	–	–	–
Ciprofloxacin	0.25	>32	0.008 to >32	68.4	10.5	21.1

Table 2 continued

	MIC_{50}	MIC_{90}	Range	*S* (%)	*I* (%)	*R* (%)
Levofloxacin	0.25	32	0.03 to >32	68.4	10.5	21.1
Nitrofurantoin	64	128	1 to 128	15.8	60.5	23.7
SXT	0.5/9.5	>32/608	0.06 to >32	65.8	–	34.2
CAZ NS *P. aeruginosa* ($n = 15$)						
Fosfomycin	64	128	2 to >256	–	–	–
Ciprofloxacin	16	32	0.125 to 32	13.3	20.0	66.7
Levofloxacin	16	>32	0.5 to >32	13.3	0.0	86.7
Nitrofurantoin	–	–	–	–	–	–
SXT	–	–	–	–	–	–
VAN NS *E. faecalis* ($n = 5$)						
Fosfomycin	NC	NC	32 to 64	100.0	0.0	0.0
Ciprofloxacin	NC	NC	>32 to >32	0.0	0.0	100.0
Levofloxacin	NC	NC	32 to >32	0.0	0.0	100.0
Nitrofurantoin	NC	NC	8 to 16	100.0	0.0	0.0
SXT	–	–	–	–	–	–

Percent susceptible not calculable due to organisms in the group without CLSI breakpoints indicated by "–"
CAZ NS ceftazidime-nonsusceptible, *ESBL* extended spectrum β-lactamase, *I* intermediate, MIC_{50} minimum concentration that inhibits 50% of isolates, MIC_{90} minimum concentration that inhibits 90% of isolates, *R* resistant, *S* susceptible, *SXT* trimethoprim/sulfamethoxazole, *VAN NS* vancomycin-nonsusceptible

[a] *P. aeruginosa* and *Proteus* spp. ($n = 184$) are intrinsically resistant to nitrofurantoin and were not included in the calculations

[b] *P. aeruginosa* and *E. faecalis* ($n = 182$) are intrinsically resistant to SXT and were not included in the calculations

[c] *E. coli* ($n = 76$), *K. pneumoniae* ($n = 59$), *K. oxytoca* ($n = 6$), and *P. mirabilis* ($n = 3$)

[d] *P. mirabilis* ($n = 141$) are intrinsically resistant to nitrofurantoin and were not included in the calculations

[e] *K. pneumoniae* ($n = 59$) and *K. oxytoca* ($n = 6$)

[f] *E. cloacae* ($n = 23$) and *E. aerogenes* ($n = 15$)

(93.4–99.4%), despite high ESBL-producing phenotype rates of 58.1% among isolates tested; ESBL-producing *E. coli* susceptibility rates for fosfomycin (93.8%) and nitrofurantoin (86.2%) were also similar to those reported here for isolates with ESBL phenotypes [23]. Over the period 2010 through to 2013, susceptibility rates of *E. coli* isolates were similarly high for fosfomycin (95.8%) in multidrug-resistant urinary isolates from a Veterans Affairs population in Boston, MA, USA [24].

The reduced susceptibility of cUTI isolates *Klebsiella* and *Enterobacter* spp. to nitrofurantoin observed in this study was also observed in a study conducted in Canada in 2001 (susceptibility rates of 69.2% and 63.0%, respectively) [25], and in a recent US study (susceptibility rates of 52.3% and 71.9%, respectively) [19]. Similarly, in the Boston area Veterans Affairs population, 64.0% of *Klebsiella* spp. were susceptible to nitrofurantoin, but in contrast to the current study slightly more than half of the multidrug-resistant isolates were susceptible to fosfomycin [24]. Ongoing surveillance is needed to assess the activity of nitrofurantoin against contemporary isolates of these organisms.

Overall, the rate of fosfomycin resistance was low in this study, as has been recently reported in general populations in the US (resistance rates of 1.5% and 3.3%) [19, 22], but not in a predominantly male population from the Boston area (overall resistance rate of 21.6%) [24]. There are several mechanisms by which bacteria become resistant to fosfomycin, including amino acid substitutions that modify the fosfomycin target MurA, mutations that affect fosfomycin transporters, and acquisition of genes that encode enzymes that inactivate fosfomycin [11]. Although fosfomycin resistance is not typically associated with β-lactamase production, one study demonstrated that the *fosA3* gene, which encodes a fosfomycin-inactivating enzyme, can be associated with $bla_{\text{CTX-M}}$ genes in *E. coli* [26]. This may explain the fosfomycin resistance observed in a few of the isolates assessed in this study. Further testing is needed to determine whether these fosfomycin- resistant pathogens encode the *fosA3* gene.

This study shares the limitations inherent to in vitro trials, including the potential limited applicability to the clinical setting. In this case, our study is further limited by relying on a subset of data from the AWARE surveillance program. The data presented here represent a subset of surveillance results of US isolates from the 2012 AWARE surveillance program, and although the data have been stratified by region and by pathogen to reflect the original database, it is possible that the susceptibility patterns of the actual isolates included in our study may have differed from the underlying AWARE data. Furthermore, the isolates that we could assess were limited to those isolates collected through the AWARE surveillance program and duplicate MIC testing was not performed. Nonetheless, the AWARE surveillance program offers a useful dataset from which to assess susceptibility patterns at a point in time. The clinical applicability of our study may be further limited as susceptibility rates may change over time or differ in other regions of the world.

CONCLUSION

The data reported in this study demonstrate that, compared with the other antibiotics tested, fosfomycin has greater in vitro activity against US UTI isolates, including Enterobacteriaceae with ESBL-phenotypes and other drug-resistant isolates. The susceptibility data for US surveillance isolates in this study should serve as a baseline for benchmarking any future changes in the susceptibility of uropathogens to fosfomycin as it becomes more widely used for the treatment of infections caused by resistant pathogens.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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