International Journal of Infectious Diseases 17 (2013) e966–e970

Contents lists available at SciVerse ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Evaluation of the in vitro activity of fosfomycin tromethamine against Gram-negative bacterial strains recovered from community- and hospital-acquired urinary tract infections in Turkey

Tulin Demir [a,*], Tuncay Buyukguclu [b]

[a] Ahi Evran University, Research and Training Hospital, Clinical Microbiology Department, 40100 Kırşehir, Turkey
[b] Ministry of Health, Public Healthcare Center, Karabuk, Turkey

ARTICLE INFO

Article history:
Received 14 March 2013
Received in revised form 17 April 2013
Accepted 18 April 2013

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Fosfomycin tromethamine
Antimicrobial resistance
Urinary tract infections

SUMMARY

Objectives: The aim of this study was to evaluate the in vitro activities of antimicrobial agents including fosfomycin tromethamine against Gram-negative isolates recovered from urine samples.
Methods: A total of 2334 strains (1562 *Escherichia coli*, 509 *Klebsiella spp*, 85 *Proteus spp*, 75 *Pseudomonas spp*, 45 *Enterobacter spp*, 37 *Acinetobacter baumannii*, 8 *Citrobacter spp*, 7 *Morganella morganii*, and 6 *Serratia spp*) were identified by VITEK 2 during the study period, November 2008 to June 2012. Antimicrobial susceptibilities of the strains were also evaluated using the Kirby–Bauer disk diffusion method, in accordance with the Clinical and Laboratory Standards Institute guidelines.
Results: Overall, 2160 (92.5%) of the isolates tested were susceptible to fosfomycin tromethamine. Higher resistance rates were observed among inpatients compared to outpatients. Resistance rates by strain were: 2.0% for *E. coli*, 4.4% for *Enterobacter spp*, 6.9% for *Klebsiella spp*, 9.4% for *Proteus spp*, 48.6% for *A. baumannii*, 56.0% for *Pseudomonas spp*, and 100% for *Morganella morganii*. All *Serratia spp* and *Citrobacter spp* strains were susceptible. Extended-spectrum beta-lactamase (ESBL)-producing isolates displayed higher fosfomycin resistance rates than negative strains (19.2% vs. 2.9%). The highest in vitro activity was detected for amikacin, piperacillin–tazobactam, and imipenem for all strains including ESBL-producers.
Conclusions: Regardless of ESBL production, the excellent activity of fosfomycin against *E. coli*, *Enterobacter spp*, *Serratia spp*, and *Citrobacter spp*, indicates that the drug is a valuable therapeutic option for urinary tract infections, even those with co-trimoxazole- and ciprofloxacin-resistant isolates, but not in ESBL-producing *Klebsiella spp*, *Pseudomonas spp*, *A. baumannii*, and *Proteus spp*. Further studies should be carried out to determine the in vivo drug activity among *Enterobacteriaceae* other than *E. coli*.

1. Introduction

The emergence and spread of multidrug-resistant (MDR) Gram-negative bacteria related to urinary tract infections (UTIs) is increasing worldwide, both in hospitals and in the community. The therapeutic option is a growing concern due to the production of extended-spectrum beta-lactamases (ESBLs) exhibiting resistance not only to cephalosporins but also quinolones and co-trimoxazole.[1–6]

Fosfomycin inhibits bacterial cell wall biogenesis by inactivating the enzyme UDP-*N*- acetylglucosamine-3-enol-pyruvyltransferase (MurA). It exhibits excellent tissue penetration and impairs adherence to the urogenital mucosa, and it is excreted unchanged in high concentrations in the urine.[1,3] With the advantages of administration as a single dose per day, a good safety profile, no effect on the anaerobic gut flora, and availability during pregnancy, this drug is a good option in the treatment of uncomplicated UTIs.[1,3,4,6–8]

Fosfomycin tromethamine (FOF), a stable salt of fosfomycin, has been found to be effective for the treatment of UTIs related to *Escherichia coli*, *Citrobacter spp*, *Enterobacter spp*, *Klebsiella spp*, *Serratia spp*, and *Enterococcus faecalis*.[3–5,7–9] Although it has been commonly prescribed in some countries in Europe and the USA for the treatment of uncomplicated UTIs for several years,[3–5,8] resistance rates have so far remained low.[4,10,11] Moreover, the drug was found to be effective against MDR and metallo-beta-lactamase (MBL)-producing *Enterobacteriaceae* strains, with susceptibility rates over 83%.[12,13]

In the present study, we aimed to determine the in vitro FOF susceptibility of Gram-negative strains recovered from urine samples and to compare its activity with the other antimicrobial agents commonly used for the treatment of UTIs.

* Corresponding author. Tel.: +90 3862134515; fax: +90 3862133398.
E-mail address: drtulin@yahoo.com (T. Demir).

1201-9712/$36.00 – see front matter
http://dx.doi.org/10.1016/j.ijid.2013.04.005

2. Materials and methods

2.1. Study design

Urine samples of 10 248 patients with clinical symptoms of UTI who were referred to the Clinical Microbiology Laboratory of Ahi Evran University Research and Training Hospital, Kırşehir (a 340-bed teaching hospital located in the central region of Turkey) during the study period of November 2008 to June 2012, were evaluated. Significant bacteriuria is defined by counts of $\geq 10^5$ cfu/ml in the patient's mid-stream urine sample. A total of 2334 bacterial strains (1562 *E. coli*, 509 *Klebsiella spp*, 85 *Proteus spp*, 75 *Pseudomonas spp*, 45 *Enterobacter spp*, 37 *Acinetobacter baumannii*, 8 *Citrobacter spp*, 7 *Morganella morganii*, and 6 *Serratia spp*) were identified by VITEK 2 Compact (bioMérieux, Marcy l'Etoile, France).

2.2. Antimicrobial susceptibility

Testing of susceptibility to ampicillin (AMP, 10 μg), amikacin (AMK, 30 μg), amoxicillin–clavulanic acid (AMC, 20/10 μg), aztreonam (ATM, 30 μg), cefepime (FEP, 30 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), ceftriaxone (CRO, 30 μg), cefuroxime (CXM, 30 μg), ciprofloxacin (CIP, 5 μg), co-trimoxazole (SXT, 1.25/23.75 μg), fosfomycin tromethamine (FOF, 200 μg), gentamicin (GEN, 10 μg), imipenem (IPM, 10 μg), and piperacillin–tazobactam (TZP, 100/10 μg) (Oxoid Ltd, Basingstoke, UK) was determined by Kirby–Bauer disk diffusion test method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines,[14] and also with the VITEK 2 Compact system. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.

ESBL screening of the isolates was performed by disk synergy test, and results were confirmed by cefotaxime, ceftazidime, cefotaxime–clavulanic acid (CTC, 30/10 μg), and ceftazidime–clavulanic acid (CZC, 30/10 μg) disks, in accordance with CLSI guidelines.[14] *E. coli* ATCC 25922 (ESBL-negative) and *Klebsiella pneumoniae* ATCC 700603 (ESBL-positive) were used as quality control strains for the phenotypic testing of ESBL production.

The minimum inhibitory concentration (MIC) for imipenem was determined by Etest method (AB Biodisk, Solna, Sweden) following the manufacturer's instructions, for strains resistant or intermediately resistant to imipenem by disk diffusion test. Additionally, the MBL Etest strip (AB Biodisk, Solna, Sweden) was used to determine MBL production for the strains resistant or intermediately resistant to imipenem. Several colonies from a 24-h culture plate were used to prepare the inoculum with a 0.5 McFarland standard density, and Mueller–Hinton agar plates were streaked using cotton swabs. The Etest MBL strips were then applied, and the plates were incubated at 35 °C in air for 16–20 h. A ratio of the MICs of the imipenem (IP) to imipenem plus ethylenediaminetetraacetic acid (EDTA) (IPI) of ≥8, or the presence of a phantom zone, i.e., an extra inhibition zone between the IP and IPI regions, or a deformation of the IP or IPI ellipses, was interpreted as being positive for MBL production.

2.3. Statistical analyses

Data were analyzed using SPSS software 15.0 (SPSS, Inc., Chicago, IL, USA). Comparisons of categorical variables were done using Chi-square tests, although Fisher's exact test was used when data were sparse. Significance was set at $p < 0.05$ using two-sided comparisons.

3. Results

A total of 2334 bacterial strains recovered from 10 248 urine samples of 434 (18.6%) inpatients and 1900 (81.4%) outpatients were included in the study. The most commonly isolated pathogens were *E. coli* (66.9%) and *Klebsiella spp* (21.8%). Identification of the strains to the species level is shown in Table 1. Of the 2334 bacterial strains, ESBL production was detected in 651 (27.9%) isolates; the distribution for *E. coli*, *Klebsiella spp*, *Enterobacter spp*, *Proteus spp*, *M. morganii*, and *Citrobacter spp* were 408 (26.1%), 124 (24.4%), 16 (35.6%), 3 (3.5%), 2 (28.6%), and 2 (25%), respectively. Among nonfermenting strains, 80% ($n = 60$) of *Pseudomonas spp* and 97.3% ($n = 36$) of *A. baumannii* strains were ESBL-producers.

Table 1
Bacterial species distribution in this study

Bacterial strain	Number of strains	%
Escherichia coli	1562	66.9%
Klebsiella spp	509	21.8%
K. pneumoniae	260	
K. oxytoca	249	
Proteus spp	85	3.6%
P. mirabilis	72	
P. vulgaris	13	
Pseudomonas spp	75	3.2%
P. aeruginosa	71	
P. luteola	2	
P. putida	1	
P. fluorescens	1	
Enterobacter spp	45	1.9%
E. cloacae	32	
E. aerogenes	9	
E. sakazakii	4	
Acinetobacter baumannii	37	1.6%
Citrobacter spp	8	0.3%
C. freundii	5	
C. koseri	3	
Morganella morganii	7	0.3%
Serratia spp	6	0.3%
S. fonticola	4	
S. marcescens	1	
S. liquefaciens	1	
Total	2334	100

Antimicrobial resistance rates of the isolates belonging to the *Enterobacteriaceae* family ($n = 2222$) tested in this study were as follows: 71.6% to ampicillin, 38.7% to co-trimoxazole, 28.2% to cefuroxime, 25.4% to ciprofloxacin, 18.7% to gentamicin, 11.8% to amoxicillin–clavulanic acid, 5.5% to piperacillin–tazobactam, 3.7% to FOF, 2.3% to amikacin, and 0.04% to imipenem. Twenty-five percent of the strains were resistant to any of the third-generation cephalosporin group. Antimicrobial resistance rates in relation to species are shown in Table 2. Imipenem was the most active agent against all strains except *Acinetobacter spp*. Overall, 33 of the isolates tested (1 *E. coli*, 1 *K. pneumoniae*, 4 *P. aeruginosa*, 26 *A. baumannii*, and 1 *Pseudomonas luteola*) showed resistance or intermediate resistance to imipenem, and all strains but one *A. baumannii* were found to be MBL-producers by MBL Etest, with MIC ratios of IP/IPI ranging from 1/16 to 1/256.

Of the 2334 strains, 2160 (92.5%) were susceptible to fosfomycin, 143 (6.1%) showed resistance, and 31 (1.3%) displayed intermediate resistance. *E. coli* strains displayed higher antimicrobial activity for fosfomycin compared to other strains ($p < 0.05$). The resistance rate was higher among inpatient strains than among outpatient strains: 17.5% vs. 5.2% (odds ratio (OR) 3.90, 95% confidence interval (CI) 2.83–5.38; $p = 0.001$). In addition, higher resistance rates were detected among inpatient compared to community strains: 7.9% vs. 4.3%, respectively, among *Enterobacteriaceae*.

In this study, the most common pathogens causing UTI were *E. coli* (66.9%) and *Klebsiella spp* (21.8%). *Klebsiella spp* strains displayed higher rates of fosfomycin resistance compared to *E. coli* strains: 10.8% vs. 2.2% (OR 5.44, 95% CI 3.51–8.46; $p = 0.001$).

Table 2
Distribution of resistance rates of all isolates by strain type (n = 2334)

Antimicrobial	*Escherichia coli*	*Klebsiella spp*	*Enterobacter spp*	*Proteus spp*	*Morganella spp*	*Serratia spp*	*Citrobacter spp*	*Pseudomonas spp*	*Acinetobacter baumannii*
Ampicillin	69.5	79.6	84.4	55.3	85.7	50.0	87.5	98.7	100.0
Amoxicillin–clavulanic acid	10.3	13.9	42.2	4.7	71.4	16.7	12.5	90.7	70.3
Amikacin	2.1	2.9	4.4	-	-	-	12.5	12.0	73.0
Cefuroxime	28.9	28.3	44.4	7.1	57.1	-	25.0	92.0	97.3
Third-generation cephalosporin	26.1	24.4	35.6	3.5	28.6	-	25.0	80.0	97.3
Ciprofloxacin	29.5	18.7	6.7	3.5	28.6	-	-	24.0	81.1
Fosfomycin	2.0	6.9	4.4	9.4	100	-	-	56.0	48.6
Gentamicin	19.6	17.7	8.9	11.8	57.1	-	12.5	20.0	75.7
Imipenem[a]	0.1	0.2	-	-	-	-	-	6.7	70.2
Co-trimoxazole	41.7	30.8	13.3	47.1	57.1	-	26.0	92.0	64.9
Piperacillin–tazobactam	4.7	9.4	2.2	1.2	-	-	-	12.0	81.1

[a] Intermediately resistant test results were evaluated as resistant.

For all antimicrobials tested, ESBL-producer *Enterobacteriaceae* strains showed lower susceptibility rates compared to non-producers ($p < 0.05$). Resistance rates for ciprofloxacin, co-trimoxazole, and gentamicin were over 45% among ESBL-producers (Table 3). Of the ESBL-positive *Enterobacteriaceae* strains (n = 555), 49 (8.8%) were resistant and 13 (2.3%) showed intermediate resistance to fosfomycin, displaying an overall susceptibility rate of 88.8%. Moreover, higher resistance to fosfomycin was observed for ESBL-positive strains compared to non-producer isolates (11.2% vs. 2.8%; p = 0.001). Fosfomycin resistance was not detected among *Serratia spp* or *Citrobacter spp*, but all *Morganella spp* strains were resistant to this drug. Regardless of ESBL production, the highest in vitro activity was detected against *E. coli* strains. Among *Klebsiella spp* strains, ESBL-producer strains showed higher resistance rates compared to non-producer isolates: 17.7% vs. 3.4%. The distribution of fosfomycin susceptibility rates of the isolates by strain type and ESBL production is shown in Table 4.

Among strains resistant to antimicrobials tested in this study, fosfomycin showed higher in vitro activity (over 80%) against *Enterobacteriaceae* strains compared to nonfermenting strains (*A. baumannii* and *Pseudomonas spp*) (below 45%).

Overall, the resistance rates of the isolates tested to co-trimoxazole and ciprofloxacin were 89.2% and 89.7%, respectively. Fosfomycin was found to be effective against strains resistant to co-trimoxazole and ciprofloxacin, displaying susceptibility rates of 94.6% and 93.3% for *Enterobacteriaceae* and 39.4% and 40.8% for nonfermenting Gram-negative bacilli.

Among *E. coli* strains, the most active agents regardless of ESBL production were imipenem (99.9%) and fosfomycin (97.8%), followed by amikacin (96.9%) and piperacillin–tazobactam (94.6%). Comparison of the in vitro efficacy of the antimicrobials by strain type and ESBL production is shown in Figure 1.

Table 3
Distribution of antimicrobial resistance rates of *Enterobacteriaceae* (n = 2222) by extended-spectrum beta-lactamase (ESBL) production

Antimicrobial agents	ESBL (%)	
	Negative (n = 1667)	Positive (n = 555)
Ampicillin	1052 (63.1)	555 (100)
Amoxicillin–clavulanic acid	113 (6.8)	187 (33.7)
Gentamicin	165 (9.9)	267 (48.1)
Amikacin	7 (0.4)	63 (11.4)
Cefuroxime	82 (4.9)	555 (100)
Piperacillin–tazobactam	26 (1.6)	114 (20.5)
Ciprofloxacin	247 (14.8)	331 (59.6)
Co-trimoxazole	547 (32.8)	319 (57.5)
Fosfomycin	46 (2.8)	62 (11.2)
Imipenem	-	2 (0.4)[a]

Intermediately resistant isolates were evaluated as resistant in the statistical analysis.
[a] Fisher's exact test.

4. Discussion

Fosfomycin is a cell wall active antimicrobial agent found to be effective against *E. coli*, *Citrobacter spp*, *Enterobacter spp*, *Klebsiella spp*, *Serratia spp*, and *E. faecalis* related UTIs.[7,13,15,16] Although it has been used for several years, resistance has remained low, at 0.3–2.8% in *E. coli*[4,11,17–19] and 7.2–28.6% in *Klebsiella spp*.[17,19] The CLSI recommends fosfomycin therapy only for the treatment of uncomplicated UTIs related to *E. coli*.[14] The explanation for this limitation is the reported discrepancies between disk diffusion and agar dilution tests observed for *Klebsiella spp* strains[19,20] in contrast to the good correlation in *E. coli* isolates.[20,21] Further studies are required to assess the activity against *Klebsiella spp* strains.

The present study compared the in vitro efficacy of FOF with that of other antimicrobials, against 2334 Gram-negative bacterial isolates representing nine species. The most common pathogens recovered from urine were *E. coli* and *Klebsiella spp*. Overall, 6.1% of the isolates tested were resistant and 1.3% showed intermediate resistance to fosfomycin. Higher rates of resistance were detected among *Klebsiella spp* compared to *E. coli* strains (10.8% vs. 2.2%; $p < 0.05$), supporting the data published previously.[17,18,22–24]

ESBL production among *Enterobacteriaceae* is a growing concern worldwide. In this study, nearly a quarter of the strains were

Table 4
Distribution of fosfomycin susceptibilities of the isolates by strain type and extended-spectrum beta-lactamase (ESBL) production

Bacterial isolate	Fosfomycin susceptibility rate (%)		
	Total	ESBL	
		Negative	Positive
Escherichia coli (n = 1562)	97.8	99.1	94.1
Klebsiella spp (n = 509)	89.2	94.2	73.3
K. pneumoniae	84.6	91.5	66.1
K. oxytoca	94	96.9	83.0
Enterobacter spp (n = 45)	93.3	93.1	93.7
E. cloacae	90.6	88.2	93.3
E. sakazakii	100	100	100
E. aerogenes	100	100	-
Proteus spp (n = 85)	89.4	91.4	50
P. mirabilis	94.4	95.7	50
P. vulgaris	61.5	66.6	-
Morganella morganii (n = 7)	-	-	-
Serratia spp (n = 6)	100	100	-
Citrobacter spp (n = 8)	100	100	100
Acinetobacter baumannii (n = 37)	35.1	100	33.3
Pseudomonas spp (n = 75)	44	80	35

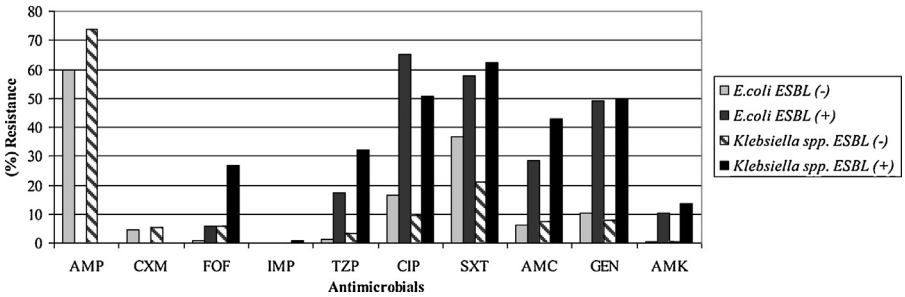

Figure 1. Antimicrobial resistance rates of the strains by extended-spectrum beta-lactamase production.

ESBL-producers. Regarding ESBL production, a marked difference in the FOF resistance rate was not detected among *E. coli* strains (0.9% vs. 5.9%), but a major distinction was observed for *Klebsiella spp* (5.7% vs. 26.6%, the latter being the ESBL-producer). In previous studies on ESBL-producers, FOF resistance rates of 0–9.1% for *E. coli*[1–3,5,19,21,23,24] and 18.7–42.4% for *Klebsiella spp*[21,23] were reported. The low level of resistance among *E. coli* strains could be explained with the drug's limited use for the treatment of uncomplicated UTIs,[4,10,11,17–19,22] suggesting that fosfomycin is the drug of choice for the treatment of UTIs, especially those caused by *E. coli*.

Co-trimoxazole is the recommended drug for the treatment of UTIs in settings where the resistance is <10–20%,[18] and quinolones are the drugs of choice if the co-trimoxazole resistance is higher than 20%.[25] Several studies have shown ciprofloxacin and co-trimoxazole to be highly active against *E. coli*, with susceptibility rates over 81–99%[17,18,20,21,26,27] and 64–82%,[17,18,21,26,27] respectively, in contrast to dramatically decreasing susceptibility rates among ESBL-producers for ciprofloxacin and co-trimoxazole, 19–36% and 33–43%, respectively.[2,5] In this study, lower susceptibility rates for ciprofloxacin and co-trimoxazole were obtained: 70.5% and 38.3% in *E. coli*, and 81.3% and 69.2% in *Klebsiella spp*, respectively. The high levels of resistance to co-trimoxazole and ciprofloxacin reported in this study and previously[18] may indicate the misuse of these drugs for both inpatients and outpatients in our country, and it is clear that ciprofloxacin and co-trimoxazole therapy should be evaluated with caution in the treatment of UTI. In agreement with some reports,[22] fosfomycin appears to be an important treatment option for UTIs associated with *E. coli* and *Klebsiella spp*, even quinolone- and co-trimoxazole-resistant strains, with susceptibility rates of 93.3% and 94.6%, respectively.

Several studies have shown that community-acquired ESBL-producing *E. coli* urinary isolates have high resistance rates to most of the currently used oral antimicrobial agents, with resistance rates of 84% for ciprofloxacin, 75% for co-trimoxazole, 15% for nitrofurantoin, and 0% for fosfomycin,[28] suggesting the use of fosfomycin and nitrofurantoin for the first-line empirical oral treatment of community-acquired uncomplicated UTIs. A single dose of FOF was found to be as effective as ciprofloxacin in the treatment of uncomplicated UTIs.[29] In a multicenter study, FOF, ciprofloxacin, and co-trimoxazole susceptibility rates were 99%, 98.3%, and 87.8%, respectively, among *E. coli* strains recovered from female patients with symptoms of uncomplicated cystitis; it was stated that co-trimoxazole and quinolones are not recommended as first-line drugs for the empiric treatment of uncomplicated cystitis because of the increasing resistance rates.[30]

Four drugs, FOF, amikacin, piperacillin–tazobactam, and imipenem, were found to have maintained high activity against ESBL-producers in this study. For FOF the explanation lies in the decreased fitness of *E. coli* after acquiring a mutation that confers resistance to this drug,[31] which allows the strains without the mutation to grow faster and displace the resistant ones. The explanation for amikacin, piperacillin–tazobactam, and imipenem is probably the fact that these drugs are used only in a hospital setting and generally not as the first-line therapy option. In contrast to many reports indicating low resistance rates to gentamicin (4–8%[18,24] for *E. coli* and 13–16%[2,5] for ESBL-positive *E. coli* strains), higher rates were detected for *E. coli* and ESBL-positive *E. coli* strains in this study (19.6% and 49%, respectively). It is clear that gentamicin should be used with caution in UTIs related to ESBL-producer *E. coli* strains.

Susceptibilities to fosfomycin of *Enterobacteriaceae* other than *E. coli* and *Klebsiella spp* were not extensively studied. In the limited number of studies available, susceptibility rates of *Proteus mirabilis*, *Proteus vulgaris*, *M. morganii*, and *Enterobacter spp* were 73.8–87.5%, 50%, 0%, and 82.9%, respectively.[10,17] Additionally, more than 90% of the *E. coli* and *Citrobacter spp*, more than 70% of *Klebsiella spp*, *Enterobacter spp*, and *P. mirabilis* strains, 31.8% of *P. aeruginosa*, and 11.1% of *Acinetobacter spp* strains were reported to be susceptible to fosfomycin.[32] Similar to previous reports,[17] all *Morganella spp* were resistant to FOF, but resistance was not detected among *Serratia spp* and *Citrobacter spp*. However, the results should be evaluated with caution because of the limited numbers of strains. Resistance rates were higher for *Pseudomonas spp* and *A. baumannii*, at 56% and 48.6%, respectively. High activity was detected for *Enterobacter spp*, with a susceptibility rate of 4.4%, similar to the rate in a previous report.[17] In contrast to reported FOF resistance rates of up to 40% for *Proteus spp*,[11,17] a lower rate, 9.4%, was detected, indicating that the drug could be an alternative therapeutic option for UTIs related with these strains. Good in vitro activity against *E. coli* and *Klebsiella spp* was detected in several studies. However it is clear that further studies should be performed to determine and evaluate the drug efficacy in vivo for strains other than *E. coli* and *Klebsiella spp*.

In this study we could not classify complicated or uncomplicated UTIs due to the lack of information in the database concerning patients' previous treatment with antibiotics, previous hospitalization, and risk factors for UTIs. This is a clear limitation of this study. However, to our knowledge, this is the first study conducted on a large scale to evaluate antimicrobial susceptibilities of Gram-negative bacterial strains other than *E. coli* and *Klebsiella spp* recovered from UTIs.

Several analyses of fosfomycin activity against *E. coli* strains over the last decade have shown excellent susceptibility rates of over 93% regardless of ESBL production,[1–3,5,12,19,21,23,24,27,33] although an increase in resistance has been reported from Spain and Japan.[5,33] It is clear that in the following years FOF use will gain importance due to the strains producing ESBL and increasing resistance to co-trimoxazole and quinolones.

In conclusion, it is clear that FOF could be an alternative treatment option for UTIs related to *E. coli* and *Klebsiella spp*, but not for ESBL-producing *Klebsiella spp*. Although in vitro data seem to encourage the prescription of FOF, further clinical studies evaluating the clinical efficacy and safety profile of this drug should be conducted.

Conflict of interest: No conflict of interest to declare.

References

1. Auer S, Wojna A, Hell M. Oral treatment options for ambulatory patients with urinary tract infections caused by extended-spectrum beta-lactamase producing *Escherichia coli*. *Antimicrob Agents Chemother* 2010;**54**:4006–8.
2. Bano J, Alcala JC, Cisneros JM, Gril F, Oliver A, Horcajada JP, et al. Community infections caused by extended-spectrum beta lactamase producing *Escherichia coli*. *Arch Intern Med* 2008;**168**:1897–902.
3. Chislett RJ, White G, Hills T, Turner DP. Fosfomycin susceptibility among extended-spectrum beta-lactamase producing *Escherichia coli* in Nottingham, UK. *J Antimicrob Chemother* 2010;**65**:1076–7.
4. Knottnerus J, Nys S, Riet G, Donker G, Geerlings SE, Stobberingh E. Fosfomycin tromethamine as second agent for the treatment of acute, uncomplicated urinary tract infections in adult female patients in The Netherlands? *J Antimicrob Chemother* 2008;**62**:356–9.
5. Oteo J, Bautista V, Lara N, Cuevas O, Arroyo M, Fernandez S, et al. Parallel increase in community use of fosfomycin and resistance to fosfomycin in extended-spectrum beta-lactamase producing *Escherichia coli*. *J Antimicrob Chemother* 2010;**65**:2459–63.
6. Schito GC. Why fosfomycin trometamol as first line therapy for uncomplicated UTI? *Int J Antimicrob Agents* 2003;**22**:79–83.
7. Falagas ME, Vouloumanou EK, Togias AG, Karadima M, Kapaskelis AM, Rafailidis PI, et al. Fosfomycin versus other antibiotics for the treatment of cystitis: a meta-analysis of randomized controlled trials. *J Antimicrob Chemother* 2010;**65**:1862–77.
8. Patel SS, Balfour JA, Bryson HM. Fosfomycin tromethamine. A review of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy as a single-dose oral treatment for acute uncomplicated lower urinary tract infections. *Drugs* 1997;**53**:637–56.
9. Lu CL, Liu C, Huang Y, Liao CH, Teng LJ, Turnidge JD, et al. Antimicrobial susceptibilities of commonly encountered bacterial isolates to fosfomycin determined by agar dilution and disk diffusion methods. *Antimicrob Agents Chemother* 2011;**55**:4295–301.
10. Kahlmeter G. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECOSENS Project. *J Antimicrob Chemother* 2003;**51**:69–76.
11. Marchese A, Gualco L, Debbia EA, Schito GC, Schito AM. In vitro activity of fosfomycin against Gram-negative urinary pathogens and the biological cost of fosfomycin resistance. *Int J Antimicrob Agents* 2003;**2**:53–9.
12. Falagas ME, Maraki S, Karageorgepoulos DE, Kastoris AC, Mavromanalakis E, Samonis G. Antimicrobial susceptibility of multidrug resistant (MDR) and extensively drug resistant (XDR) *Enterobacteriaceae* isolates to fosfomycin. *Int J Antimicrob Agents* 2010;**35**:240–3.
13. Pullukcu H, Tasbakan M, Sipahi OR, Yamazhan T, Aydemir S, Ulusoy S. Fosfomycin in the treatment of extended-spectrum beta-lactamase producing *Escherichia coli* related lower urinary tract infections. *Int J Antimicrob Agents* 2007;**29**:62–5.
14. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-first informational supplement M100-S21. Wayne, PA: CLSI; 2011.
15. Bonfiglio G, Mattina R, Lanzafame A, Cammarata E, Tempera G. Fosfomycin tromethamine in uncomplicated urinary tract infections: a clinical study. *Chemotherapy* 2005;**51**:162–6.
16. Matsumoto T, Muratani T, Nakahama C, Tomono K. Clinical effects of 2 days of treatment by fosfomycin calcium for acute uncomplicated cystitis in women. *J Infect Chemother* 2011;**17**:80–6.
17. Alhambra A, Cuadros JA, Cacho J, Gomez-Garces JL, Alos JI. In vitro susceptibility of recent antibiotic resistant urinary pathogens to ertapenem and 12 other antibiotics. *J Antimicrob Chemother* 2004;**53**:1090–4.
18. Arslan H, Azap SK, Ergönül Ö, Timurkaynak F. Risk factors for ciprofloxacin resistance among *E. coli* strains isolated from community-acquired urinary tract infections in Turkey. *J Antimicrob Chemother* 2005;**56**:914–8.
19. de Cueto M, Hernandez JR, Lopez-Cerero L, Morillo C, Pascual A. Activity of fosfomycin against extended-spectrum beta-lactamase producing *E. coli* and *K. pneumoniae*. *Enferm Infecc Microbiol Clin* 2006;**24**:613–6.
20. Farrell DJ, Morrissey I, De Rubeis D, Robbins M, Felmingham M. A UK multicenter study of the antimicrobial susceptibility of bacterial pathogens causing urinary tract infection. *J Infect* 2003;**46**:94–100.
21. Liu HY, Lin HC, Lin YC, Yu SH, Wu WH, Lee YJ. Antimicrobial susceptibilities of urinary extended-spectrum beta-lactamase producing *E. coli* and *K. pneumoniae* to fosfomycin and nitrofurantoin in a teaching hospital in Taiwan. *J Microbiol Immunol Infect* 2011;**44**:364–8.
22. Ko KS, Suh JY, Peck KR, Lee MY, Oh WS, Kwon KT, et al. In vitro activity of fosfomycin against ciprofloxacin-resistant or extended-spectrum beta lactamase producing *Escherichia coli* isolated from urine and blood. *Diagn Microbiol Infect Dis* 2007;**58**:111–5.
23. Falagas ME, Kastoris AC, Kapaskelis AM, Karageorgepoulos DE. Fosfomycin for the treatment of multidrug resistant, including extended-spectrum beta-lactamase producing, *Enterobacteriaceae* infections: a systematic review. *Lancet Infect Dis* 2010;**10**:43–50.
24. Hernandez MS, Garcia JA, Munoz JL. In vitro activity of fosfomycin against ESBL-producing *Enterobacteriaceae* of urinary origin. *Rev Esp Quimioter* 2009;**22**:25–9.
25. Biondo CM, Rocha JL, Tuon FF. Fosfomycin in vitro resistance of *E. coli* from the community. *Braz J Infect Dis* 2011;**15**:96.
26. Fuchs PC, Barry AL, Brown SD. Fosfomycin tromethamine susceptibility of outpatient urine isolates of *E. coli* and *E. faecalis* from ten North American medical centers by three methods. *J Antimicrob Chemother* 1999;**43**:137–40.
27. Wachino J, Yamane K, Suzuki S, Kimura K, Arakawa Y. Prevalence of fosfomycin resistance among CTX-M producing *Escherichia coli* clinical isolates in Japan and identification of novel plasmid mediated fosfomycin modifying enzymes. *Antimicrob Agents Chemother* 2010;**54**:3061–4.
28. Meier S, Weber R, Zbinden R, Ruef C, Hasse B. Extended-spectrum beta-lactamase producing Gram-negative pathogens in community-acquired urinary tract infections: an increasing challenge for antimicrobial therapy. *Infection* 2011;**39**:333–40.
29. Ceran N, Mert D, Kocdogan FY, Erdem I, Adalati R, Ozyurek S, et al. A randomized comparative study of single-dose fosfomycin and 5-day ciprofloxacin in female patients with uncomplicated lower urinary tract infections. *J Infect Chemother* 2010;**16**:424–30.
30. Neuzillet Y, Naber KG, Schito G, Gualco L, Botto H, French results of the ARESC study. Clinical aspects and epidemiology of antimicrobial resistance in female patients with cystitis. Implications for empiric therapy. *Med Mal Infect* 2012;**42**:66–75.
31. Alós JI, García-Peña P, Tamayo J. Biological cost associated with fosfomycin resistance in *Escherichia coli* isolates from urinary tract infections. *Rev Esp Quimioter* 2007;**20**:211–5.
32. Garcia-Rodriguez JA, Trujillano Martin I, Baquero F, Cisterna R, Gobernado M, Linares F, et al. In vitro activity of fosfomycin trometamol against pathogens from urinary tract infections: a Spanish multicenter study. *J Chemother* 1997;**9**:394–402.
33. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. *Clin Infect Dis* 1999;**29**:745–58.