Pathogens and Disease, 77, 2019, ftz022

doi: 10.1093/femspd/ftz022
Advance Access Publication Date: 16 April 2019
Research Article

RESEARCH ARTICLE

Fosfomycin tromethamine activity on biofilm and intracellular bacterial communities produced by uropathogenic *Escherichia coli* isolated from patients with urinary tract infection

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One sentence summary: Fosfomycin tromethamine reduces uropathogenic *Escherichia coli* biofilm, without activity on intracellular bacterial communities within the bladder.

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Editor: Tom Coenye

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ABSTRACT

Fosfomycin tromethamine (FT), an old antibiotic revived as a new strategy to overcome antibiotic resistance, is an excellent option for the treatment of lower urinary tract infection (UTI). During UTI, *Escherichia coli* produces biofilms and could invade the bladder epithelial cells, developing intracellular bacterial communities (IBC). The present work aimed to evaluate the activity of FT on biofilms and IBC from clinical isolates of *E. coli*. A total of 38 *E. coli* clinical UTI isolates previously characterized as biofilm and IBC producers were studied. FT susceptibility was evaluated and its activity on 48 h biofilm was determined by microtiter plate-based biofilm assay comparing three different antibiotic concentrations. Two UPEC strains were selected to evaluate FT activity on IBC *in vitro* using T24 bladder cells. The survival percentage of intracellular bacteria after 24 h exposure to FT was calculated and compared to the percentage of intracellular bacteria without antibiotic. All the strains were susceptible to FT. FT produced a significant reduction of biofilms at the three concentrations tested, compared to the control. However, no statistically effect on IBC was observed after 24 h of fosfomycin exposure in cell culture. FT is a good option for bacterial biofilm reduction within UTI. However, it does not affect IBC.

Keywords: fosfomycin tromethamine; intracellular bacterial communities; biofilms; uropathogenic *E. coli*; urinary tract infections; antibiotic resistance

Received: 13 April 2019; **Accepted:** 15 April 2019

INTRODUCTION

Antibiotic resistance is a worldwide emerging problem. Therefore, reviving old and neglected antibiotics is regarded as a new strategy to overcome antibiotic resistance and to expand treatment options.

Fosfomycin is an 'old' antibiotic isolated for the first time in 1969 from cultures of *Streptomyces* spp., which has been reintroduced in many countries for the treatment of different infections. Fosfomycin represents its own class of antibiotics and acts inhibiting the early stages of bacterial cell wall biosynthesis in an irreversible way (Hendlin *et al.* 1969; Popovic *et al.* 2010). Different fosfomycin formulations are available: a hydrophilic disodium salt (fosfomycin di-sodium) for parenteral administration and fosfomycin tromethamine (FT) for oral administration (Popovic *et al.* 2010; Bergan 1990).

It is a broad-spectrum antibiotic with activity against a wide range of microorganisms, including Gram-negative bacteria like *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter spp., Citrobacter spp., and Salmonella typhi* and Gram-positive as *Enterococcus spp., Streptococcus spp.*, and *Staphylococcus spp*. (Michalopoulos, Livaditis and Gougoutas 2011; Barry and Brown 1995). It is also active against Multidrug-resistant Enterobacteriaceae, such as extended-spectrum beta-lactamases and carbapenemases producers [Michalopoulos, Livaditis and Gougoutas 2011; Barry and Brown 1995; Dijkmans *et al.* 2017; Aris *et al.* 2018; Seitz, Stief and Waidelich 2017). FT is an excellent option for the treatment of lower urinary tract infection (UTI), with the advantage of single-dose posology, high urine concentrations during 48 h, and rare adverse effects (Dijkmans et al. 2017; Aris *et al.* 2018; Seitz, Stief and Waidelich 2017).

UTI is a common infection in children and adults, being Uropathogenic *E. coli* (UPEC) the most common etiological agent (Habib 2012; Robino *et al.* 2014a). Recurrent UTI is a common problem. Approximately, 20–40% of children and women, respectively, would have another UTI during the next 12 months after the previous episode (Elder 2009; Habib 2012; Garin *et al.* 2006). Adherence to the host urothelial cells is one of the first steps in the pathogenesis of UPEC UTI. The adhesion of FimH (the adhesin of type 1 pili) to uroplakin or integrins induces a reorganization of the cytoskeleton allowing UPEC internalization by an endocytic pathway. Once within the cell, bacteria can escape from the endocytic vesicles to the cytoplasm and replicate establishing structures similar to biofilms, called intracellular bacterial communities (IBC) (Justice *et al.* 2004; Andersen *et al.* 2004, 2012). Internalization may protect the bacteria from antibiotics, neutrophil influx,and shear stress. Intracellular bacteria could emerge from these reservoirs, usually adopting a filamentous morphology and eventually initiating a new IBC cycle (Justice *et al.* 2004; Andersen et al. 2004, 2012). After infection, UPEC may persist in the urinary tract in different situations, as IBC or quiescent intracellular reservoirs (QIRs) and forming biofilms on biological surfaces (Robino et al. 2013; Robino *et al.* 2014b; Rosen*et al.* 2007 ; Sanchez *et al.* 2013).

A biofilm is defined as a microbial-derived sessile community characterized by cells that are irreversibly attached to a substratum or interface on each other and embedded in a matrix of extracellular polymeric substances that they have produced (Flemming and Wingender 2010). Bacteria within the biofilm are in a different replication state (Soto 2014). These characteristics may prevent bacteria eradication during antibiotic treatment.

Antibiotic resistance in biofilms has been widely studied in the last years, proposing that it is an adaptive and reversible situation, demonstrated by the recovery of the original susceptibility when bacteria return to a planktonic state (Keren *et al.* 2004). It is proposed that antibiotic resistance in the biofilm occurs due to different mechanisms, like a limitation of antibiotic diffusion through the matrix, horizontal transmission of resistance genes, inactivation of the antibiotic by changes in metal ion concentrations and pH values, and the metabolic inactive bacterial status. The level of resistance depends on the biofilm formation stage, being the bacteria in the initial reversible step more susceptible (Soto 2014). However, some cephalosporins, amikacin and ciprofloxacin, at concentrations achieved in human urine after a standard UTI treatment dose, produced a reduction on UPEC biofilm '*in vitro*' models (González *et al.* 2017).

Treatment for IBC eradication is not well established. Schilling *et al.* (2002) showed that a long-term antibiotic therapy with trimethoprim-sulfamethoxazole, was unable to eradicate persistent bacteria from the bladder. Blango and Mulvey (2010) suggested that UPEC IBC can persist in the face of treatment with multiple antibiotics. Recently, Liu *et al.* (2016) have demonstrated the persistence of UPEC within the bladder epithelial cells in female patients with recurrent UTI despite sensitive antibiotic therapy.

The present work aimed to evaluate the '*in vitro*' activity of FT on biofilm and IBC eradication from clinical isolates of UPEC.

MATERIALS AND METHODS

Bacterial strains and bladder cell culture

A total of 38 strains of UPEC isolated from children with UTI, and previously characterized as biofilm producers (González *et al.* 2017), were included for the FT activity on biofilm assay.

Two of these UPEC strains, Ec7U and Ec144U (previously characterized as IBC producers (Robino 2013, 2014) were selected for the study of FT activity on IBC '*in vitro*'. For the invasion and IBC assay, the human bladder epithelial cell line T24 (ATCC HTB-4) was used. The cell line was maintained at 37°C and 5% CO2 in DMEM medium supplemented with 10% fetal bovine serum (Gibco).

Antibiotic susceptibility assay

Fosfomycin susceptibility was assessed using the disk diffusion assay using 200 μg fosfomycin disk containing 50 μg of glucose-6-phosphate, according to the Clinical Laboratory Standard Institute (CLSI 2018). Minimal inhibition concentration (MIC) was tested by agar dilution using agar media supplemented with 25 μg/ml of glucose-6-phosphate (CLSI 2018). The minimal biofilm eradication concentration (MBEC) was determined using the Calgary Biofilm Device. Biofilm assay was performed as described later (see point 'Antibiotic assay in biofilm'). After 48 h, biofilm was exposed to serial dilutions of fosfomycin tromethamine in Luria–Bertani Broth (from 1024 to 8 μg/ml) and incubated for 24 h more. The ODs were read at $\lambda = 650$ nm for planktonic cells and at $\lambda = 590$ for the biofilm biomass.

Antibiotic assay in biofilm

Biofilm formation assays were performed as previously described with a few modifications (Pratt and Kolter 1998). The strains were grown overnight in Luria–Bertani (LB) broth at 37°C under static conditions. Aliquots of 20 μl from overnight

cultures were inoculated in 180 μl of LB in 96 flat-bottomed well, polystyrene microtiter plates (Deltalab) and incubated for 48 h at 37°C without shaking. The optical densities (ODs) were read at $\lambda = 600$ nm for bacterial growth. Planktonic bacteria were removed, and fresh LB broth was added to each well with or without fosfomycin at the following concentrations: 0, 300, 700 and 1500 μg/ml. The antibiotic concentrations used were selected according to the concentration that fosfomycin achieves in urine in humans after 3 g oral dose (dose usually used in humans for UTI treatment) (Zhanel, Walkty and Karlowsky 2016). These concentrations by far exceeds the MIC either in susceptible and resistance strains (CLSI 2018). After an additional 24 h of incubation, the wells were washed with PBS three times and stained with 200 μl of 0.1% crystal violet for 15 min at room temperature. Then, the plates were washed to remove the dye excess, and CV was solubilized with 200 μl of 95% ethanol. The ODs were read at $\lambda = 590$ nm for stained biofilms using a Microplate Reader (Varioskan, Thermo Scientific, Waltham,Massachusetts, USA). The results are the mean of 3 independent experiments. In each experiment, the strains were assayed in triplicate and the means and standard error were calculated for all experiments.

Live and dead assay on biofilm

Ec7U and Ec144U were selected for live and dead assay.

The biofilm was produced on a glass cover, with and without FT (as described above in biofilm and antibiotic assay), and stained with a solution containing 10 μM Green-fluorescent nucleic acid stains (Syto9, Molecular Probes, Invitrogen, Waltham,Massachusetts, USA), 5 μg/ml Propidium iodide nucleic acid stain (Molecular Probes, Invitrogen,) and 2 μg/ml Hoescht 33 342 (Molecular Probes, Invitrogen, Waltham,Massachusetts, USA) for 15 min, in a humid chamber in the dark. After 3 washes with PBS, slides were fixed with 4% of paraformaldehyde for 15 minutes. Slides were analyzed using laser confocal microscopy as described above.

Cellular invasion, IBC and antibiotic eradication assay

T24 bladder epithelial cells were seeded into 24-well plates and grown to 1×10^5 cells/well, in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum. Bacteria were then added to the medium using a multiplicity of infection of 15 bacteria per host cell, for 2 h at 37°C. After 2 h incubation at 37°C, samples were washed three times with PBS containing Ca^{2+} and Mg (PBS2) to remove non-adherent bacteria. Monolayers were then incubated for another 2 h with complete DMEM medium with 100 μg/ml of gentamicin to kill extracellular bacteria. Following additional washes with PBS2, fresh medium containing a lower concentration of gentamicin (10 μg/ml) was added, and incubation was continued for another 18 h. Then, the different antibiotic concentrations were added and incubated for another 10 h.

After final washes in PBS2, host cells were lysed in PBS plus 0.4% Triton X-100 and bacteria present within the lysates were enumerated by plating serial dilutions on LB agar plates. Experiments were performed in triplicate.

Confirmation of FT activity over IBC and biofilm was made by confocal laser scanning microscopy. Immunofluorescence staining of IBC was performed according to the protocol previously published (Robino 2014b) using specific rabbit anti-*E. coli* antibody coupled to fluorescein isothiocyanate (Abcam, Biotech, Life Science, Eugene, USA), anti-Actin (Rhodamine-Phalloidin) and Hoescht 33258 (molecular probes, Waltham,Massachusetts, USA). Slides were fixed with 4% of paraformaldehyde for 15 minutes. Acquisition and processing of 3-D image stacks were performed using a BX61 confocal microscopy (Olympus, Shinjuku, Tokyo, Japan) as described before (Schlapp *et al.* 2011) using 350/460, 488/520 and 543/565 excitation/emission wavelengths. Acquisition step size was of 0.3 μm in the z-axis and 1024 × 1024 pixels in the x–y plane with a pixel size of 70 nm. The 3-D image stacks were deconvolved using the Huygens Software and were reconstructed using Volocity 3-D Image Analysis Software (PerkinElmer, Waltham,Massachusetts, USA).

Cytotoxicity assay of the different antibiotic concentrations on the monolayer cell

Cells were incubated during 10 h with the different antibiotic concentrations, in 6 wells plates with coverslips. After incubation and 3 washes with PBS, staining was performed as previously reported by Kabakov, Kudryavtsev and Gabai (2011) using 5 μg/ml of propidium iodide (PI) and 1 μg/ml of Hoescht 33342. A total of 20 fields were analyzed for each experiment. The dead cells percentage was calculated and the results were compared using the Mann–Whitney statistic test and a *P*-value ≤ 0.05 was considered significant.

Statistical analyses

The differences between the treatments groups were first assessed using the Kruskal–Wallis test, and the differences between pairs of groups were further assessed using the Mann–Whitney *U* test.

RESULTS

FT susceptibility and its effect on established biofilm

All the *E. coli* strains (38) used in this study were susceptible to FT by the disk diffusion assay. The MIC for all the strains was between 0.5 and 2 μg/ml and the MBEC from 8 to 128 μg/ml.

The mean OD associated with biofilm formed in the culture medium without antibiotic was 0.53 (Standard error [SE], 0.07). The mean OD after 300 μg/ml FT exposure was 0.196 (SE 0.043; $P < 0.000$), at 700 μg/m 0.128, (SE 0.025, $P < 0.000$) and at 1500 μg/ml 0.165 (SE 0.035, $P < 0.000$). The three-fosfomycin concentrations showed a significant reduction of the biofilm compared with the condition without antibiotic. Fig. 1 compares the effect of fosfomycin at 0, 300, 700 and 1500 μg/ml concentrations on all strains.

When biofilms were formed without the antibiotic, most of the bacteria were live and the presence of microcolonies, the basic structure of the biofilm, was observed. When the medium was supplemented with 300 μg/ml of FT, a decrease in the volume of live and dead bacteria was observed and the microcolonies were more sparsely containing fewer bacteria. When 700 and 1500 μg/ml FT concentrations were used, a decrease of both live and dead bacteria was observed, without the presence of microcolonies (Figs 2 and 3).

Fosfomycin activity on intracellular bacteria

Two IBC-producer UPEC isolates (EcU7 and EcU144) were selected to assess the ability of fosfomycin to eradicate intracellular bacteria.

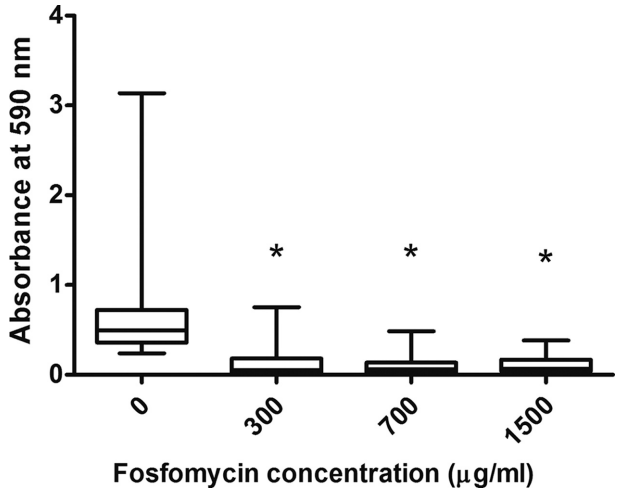

Figure 1. Biofilm quantification with different fosfomycin tromethamine concentrations. Boxplot comparing the effect of fosfomycin tromethamine at 0, 300, 500 and 1000 μg/ml concentrations.

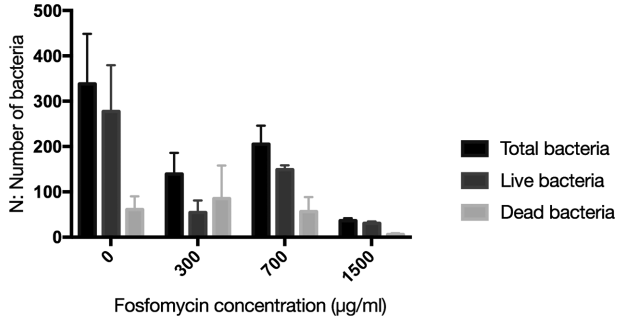

Figure 2. Effect of different concentrations of fosfomycin on bacterial number in biofilm analyzed with LIVE/DEAD stain. A reduction of bacterial volume, in live and dead groups, is observed with different fosfomycin concentrations compared to no antibiotic.

After monolayer epithelial cells were exposed to EcU7 and EcU144 *E. coli* strains, extracellular bacteria were killed by gentamicin (which cannot cross host cellular membranes). Then, cells were incubated in the presence of FT at three different concentrations that were similar to the levels achieved in urine after a 3 g dose (dose usually used in adults).

No effects on intracellular bacteria were observed at the 3 antibiotic tested concentrations. The averages of intracellular bacteria recovered after no antibiotic exposure (except gentamicin) and fosfomycin at 300, 700 and 1500 μg/ml are shown in Table 1.

When the images of invasion assay were analyzed by laser confocal microscopy, IBC could be detected, both with and without fosfomycin exposure (Fig. 4).

Fosfomycin cytotoxicity on the cell monolayer

The cytotoxic effect of different concentrations of FT on the cell culture monolayer was assessed. When a FT concentration of 300 μg/ml was used, the percentage of surviving cells was similar to the monolayer incubated without the antibiotic (99.9%). However, at 700 μg/ml of fosfomycin a low cytotoxic effect was observed (95.4%) but at 1500 μg/ml cytotoxicity was statistically significant compared to untreated controls (47.1%) (Fig. 5).

DISCUSSION

The reincorporation of 'neglected antibiotics', such as fosfomycin, for the treatment of UTI, is one of the strategies proposed to tackle the burden of antimicrobial resistance.

An excellent bioavailability in the urinary tract after a single dose and unfrequent side effects make the use of fosfomycin suitable for the treatment of cystitis (Dijkmans *et al.* 2017; Aris *et al.* 2018; Seitz, Stief and Waidelich 2017).

Many clinical guidelines, such as NICE (National Institute for Health and Care Excellence) and PHE (Public Health England) guidelines, include FT for the treatment of uncomplicated UTI (defined as no fever/flank pain) caused by ESBL-producing *E. coli* in adults and pregnant woman (National Institute of Health and Care Excellence 2015; Public Health England 2015). The Infectious Disease Society of America recommends its use in lower urinary tract infection for women (Gupta *et al.* 2011). Although most of the guidelines include its use for the treatment of ESBL-producer microorganisms, FT is frequently prescribed for not complicated cystitis. In a retrospective study, evaluating the indications of FT according to the clinical guidelines, only 41% of patients who received FT met the guidelines criterion (Matthews *et al.* 2016).

Despite its increasing use, susceptibility levels have remained relatively stable (98–100%), being the selection of resistant strains more frequent in non-*E. coli* Enterobacteriaceae than in *E. coli* (Matthews *et al.* 2016; Vardakas *et al.* 2016). In Uruguay, oral FT was introduced a few years ago in the vademecum, although susceptibility tests are not performed routinely in clinical microbiology laboratories so far.

In the collection of strains studied in the present study, no resistance to FT was detected.

A biofilm is proposed to be an antibiotic resistance mechanism due to different conditions, including metabolically inactive bacterial populations within the biofilm, limitation of antibiotic diffusion through the matrix and transmission of resistance genes within the community (Soto 2014). Different strategies have been proposed to control microbial biofilm formation such as the use of compounds that prevents the formation of the components of adhesin systems or blocking their functions, which are responsible for the initial steps of biofilm formation and suppression of biofilm formation at the maturation stage by affecting quorum sensing or the effect of cyclic diguanosine monophosphate (c-di-GMP)-dependent systems. Another strategies proposed to control bacterial biofilms are the use of bacteriophages and proteolytic enzymes that promote biofilm dispersion like DNase or alginate lyase (Plakunov *et al.* 2017). However, these strategies require extensive evaluation to pass through clinical trials to be used in humans.

Recently, González *et al.* (2017) studied the activity of different antibiotics, including ampicillin, cephalothin, ceftriaxone, ceftazidime, amikacin and ciprofloxacin on biofilms formed by UPEC. Except ampicillin, the other tested antibiotics induced a significant reduction of biofilm biomass, even in resistant strains.

Rodriguez-Martínez, Ballesta and Pascual (2007) studied the activity of fosfomycin on *E. coli* biofilm produced on catheter surfaces, concluding that fosfomycin was able to kill more than 96% of viable bacteria, partially due to their high penetration rate into a mature biofilm, but it was not able to sterilize the catheter surface completely. Blango and Mulvey (2010) found that fosfomycin not only inhibited the biofilm growth but also promoted the disassemble preexisting biofilm communities. Our results are consistent with these studies, showing that FT reduces the

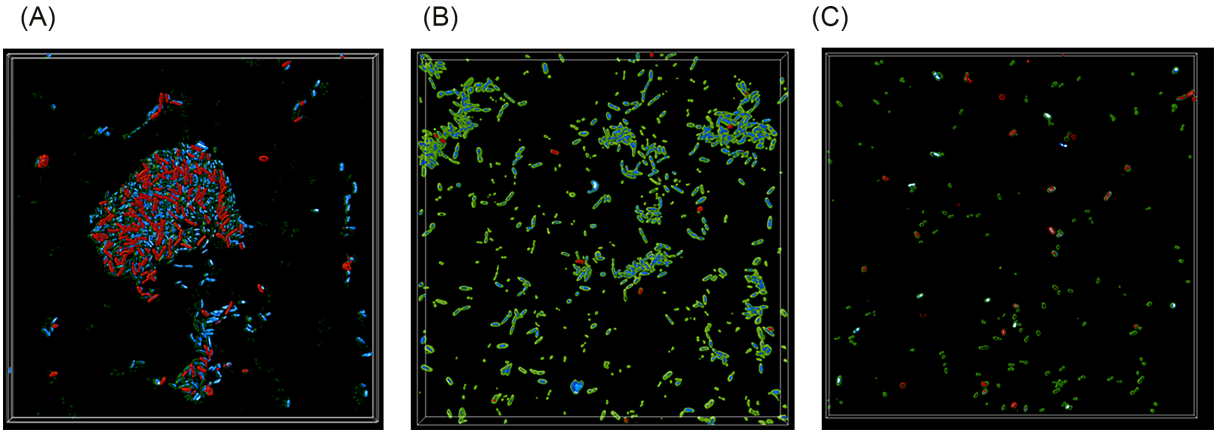

Figure 3. Effect of different concentrations of fosfomycin on bacterial biofilm analyzed with LIVE/DEAD stain. The biofilm was stained with a solution containing Syto9 (green), Propidium iodide nucleic acid stain (red) and Hoescht 33342 (blue). A reduction of bacterial volume and number, in live and dead groups, and microcolonies disruption using 300 μg/ml **(B)** and 1500 μg/ml **(C)** of fosfomycin tromethamine is observed compared with no fosfomycin tromethamine **(A)**.

Table 1. Percentage of infection in bladder epithelial cell line (T24) of two UPEC isolates after different concentrations to fosfomycin compared to the percentage of infection without antibiotics.

Strain	Fosfomycin concentration (μg/ml)	Mean (SE)	P-value
7	0	0.971 (0.13)	-
	300	0.747 (0.42)	0.6631
	500	0.399 (0.23)	0.1102
	1500	0.367 (0.22)	0.0591
144	0	3.010 (0.93)	-
	300	3.229 (0.27)	1.0000
	700	2.708 (0.98)	0.8857
	1500	1.735 (0.91)	0.3429

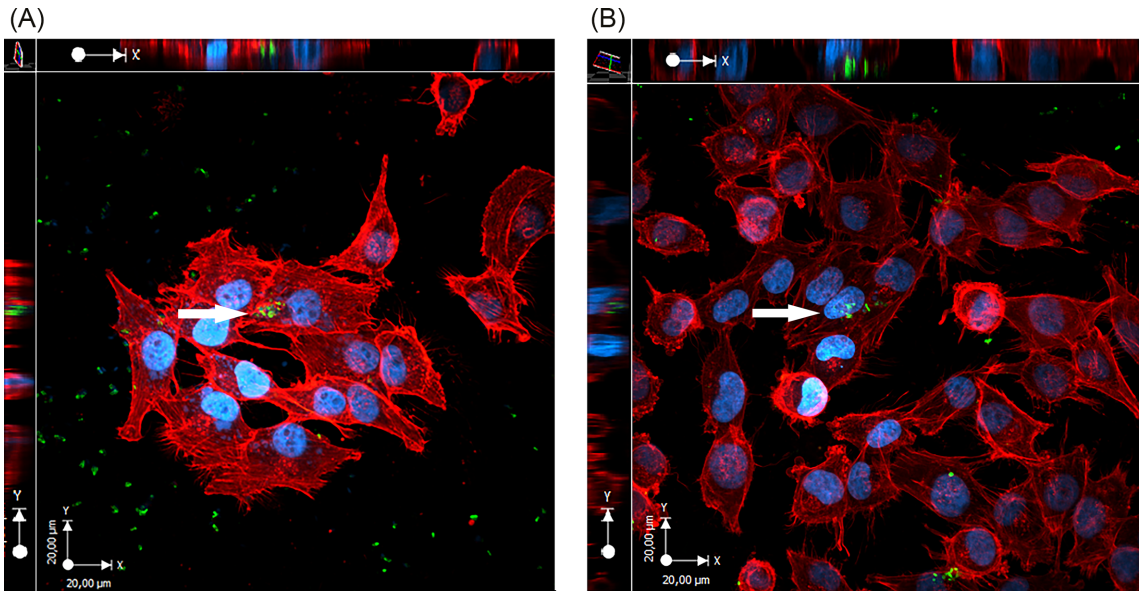

Figure 4. Image of IBC *in vitro* in T24 cells infected with UPEC stained with DAPI for detection of DNA (blue), anti-actin (red) and *E. coli* (green). The 3D images of the stacks, where the 3 channels converge, reveal a small group of intracellular bacteria at the perinuclear level (arrow). In the central square the plane xy is observed, in the upper zone the zx plane and to the left the zy plane. IBC was defined as ≥5 bacterial clusters in the cellular interior. **(A)** Infected cells with Ec7U and treated for 10 h with 300 μg/ml. **(B)** Infected cells Ec144U and treated for 10 h with 700 μg/ml. Despite the antibiotic treatment, we found IBC with or without Fosfomycin.

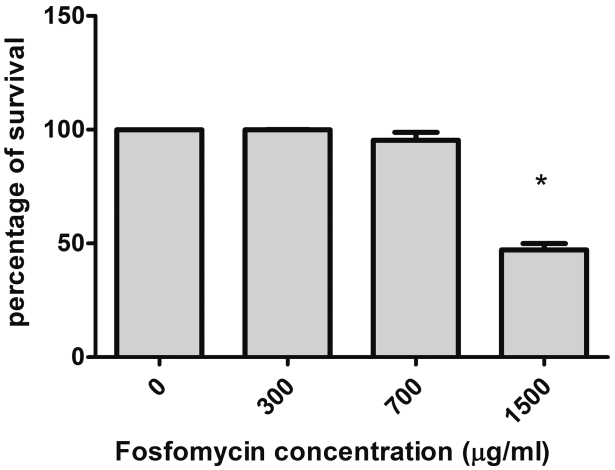

Figure 5. Cytotoxic effect of fosfomycin over the cell monolayer. Cytotoxic effects of fosfomycin used at three concentrations on T24 bladder cells were determined after 10 h exposure and observed by immunofluorescence microscopy. Results indicate the means ± . ∗ Significant differences compared to the control without antibiotic (P-value ≤0.05).

biofilm biomass at 300, 700 and 1500 μg/ml. Even though its effect on the biofilm biomass reduction, viable bacteria that withstood the antibiotic treatment were detected using the Live/Dead staining.

Another bottleneck in UTI treatment is IBC eradication. Antibiotics frequently used for UTI treatment, such as beta-lactams and aminoglycosides do not reach adequate intracellular concentrations. Fluoroquinolones reach high intracellular concentrations; however, do not show a significant effect on IBC eradication in UTI mouse models (Blango and Mulvey 2010). In this work, the effectiveness of FT against intracellular UPEC was assessed using a cell culture assay. FT concentrations tested were those commonly achieved in the human urine after a 3 g single dose. In these conditions, FT did not show any effect on intracellular UPEC compared to the control without antibiotics other than membrane-impermeable gentamicin.

One of the hypotheses raised is that fosfomycin may not concentrate well at the intracellular level because it is a hydrophilic molecule (Dijkmans *et al.* 2017). However, fosfomycin demonstrated intracellular antimicrobial activity on *Listeria monocytogenes* and *Salmonella*, '*in vitro*' in HeLa cells (Okada, Nishio and Danbara 2003).

At 1500 μg/ml, fosfomycin exerted a significant cytotoxic effect over the cell culture monolayer. However, non-intracellular effect over IBC was observed at this concentration, demonstrating that cytotoxicity does not correlate with intracellular bacterial killing. The quiescent nature of UPEC within IBC may also contribute to fosfomycin resistance thus its wall synthesis inhibitor mechanisms requires the bacteria being in a replicating state.

Considering that classic antimicrobials do not seem to have an effect on IBC eradication, other alternative molecules such as mannosides, small-molecular weight compounds that specifically inhibit adhesion of the FimH type 1 pilus of UPEC have been proposed for IBC prevention (Cusumano *et al.* 2011). Another novel proposed therapeutic strategy is the use of chitosan, a bladder cell exfoliant, to deplete UPEC reservoirs. Recently Erman *et al.* showed that the repeated use of chitosan in combination with ciprofloxacin completely eradicates UPEC from the urinary tract and prevents bacteriuria recurrence (Erman et al. 2017). New intracellular drug delivery systems could also be an exciting studying area for intracellular bacterial treatment.

CONCLUSIONS

Fosfomycin tromethamine is a good option for lower urinary tract infections treatment, with high susceptibility rates and effect on bacterial biofilm reduction. However, it does not affect intracellular bacterial reservoirs eradication, which may be a cause of urinary tract infection recurrence.

FUNDING

This work was supported by Comisión Sectorial de Investigación Científica (CSIC iniciación 2017), UdelaR, Uruguay.

Conflict of interest. None declared.

REFERENCES

Andersen GG, Dodson K, Hooton T *et al.* Intracellular bacterial communities of uropathogenic *Escherichia coli* in urinary tract pathogenesis. *Trend Microbiol* 2004;**12**:424–30.

Andersen TE, Khandige S, Madelung M *et al.* *Escherichia coli* uropathogenesis in vitro: invasion, cellular escape, and secondary infection analyzed in a human bladder cell infection model. *Infect Immun* 2012;**80**:1858–67.

Aris P, Boroumand MA, Rahbar M *et al.* The activity of fosfomycin against extended-spectrum beta-lactamase-producing isolates of Enterobacteriaceae recovered from urinary tract infections: a single-center study over a period of 12 years. *Microb Drug Resist* 2018;**24**:607–12.

Barry AL, Brown SD. Antibacterial spectrum of fosfomycin trometamol. *J Antimicrob Chemother* 1995;**35**:228–30.

Bergan T. Degree of absorption, pharmacokinetics of fosfomycin trometamol and duration of urinary antibacterial activity. *Infection* 1990;**18**:65–9.

Blango MG, Mulvey MA. Persistence of uropathogenic *Escherichia coli* in the face of multiple antibiotics. *Antimicrob Agents Chemother* 2010;**54**:1855–63.

CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute, 2018.

Cusumano CK, Pinkner JS, Han Z *et al.* Treatment and prevention of urinary tract infection with orally active FimH inhibitors. *J Sci Transl Med* 2011;**3**:109ra115.

Dijkmans AC, Zacarías NVO, Burggraaf J *et al.* Fosfomycin: pharmacological, clinical and future perspectives. *Antibiotics* 2017;**6**:E24.

Elder S. Infecciones del tracto urinario. In: Kliegman RM, Jenson HB, Behrman RE, Stanton BF (eds). *Nelson. Tratado de Pediatría*. Madrid, Spain: Elsevier, 2009, 2223–8.

Erman A, Hergouth VK, Blango MG *et al.* Repeated treatments with Chitosan in combination with antibiotics completely eradicate uropathogenic *Escherichia coli* from infected mouse urinary bladders. *J Infect Dis* 2017;**216**:375–81.

Flemming HC, Wingender J. The biofilm matrix. *Nat Rev Microbiol* 2010;**8**:623–33.

Garin EH, Olavarria F, Garcia Nieto V *et al.* Clinical significance of primary vesicoureteral reflux and urinary antibiotic prophylaxis after acute pyelonephritis: a multicenter, randomized, controlled study. *Pediatrics* 2006;**117**:626–32.

González MJ, Robino L, Iribarnegaray V *et al.* Effect of different antibiotics on biofilm produced by uropathogenic *Escherichia*

coli isolated from children with urinary tract infection. *Pathog Dis* 2017;**75**,DOI: 10.1093/femspd/ftx053.

Gupta K, Hooton TM, Naber KG *et al.* International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the infectious diseases society of America and the European Society for Microbiology and infectious diseases. *Clin Infect Dis* 2011;**52**:103–20.

Habib S. Highlights for management of a child with a urinary tract infection. *Int J Pediatr* 2012;**1**:943653

Hendlin D, Stapley EO, Jackson M *et al.* Phosphonomycin, a new antibiotic produced by strains of Streptomyces. *Sci* 1969;**16**:122–23.

Justice SS, Hung C, Theriot JA *et al.* Differentiation and developmental pathways of uropathogenic *Escherichia coli* in urinary tract pathogenesis. *Proc Natl Acad Sci USA* 2004;**101**:1333–8.

Kabakov AE, Kudryavtsev VA, Gabai VL. Determination of cell survival or death. In: *Calderwood S., Prince T. (eds)*. *Molecular Chaperones*, Methods in Molecular Biology (*Methods and Protocols*), New York 2011, 231–44.

Keren I, Shah D, Spoering A *et al.* Specialized persister cells and the mechanism of multidrug tolerance in *Escherichia coli*. *J Bacteriol* 2004;**186**:8172–80.

Liu SC, Han X, Shi M *et al.* Persistence of uropathogenic *Escherichia coli* in the bladders of female patients with sterile urine after antibiotic therapies. *J Huazhong Univ Sci Technol Med Sci* 2016;**36**:710–15.

Matthews P, Barrett L, Warren S *et al.* Oral fosfomycin for treatment of urinary tract infection: a retrospective cohort study. *BMC Infect Dis* 2016;**16**:556.

Michalopoulos AS, Livaditis IG, Gougoutas V. The revival of fosfomycin. *Int J Infect Dis* 2011;**15**:732–39.

National Institute of Health and Care Excellence. Fosfomycin for treating urinary tract infections caused by bacteria that are resistant to more than one antibiotic. https://www.nice.org.uk/guidance/esuom17/resources/fosfomycin-for-treating-urinary-tract-infections-caused-by-bacteria-that-are resistant-to-more-than-one-antibiotic-17481397957(September 2015, date last accessed).

Okada N, Nishio M, Danbara H. Intracellular activity of fosfomycin against two distinct enteropathogenic bacteria, *Salmonella enterica* and *Listeria monocytogenes*, alive inside host cells. *Chemotherapy* 2003;**49**:49–5.

Plakunov V, Martyanov S, Teteneva N *et al.* Controlling of microbial biofilms formation: anti- and probiofilm agents. *Microbiology* 2017;**86**:423–38.

Popovic M, Steinort D, Pillai S *et al.* Fosfomycin: an old, new friend? *Eur J Clin Microbiol Infect Dis* 2010;**29**:127–42.

Pratt LA, Kolter R. Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis, and type I pili. *Mol Microbiol* 1998;**30**:285–93.

Public Health England. Management of infection guidance for primary care for consultation and local adaptation. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/524984/Management_of_infection_guidance_for_primary_care_for_consultation_and_local_adaptation.pdf(September 2015, date last accessed)

Robino L, Scavone P, Araujo L *et al.* Detection of intracellular bacterial communities in a child with *Escherichia coli* recurrent urinary tract infections. *Pathog Dis* 2013;**68**:78–81.

Robino L, García-Fulgueiras V, Araujo L *et al.* Urinary tract infection in Uruguayan children: aetiology, antimicrobial resistance, and uropathogenic *Escherichia coli* virulotyping. *J Glob Antimicrob Re* 2014a;**2**:293–98.

Robino L, Scavone P, Araujo L *et al.* Intracellular bacteria in the pathogenesis of *Escherichia coli* urinary tract infection in children. *Clin Infect Dis* 2014b;**59**:158–64.

Rodríguez-Martínez JM, Ballesta S, Pascual A. Activity and penetration of fosfomycin, ciprofloxacin, amoxicillin /clavulanic acid and co-trimoxazole in *Escherichia coli* and *Pseudomonas aeruginosa* biofilms. *Int J Antimicrob Agents* 2007;**30**:366–68.

Rosen DA, Hooton TM, Stamm WE *et al.* Detection of intracellular bacterial communities in human urinary tract infection. *PLoS Med* 2007;**4**:e329.

Sanchez CJ, Jr, Mende K, Beckius ML *et al.* Biofilm formation by clinical isolates and the implications in chronic infections. *BMC Infect Dis* 2013;**13**:47.

Schlapp G, Scavone P, Zunino P *et al.* Development of 3D architecture of uropathogenic *Proteus mirabilis* batch culture biofilms: a quantitative confocal microscopy approach. *J Microbiol Methods* 2011;**87**:234–40.

Schilling JD, Lorenz RG, Hultgren SJ. Effect of trimethoprim-sulfamethoxazole on recurrent bacteriuria and bacterial persistence in mice infected with uropathogenic *Escherichia coli*. *Infect Immun* 2002;**70**:7042–49.

Seitz M, Stief C, Waidelich R. Local epidemiology and resistance profiles in acute uncomplicated cystitis (AUC) in women: a prospective cohort study in an urban urological ambulatory setting. *BMC Infect Dis* 2017;**17**:685.

Soto SM. Importance of biofilms in urinary tract infections: new therapeutic approaches. *Adv Biol* 2014;543974.

Vardakas K, Legakis N, Triarides N *et al.* Susceptibility of contemporary isolates to fosfomycin: a systematic review of the literature. *Int J Antimicrob Agents* 2016;**47**:269–85.

Zhanel G, Walkty A, Karlowsky J. Fosfomycin: a first-line oral therapy for acute uncomplicated cystitis. *Can J Infect Dis Microbiol* 2016;2082693.