International Journal of Antimicrobial Agents 54 (2019) 435–441

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

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

Urinary antibacterial activity of fosfomycin and nitrofurantoin at registered dosages in healthy volunteers

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ARTICLE INFO

Article history:
Received 14 May 2019
Accepted 21 July 2019

Editor: Matthew Falagas

KEYWORDS:
Fosfomycin
Nitrofurantoin
Urinary tract infection
Pharmacokinetics
Pharmacodynamics
Healthy volunteers

ABSTRACT

Given emerging uropathogen resistance to more recent antibiotics, old antibiotics used for uncomplicated urinary tract infection (UTI) warrant re-examination. In this study, the urinary antibacterial activities of fosfomycin and nitrofurantoin were investigated by determining the urinary inhibitory titre and urinary bactericidal titre against uropathogens in urine samples from female volunteers following administration of single-dose fosfomycin (3 g) or nitrofurantoin (50 mg q6h or 100 mg q8h). Urine samples were collected over 48 h (fosfomycin) or 6 or 8 h (nitrofurantoin), with drug levels quantified with every void. Fosfomycin concentrations ranged from <0.75 mg/L [lower limit of quantification (LLOQ)] to 5729.9 mg/L and nitrofurantoin concentrations ranged from <4 mg/L (LLOQ) to 176.3 mg/L (50 mg q6h) or 209.4 mg/L (100 mg q8h). There was discrepancy in the response to fosfomycin between *Escherichia coli* and *Klebsiella pneumoniae*, with fosfomycin displaying strong bactericidal activity for 48 h against *E. coli* but moderate bactericidal activity for 18 h against *K. pneumoniae*. This effect was not related to the strain's baseline minimum inhibitory concentration but rather to the presence of a resistant subpopulation. Maximum titres of nitrofurantoin were obtained during the first 2 h, but no antibacterial effect was found in most samples regardless of the dose. In the rare samples in which antibacterial activity was detectable, titres were comparable for both species tested. These findings confirm doubts regarding fosfomycin administration in UTIs caused by *K. pneumoniae* and reveal a discrepancy between nitrofurantoin's measurable ex vivo activity and its clinical effect over multiple dosing intervals.

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1. Introduction

Fosfomycin and nitrofurantoin are recommended first-line antimicrobial agents for urinary tract infections (UTIs), the most common bacterial infection among otherwise healthy women [1]. Although antimicrobial resistance among uropathogens is increasing, it remains relatively low to fosfomycin and nitrofurantoin [2–4]. Despite their use over several decades, the pharmacokinetic (PK) and pharmacodynamic (PD) properties of these antibiotics remain poorly defined, although such information is essential for therapy optimisation and for the prevention of resistance emergence [5,6]. Whilst new data are beginning to emerge on the PK [7–11] and PD properties [12,13] of both drugs, most in vitro PD studies have been conducted in a non-biological matrix and/or did not take into account drug concentration changes over time ex vivo, thus limiting the clinical translation of these results.

A method to address these limitations is the determination of the urinary antibacterial activity of antimicrobial agents in which ex vivo PK data are used within a static in vitro model [14–16]. The urinary antibacterial activity of an antimicrobial agent is described by the urinary inhibitory titre (UIT) and urinary bactericidal titre (UBT). These are measures of antibacterial activity over time in urine, the relevant biological matrix, thus providing in vitro data that more closely reflects the clinical scenario by describing antibiotic activity against the pathogen within the host's environment.

In this study, the urinary antibacterial activities of fosfomycin and nitrofurantoin were determined against common uropathogens

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https://doi.org/10.1016/j.ijantimicag.2019.07.018
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following administration of registered doses for the treatment of UTI in order to evaluate the effectiveness of these drugs.

2. Materials and methods

2.1. Study design, subjects, drug administration and sample collection

Urine samples to determine the UIT and UBT were obtained in two previous studies evaluating the PK properties of both fosfomycin and nitrofurantoin [7,8]. Briefly, the fosfomycin urinary PK study was a single-centre study examining the urinary pharmacokinetics following a single oral 3 g dose of fosfomycin trometamol (Monuril®; Zambon Nederland B.V., Amersfoort, the Netherlands) in 40 healthy female volunteers [7]. Fosfomycin was administered under supervision of one of the researchers. Urine samples were collected in a home setting over 48 h with every void and then two times daily from 48 h until 7 days after administration. For the present study, only samples collected in the first 48 h were used. There were no dietary restrictions prior to or after drug administration. Samples were kept in home freezers until handed to investigators. The nitrofurantoin PK study was a single-centre study in which macrocrystalline nitrofurantoin was administered at either 50 mg every 6 h (q6h) (Furadantine® MC; Mercury Pharma Ltd., Croydon UK) or 100 mg every 8 h (q8h) (Furadantine® retard; Mercury Pharma Ltd.) in a crossover design to 12 healthy female volunteers [8]. The drug was administered with food and administration began in a home setting 24 h prior to sample collection in order to achieve steady-state. The last dose was administered in the hospital at the start of an 8-h visit during which urine samples were collected for 6 h or 8 h depending on the assigned dosing interval. Volunteers were instructed to protect the nitrofurantoin samples from daylight using aluminium foil to avoid photodegradation of the drug.

Total volume, pH and time of each sample were recorded both for fosfomycin and nitrofurantoin samples prior to storage at −80 °C. The stability of the samples under these conditions was confirmed during validation of the analytical methods [17,18]. Drug levels were quantified using ultrahigh performance liquid chromatography (UHPLC) with tandem mass spectrometry (MS/MS) detection for fosfomycin or using ultraviolet (UV) detection for nitrofurantoin. Both methods were validated according to US Food and Drug Administration (FDA) guidelines as described elsewhere [17,18].

2.2. Test organisms and minimum inhibitory concentrations (MICs)

Isolates were obtained from clinical sources (except the *Escherichia coli* ATCC reference strain) and were selected with a range of fosfomycin and nitrofurantoin MICs (Table 1). Fosfomycin susceptibility was determined by agar dilution using 10^4 CFU/spot of each isolate inoculated on Mueller–Hinton II agar (BD Diagnostics, Franklin Lakes, NJ, USA) containing 25 mg/L glucose-6-phosphate (G6P) (Sigma, Taufkirchen, Germany) and fosfomycin (InfectoPharm, Heppenheim, Germany) following Clinical and Laboratory Standards Institute (CLSI) recommendations at a concentration range of 0.25–1024 mg/L. Isolates were tested in triplicate. Nitrofurantoin susceptibility was determined by broth microdilution according to ISO guidelines [19].

Fosfomycin-containing urine samples from volunteers were divided into two sets to allow for the limited volume of material. Set 1 consisted of samples from the initial 20 volunteers and the second set consisted of those from the remaining 20 volunteers. Both sets were tested against two *E. coli* strains, two *Klebsiella pneumoniae* strains and the ATCC strain (Table 1). All strains were used for testing the nitrofurantoin samples.

Table 1
Minimum inhibitory concentrations (MICs) of fosfomycin and nitrofurantoin

Test strain	Source	MIC (mg/L)[a]	
		Fosfomycin	Nitrofurantoin
Escherichia coli			
ATCC 25922	Laboratory strain	1	16
51[b]	Blood	2	32
03[b]	Urine	0.25	16
1231	Urine	16	512
4807	Rectal swab	32	16
Klebsiella pneumoniae			
58[b]	Urine	8	64
20[b]	Rectal swab	32	256
31865	Blood	2	128
55	Sputum	4	256

[a] The MIC represents the modal value based on the results of agar dilution (fosfomycin) or microdilution (nitrofurantoin) performed in triplicate.
[b] Strains used for set 1 of the urine samples from volunteers 1–40 in the fosfomycin study.

2.3. Determination of urinary inhibitory titres and urinary bactericidal titres

All urine samples were filtered before analysis by centrifugation (10 min at 13 000 rpm) using an Amicon® Ultra-0.5 Centrifugal Filter Unit with a 10 kDa cut-off Ultracel-10 membrane (UFC5010BK; Merck, Amsterdam, the Netherlands). The large volumes of antibiotic-free urine were filtered over 0.2 µm bottle-top vacuum filters (CLS430756; Corning, Taufkirchen, Germany). UITs and UBTs were determined by microdilution. Urine samples underwent serial two-fold dilution in antibiotic-free urine from healthy volunteers such that the first well of the microtitre plate contained a 2-times diluted sample. The final bacterial inoculum within the microtitre tray was approximately 2.5×10^5 CFU/mL. Inoculated plates were incubated for 18 ± 2 h at 35 ± 2 °C, after which every well was checked visually for growth. The UIT represents the bacteriostatic activity and was defined as the highest dilution that inhibited visible growth. The UBT represents the bactericidal activity and was defined as the absence of bacterial growth following subculture from the microtitre tray onto an antibiotic-free tryptic soy agar (TSA) plate supplemented with 5% sheep blood (254087; Becton Dickinson, Franklin Lakes, NJ, USA). The limit of detection was 50 CFU/mL. TSA plates were incubated for 18 ± 2 h at 35 ± 2 °C. The UBT was defined as the highest dilution of the sample that still exhibited bactericidal activity. Comparable UITs and UBTs reflect antibiotic bactericidal activity, whilst a UIT exceeding the UBT reflects bacteriostatic activity. UITs and UBTs are presented as reciprocal values of the titres and could therefore range from <2 (no antibacterial activity observed) to 1024, with higher titres indicating greater antibacterial activity.

2.4. Determination of fosfomycin-resistant subpopulations

To determine the presence of fosfomycin low-level resistant or high-level resistant (HLR) subpopulations, isolates were cultured overnight both in Mueller–Hinton broth and in antibiotic-free urine using a starting inoculum of 2.5×10^5 CFU/mL. Quantitative cultures were then performed in parallel on antibiotic-free Mueller–Hinton agar (MHA) and MHA supplemented with 25 mg/L G6P together with 64 mg/L or 512 mg/L fosfomycin. Total bacterial density as well as the comparative density of any growth on the fosfomycin-containing media was determined by plating 20 µL from a serial 10-fold dilution of the incubated liquid medium. Growth capacity and resistant subpopulation proportions were compared between Mueller–Hinton broth and urine.

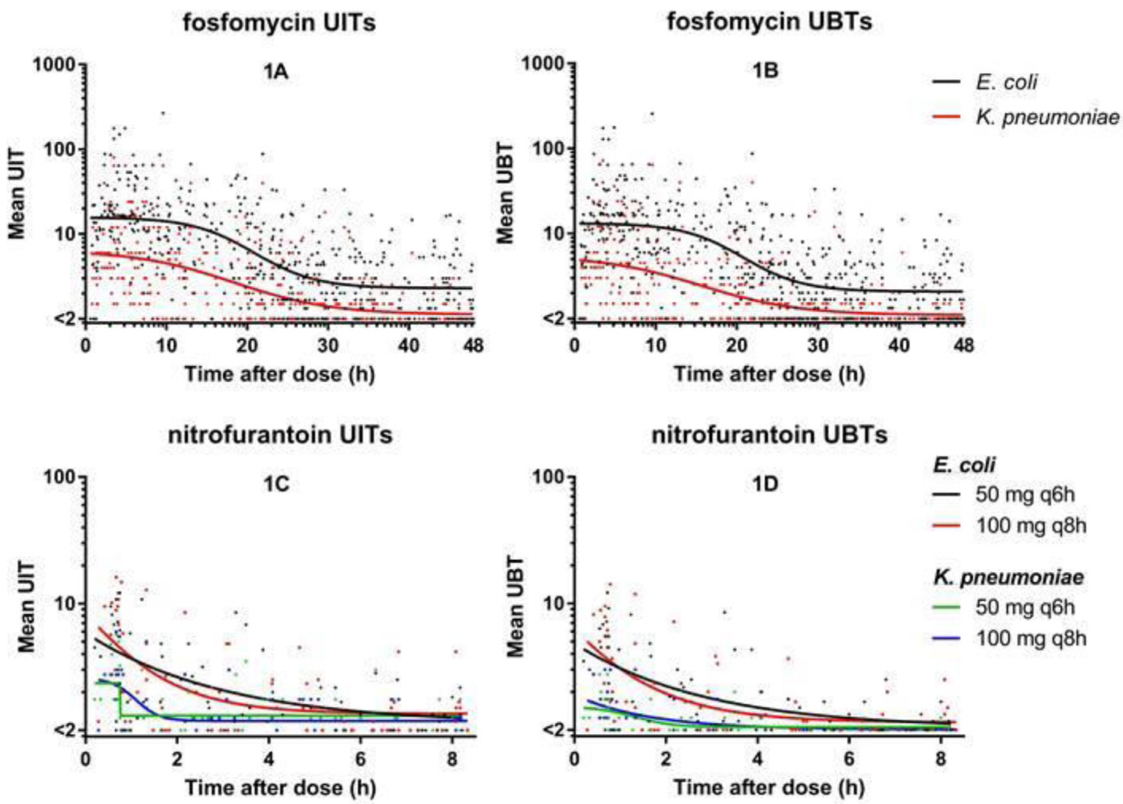

Fig. 1. (A) Urinary inhibitory titres (UITs) and (B) urinary bactericidal titres (UBTs) of fosfomycin for *Escherichia coli* and *Klebsiella pneumoniae* for all samples. (C) UITs and (D) UBTs of nitrofurantoin for both dosing regimens for *E. coli* and *K. pneumoniae*. Each dot represents the mean UIT or UBT for each sample for the *E. coli* and *K. pneumoniae* strains, respectively.

MHA plates were incubated overnight at 35 ± 2 °C. The limit of detection was considered to be 1.4 $\log_{10}$ CFU/mL. This additional analysis was performed for fosfomycin based on previous studies in which a resistant subpopulation was identified in susceptible Enterobacteriales [9,20].

2.5. Statistical analysis

Microsoft Excel 2013 (Microsoft Corp., Redmond, WA, USA), IBM SPSS Statistics v.24 (IBM Corp., Armonk, NY, USA) and GraphPad Prism v.7.0 (GraphPad Software Inc., San Diego, CA, USA) were used for processing the data. Fosfomycin samples were grouped in 6-h time intervals, and nitrofurantoin samples were grouped in 2-h time intervals. The median and range of UITs and UBTs were calculated for each interval. The area under the inhibitory titre–time curve (AUIT) and the area under the bactericidal titre–time curve (AUBT) were calculated to give an indication of the inhibitory and bactericidal activity for each strain using the trapezoidal rule [14]. A period of 48 h was considered for fosfomycin and 6 h or 8 h for nitrofurantoin for the 50 mg q6h and 100 mg q8h dosing regimens, respectively. Titre values were compared using a two-sided Wilcoxon matched-pairs rank test ($P < 0.0001$) to compare the titres of the two species, and a one-sided Wilcoxon matched-pairs rank test ($P < 0.0001$) was used to compare the UIT values and the UBT values per time interval. The d'Agostino–Pearson test was used to check the normal distribution of the data. Untransformed data were used for statistical analysis. Titre values of <2 were transformed into 1 for statistical analysis. The UBT in the most concentrated sample was used to calculate the percentage of volunteers in whom bactericidal activity (UBT ≥ 2) could be measured.

3. Results

3.1. Subjects and urine samples

Volunteers in both studies were Caucasian females with a mean ± standard deviation (S.D.) age of 24.3 ± 7.9 years and 28.5 ± 7.9 years in the fosfomycin and nitrofurantoin groups, respectively. A more detailed overview of volunteer characteristics can be found in the original studies [7,8]. The number of samples collected by the volunteers varied from 6–19 for fosfomycin and 3–9 for nitrofurantoin because they were not instructed to follow a voiding schedule. Fosfomycin urinary concentrations ranged from <0.75 mg/L [lower limit of quantification (LLOQ)] to 5729.9 mg/L and did not differ significantly between the two sets ($P < 0.05$; Supplementary Table S1). Nitrofurantoin concentrations ranged from <4 mg/L (LLOQ) to 176.3 mg/L (nitrofurantoin 50 mg q6h) and from <4 mg/L to 209.4 mg/L (nitrofurantoin 100 mg q8h) (Supplementary Table S1). Nitrofurantoin concentrations were slightly higher for the 100 mg dose but peak concentrations (C_{max}) were almost equal (mean ± S.D. C_{max} of 94.4 ± 47.8 mg/L for 50 mg q6h vs. 94.1 ± 49.9 mg/L for 100 mg q8h).

3.2. (A)UITs and (A)UBTs

3.2.1. Fosfomycin

The high interindividual variability in urinary drug concentrations was reflected by the wide range in UITs and UBTs [7]. For *E. coli*, fosfomycin UITs ranged from <2 to 256 and maximum titres were obtained during the first 12 h after dosing (Fig. 1A; Supplementary Table S2). Likewise, UBTs ranged from <2 to 512 and were comparable with the UITs for *E. coli*. Thus, fosfomycin was bactericidal against *E. coli* (Fig. 1B; Table 2). There was still reasonable

Table 2
UBTs and $AUBT_{0-48h}$ values for fosfomycin over time for each strain

Strain (MIC in mg/L)	UBT [median (range)] for the indicated time period								$AUBT_{0-48h}$ [median (range)]
	0–6 h	6–12 h	12–18 h	18–24 h	24–30 h	30–36 h	36–42 h	42–48 h	
Escherichia coli									
ATCC 25922 (1)	16 (<2–256)	16 (<2–512)	16 (<2–128)	4 (<2–128)	4 (<2–64)	2 (<2–64)	3 (<2–32)	2 (<2–32)	152 (97–303)
51 (2)	8 (<2–64)	4 (<2–32)	4 (<2–16)	4 (<2–16)	2 (<2–16)	2 (<2–8)	2 (<2–8)	2 (<2–4)	115 (53–163)
03 (0.25)	16 (<2–64)	16 (4–64)	16 (2–32)	8 (<2–32)	3 (<2–16)	2 (<2–16)	2 (<2–8)	2 (<2–8)	143 (101–192)
1231 (16)	2 (<2–16)	2 (<2–8)	<2 (<2–8)	<2 (<2–8)	<2 (<2–4)	<2 (<2–4)	<2 (<2–4)	<2 (<2–2)	63 (46–112)
4807 (32)	16 (<2–256)	16 (<2–256)	16 (<2–64)	6 (<2–128)	3 (<2–32)	2 (<2–32)	3 (<2–16)	2 (<2–16)	162 (88–275)
Klebsiella pneumoniae									
58 (8)	2 (<2–8)	<2 (<2–16)	<2 (<2–2)	<2 (<2–4)	<2 (<2–2)	<2 (<2–2)	<2 (<2–4)	<2 (<2–2)	47 (39–102)
20 (32)	4 (<2–32)	2 (<2–32)	<2 (<2–8)	2 (<2–16)	<2 (<2–2)	<2 (<2–2)	<2 (<2–2)	<2 (<2–2)	69 (44–114)
31865 (2)	8 (<2–128)	8 (<2–64)	4 (<2–64)	2 (<2–64)	<2 (<2–32)	<2 (<2–16)	<2 (<2–8)	<2 (<2–4)	106 (74–218)
55 (4)	4 (<2–16)	2 (<2–64)	<2 (<2–16)	<2 (<2–16)	<2 (<2–4)	<2 (<2–8)	<2 (<2–4)	<2 (<2–2)	68 (44–153)

UBT, urinary bactericidal titre; $AUBT_{0-48h}$, area under the bactericidal titre–time curve from 0–48 h; MIC, minimum inhibitory concentration.

bactericidal activity after 48 h for *E. coli* because UBTs were ≥2 in the majority (95%) of samples. The only exception was *E. coli* strain 1231 (MIC = 16 mg/L) where UITs and UBTs did not exceed 2 for the full 48 h. The AUIT from 0–48 h ($AUIT_{0-48h}$) values between the five *E. coli* strains were comparable, again with the exception of *E. coli* 1231 (Supplementary Table S2). The same is true for the AUBT from 0–48 h ($AUBT_{0-48h}$) values (Table 2). The difference in $AUIT_{0-48h}$ and $AUBT_{0-48h}$ values between the *E. coli* strains did not reflect their varying baseline MICs to fosfomycin (Table 1).

UITs for *K. pneumoniae* ranged from <2 to 128 and maximum titres were found during the first 6-h time period (Fig. 1A; Supplementary Table S2). UITs and UBTs were comparable, reflecting the bactericidal activity of fosfomycin in *K. pneumoniae* (Fig. 1B; Table 2). In contrast to *E. coli*, no antibacterial activity of fosfomycin in *K. pneumoniae* was observed in the majority (86%) of samples throughout the complete 48 h. Where an antibacterial effect was detected, it was bactericidal in the majority (90%) of samples but was present only during the first 18 h after administration. UITs and UBTs declined dramatically after that 18-h time point. UITs and UBTs for *K. pneumoniae* were significantly lower than those for *E. coli* ($P < 0.0001$ for all time intervals) (Fig. 1A,B). $AUIT_{0-48h}$ and $AUBT_{0-48h}$ values ranged from 47–110 and were independent of the strain's baseline MIC for fosfomycin (Table 2; Supplementary Table S2).

3.2.2. Nitrofurantoin

For *E. coli*, nitrofurantoin UITs ranged from <2 to 16 for the 50 mg q6h regimen and from <2 to 32 for the 100 mg q8h regimen and were generally within the same range for both dosing regimens (Fig. 1C; Supplementary Table S3). Maximum titres were obtained within the first 2 h after administration. UBTs for *E. coli* were comparable with the UIT values, demonstrating bactericidal activity of nitrofurantoin against *E. coli* (Fig. 1D; Table 3). After 2 h, no detectable antibacterial activity was found in the majority of samples (titres of <2).

For *K. pneumoniae,* nitrofurantoin UITs ranged from <2 to 16 for both dosing regimens and maximum titres were found in the first 2 h after administration (Fig. 1C; Supplementary Table S3). UBTs ranged from <2 to 8 and did not differ between dosing regimens (Fig. 1D; Table 3). UITs and UBTs were comparable in these first two 2 h, again reflecting the bactericidal activity of nitrofurantoin in the few samples in which antibacterial activity was detectable.

The UITs and UBTs were higher for *E. coli* compared with those for *K. pneumoniae* for both dosing regimens (Fig. 1C,D). Similar to fosfomycin activity, the AUIT and AUBT values were found to be independent of the baseline nitrofurantoin MICs of the isolates (Table 3; Supplementary Table S3). This is true for both dosing regimens.

3.2.3. Bactericidal effect in the samples and in volunteers

To correlate with clinical antibiotic effectiveness for UTI treatment, the percentage of volunteers in which bactericidal activity was found was calculated for sequential time intervals for the five *E. coli* and four *K. pneumoniae* strains. Fig. 2 demonstrates these percentages over time; a higher percentage reflects a more effective treatment.

Considering fosfomycin, the percentages for *E. coli* were higher than for *K. pneumoniae.* Bactericidal activity against *E. coli* was found in a mean of 90% of the volunteers during 24 h, but this declined to <60% thereafter. This applied to all *E. coli* strains with the exception of *E. coli* strain 1231 against which fosfomycin was not bactericidal in the least diluted sample in 50% of volunteers (Fig. 2A). This finding was supported by the detection of a resistant subpopulation in this isolate (Section 3.3). Against *K. pneumoniae*, bactericidal activity of fosfomycin was found in only a mean of 60% of the volunteers during the first 18 h after administration (Fig. 2A). Percentages declined quickly thereafter to <20% beyond 24 h after dosing. Thus, fosfomycin remained bactericidal against *K. pneumoniae* isolates after 24 h in a very small number of volunteers.

Nitrofurantoin was bactericidal in *E. coli* ATCC 25922, *E. coli* 51 and *E. coli* 03 regardless of the administered dose (Fig. 2B,C). However, this bactericidal activity was only found in 0–50% of volunteers. Percentages of >60% were found only in the first 2 h after administration. In *K. pneumoniae*, percentages never exceeded 17% in strains 58, 20 and 31865, independent of the administered dose. Only in *K. pneumoniae* 55 was bactericidal activity found in approximately 40% (100 mg q8h) and 60% (50 mg q6h) of volunteers. These percentages remained consistent over the 8-h urine collection time period for this strain.

3.3. Fosfomycin-resistant subpopulation

Only one of five *E. coli* isolates had a detectable fosfomycin-resistant subpopulation when grown both in standard laboratory medium and in human urine, whereas all of the *K. pneumoniae* isolates had a detected resistant subpopulation (Table 4). The resistant subpopulation detected in *E. coli* 1231 and in the *K. pneumoniae* isolates had fosfomycin MICs of >1024 mg/L after subculturing off the fosfomycin-containing MHA onto TSA. This result is consistent with the low antibacterial activity of fosfomycin in these strains.

4. Discussion

Whilst fosfomycin exhibited bactericidal activity for ≥48 h against *E. coli*, no antibacterial activity was detected in the majority of *K. pneumoniae* samples. In contrast to fosfomycin, nitrofurantoin showed low antibacterial activity in both species

Table 3
UBTs and $AUBT_{0-6h}$ or $AUBT_{0-8h}$ values for nitrofurantoin over time for each strain

Dose/strain (MIC in mg/L)	UBT [median (range)] for the indicated time period				$AUBT_{0-6h}$ or $AUBT_{0-8h}$ [median (range)]
	0–2 h	2–4 h	4–6 h	6–8 h	
Nitrofurantoin 50 mg q6h					
Escherichia coli					
ATCC 25922 (16)	8 (<2–16)	2 (<2–16)	<2 (<2–4)	<2 (<2–4)	18 (10–31)
51 (32)	4 (<2–16)	<2 (<2–8)	<2 (<2–2)	<2 (<2–2)	14 (9–25)
03 (16)	4 (<2–16)	<2 (<2–8)	<2 (<2–2)	<2 (<2–2)	15 (9–25)
1231 (512)	<2 (<2–<2)	<2 (<2–<2)	<2 (<2–<2)	<2 (<2–<2)	9 (9–22)
4807 (16)	2 (<2–8)	<2 (<2–4)	<2 (<2–<2)	<2 (<2–2)	12 (8–11)
Klebsiella pneumoniae					
58 (64)	2 (<2–8)	<2 (<2–4)	<2 (<2–8)	<2 (<2–2)	11 (7–9)
20 (256)	<2 (<2–2)	<2 (<2–2)	<2 (<2–<2)	<2 (<2–<2)	9 (9–17)
31865 (128)	<2 (<2–<2)	<2 (<2–<2)	<2 (<2–<2)	<2 (<2–<2)	9 (7–9)
55 (256)	<2 (<2–2)	<2 (<2–<2)	<2 (<2–<2)	<2 (<2–<2)	9 (7–11)
Nitrofurantoin 100 mg q8h					
E. coli					
ATCC 25922 (16)	4 (<2–32)	2 (<2–16)	<2 (<2–8)	<2 (<2–4)	15 (10–25)
51 (32)	2 (<2–16)	<2 (<2–8)	<2 (<2–2)	<2 (<2–2)	12 (8–21)
03 (16)	2 (<2–16)	<2 (<2–8)	<2 (<2–2)	<2 (<2–4)	13 (11–24)
1231 (512)	<2 (<2–<2)	<2 (<2–<2)	<2 (<2–<2)	<2 (<2–<2)	9 (8–21)
4807 (16)	<2 (<2–8)	<2 (<2–4)	<2 (<2–2)	<2 (<2–2)	11 (7–11)
K. pneumoniae					
58 (64)	2 (<2–8)	<2 (<2–4)	<2 (<2–4)	<2 (<2–<2)	11 (7–9)
20 (256)	<2 (<2–2)	<2 (<2–<2)	<2 (<2–<2)	<2 (<2–<2)	9 (7–20)
31865 (128)	<2 (<2–2)	<2 (<2–<2)	<2 (<2–<2)	<2 (<2–<2)	9 (7–10)
55 (256)	<2 (<2–2)	<2 (<2–<2)	<2 (<2–<2)	<2 (<2–<2)	9 (7–10)

UBT, urinary bactericidal titre; $AUBT_{0-6h}$, area under the bactericidal titre–time curve from 0–6 h; $AUBT_{0-8h}$, area under the bactericidal titre–time curve from 0–8 h; MIC, minimum inhibitory concentration; q6h, every 6 h; q8h, every 8 h.

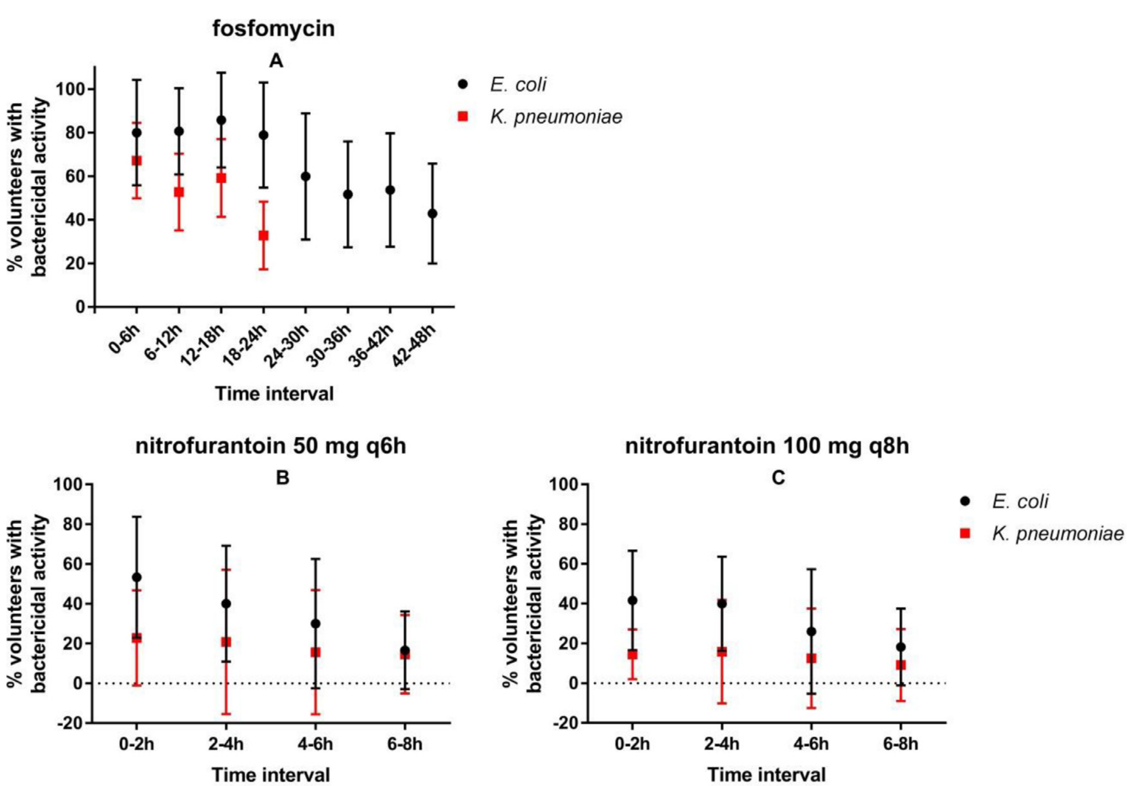

Fig. 2. Percentage of volunteers where a bactericidal effect was found for (A) fosfomycin during 48 h and (B,C) nitrofurantoin 50 mg q6h during 6 h (B) or nitrofurantoin 100 mg q8h during 8 h (C) in *Escherichia coli* and *Klebsiella pneumoniae*. Data are the mean ± standard deviation percentage for both species (*y*-axis) of the total number of volunteers that produced urine samples in the considered time interval (*x*-axis). Because bactericidal activity in *K. pneumoniae* was found in only a small number of volunteers (range 1–3 volunteers) in the time intervals after 24 h, these percentages are negligibly small and are therefore not presented in part (A). q6h, every 6 h; q8h, every 8 h.

Table 4
Presence of a fosfomycin low-level resistant (LLR) and/or high-level resistant (HLR) subpopulation of the strains in urine or Mueller–Hinton broth (MHB)

Strain (MIC in mg/L)	LLR/HLR subpopulation present	
	Urine	MHB
Escherichia coli		
ATCC 25922 (1)	No	No
51 (2)	No	No
03 (0.25)	LLR	LLR
1231 (16)	HLR	HLR
4807 (32)	LLR	LLR
Klebsiella pneumoniae		
58 (8)	HLR	HLR
20 (32)	LLR	HLR
31865 (2)	HLR	HLR
55 (4)	HLR	HLR

MIC, minimum inhibitory concentration.

regardless of the administered dose, although only one dose interval was examined among the many intervals intended with a course of nitrofurantoin.

In general, fosfomycin exhibited bactericidal activity as demonstrated by comparable UIT and UBT values. The duration of activity was strongly species-dependent, with ≥48 h for *E. coli* and only 18 h for *K. pneumoniae*. Indeed, 48-h antibacterial activity against *K. pneumoniae* could be demonstrated in only a small subset. These findings are supported by earlier in vitro research demonstrating that fosfomycin was not able to reliably kill *K. pneumoniae* isolates [9,20].

It was suggested that fosfomycin is able to kill (or at least inhibit the growth of) *E. coli*, but re-growth occurs thereafter. The extent of re-growth depends on the presence of a resistant subpopulation and this is not predicted based on the baseline fosfomycin MIC for the strain [21]. This is corroborated by the finding in the current study that bactericidal activity against *E. coli* over 24 h was found in approximately 90% of volunteers, but quickly fell below 60% thereafter. This was true for all *E. coli* strains, with the exception of *E. coli* 1231, the strain harbouring a HLR subpopulation. For *K. pneumoniae*, moderate (or almost totally absent) antibacterial activity of fosfomycin was found in the majority of samples, confirming other reports [9,10,22]. All *K. pneumoniae* strains had a HLR subpopulation. This may be more a matter of intrinsic rather than acquired resistance following antibiotic exposure [9,23]. These findings suggest that a single 3 g fosfomycin dose may be sufficient for UTIs caused by *E. coli* without HLR subpopulations, and that fosfomycin is inappropriate for UTIs caused by *K. pneumoniae* regardless of the MIC of the strain and the fosfomycin dose.

Maximum UITs and UBTs of nitrofurantoin were obtained in the first 2-h time interval. Titres were low but comparable for both species tested, demonstrating reasonable bactericidal activity of nitrofurantoin only in the first 2 h, confirming a previous report describing early activity against extended-spectrum β-lactamase (ESBL)-producing pathogens such as *E. coli* and *K. pneumoniae* [12]. No significant differences in antibacterial activity between the two dosing regimens were found; the slightly higher urinary concentrations of nitrofurantoin after 100 mg versus 50 mg did not result in more antibacterial activity in our experiment [8].

The major advantage of the method used here is that it is an ex vivo model combining patient-related PK properties of a drug with its PD effect. The ex vivo results obtained with this method may therefore reflect the antimicrobial clinical effectiveness against uropathogens better than most other ex vivo/in vitro methods. This is important as bacterial growth ex vivo/in vitro can be different from that in humans [24]. Yet we found bactericidal activity of nitrofurantoin in <50% of volunteer samples, and only for a short period of time. This contrasts with the bactericidal activity of fosfomycin, which was detected in 90% of volunteer samples. These results are in conflict with what was found in a recent randomised clinical trial comparing 5 days of nitrofurantoin (100 mg q8h) with single-dose fosfomycin (3 g) for acute lower UTI [25] in which 70% of those receiving nitrofurantoin had clinical success versus only 58% of those receiving fosfomycin. Microbiological resolution was achieved in 74% versus 63%, respectively. There is thus discrepancy between the ex vivo activity of nitrofurantoin in a single dosing interval (and also, but to a smaller extent, of fosfomycin) and its clinical efficacy.

There are several possible factors that could explain this discrepancy. Fosfomycin requires G6P to enter bacterial cells to exert its antibacterial activity, therefore it is standard practice to add 25 mg/L G6P to the laboratory medium when performing in vitro experiments with fosfomycin [26]. Because human urine normally does not contain G6P in significant amounts, the ex vivo antibacterial activity was measured without adding G6P. It should be noted, however, that the baseline MICs for fosfomycin were measured in the presence of G6P, as per the reference standard for fosfomycin susceptibility testing [27]. This could party explain the discrepancy between the fosfomycin MICs at baseline and the urinary antibacterial activity. For nitrofurantoin, its activity was investigated during only one dosing interval of a drug intended to be administered over ≥5 days. It would therefore seem likely that the short period of antibacterial activity found would be sufficient to achieve clinical success in the majority of patients when administered as a course of multiple oral doses. The cumulative effect of the full nitrofurantoin course after repetitive dosing has not been investigated, such that the current results would underestimate the effect of the antimicrobial agent. Second, the bactericidal activity was considered only when calculating the percentages of bactericidal success, but whether pathogen killing is needed to achieve clinical success is questionable. Bacteriostatic activity, or a bactericidal effect during a short period of time (e.g. <2 h), might be sufficient to promote clinical success, in particular because of the natural urodynamics of regularly voiding episodes during which uropathogens are flushed out together with the urine. Finally, the percentage of bactericidal success gives an underestimation of daily clinical practice since we were not able to measure the antibacterial activity in the undiluted sample owing to limited sample volumes.

5. Conclusion

Strong bactericidal activity of fosfomycin against *E. coli* over ≥48 h after administration and moderate bactericidal activity against *K. pneumoniae* over 18 h was found. High-level resistant subpopulations were found in all *K. pneumoniae* strains and in one *E. coli* strain, a finding that further supports the likelihood of intrinsic resistance of *K. pneumoniae* against fosfomycin and highlights that MIC measurements might not be the best measure for predicting the ex vivo activity of fosfomycin. Titres of nitrofurantoin were comparable both for *E. coli* and *K. pneumoniae*, demonstrating moderate bactericidal activity in the first 2 h after dosing. In the majority of subsequent samples, however, no antibacterial activity was detected regardless of the administered dose. This finding is in contrast to the well-observed clinical effects of nitrofurantoin over multiple dosing intervals. The current findings reveal a discrepancy between nitrofurantoin's measurable ex vivo activity in a single dosing interval time period and its clinical effectiveness. For fosfomycin, the current findings suggest that the current single-dose approach to fosfomycin administration in UTIs caused by *E. coli* without HLR may be sufficient, but confirm doubts on the use of fosfomycin in general in UTIs caused by *K. pneumoniae*.

Funding

This work was supported in part by the European Commission's FP7 AIDA project [Preserving old antibiotics for the future, Health-F3-2011- 278348].

Competing interests

None declared.

Ethical approval

Ethical approval was given by the local ethical committees [Erasmus Medical Center (MEC-2016-121) and Geneva University Hospital (13-036)] as mentioned in the papers of the original publications of both healthy volunteer studies [7,8].

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.07.018.

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