



Suppression of biofilm formation and survival of clinical isolates of uropathogens within epithelial cells under the effect of Fluorothiazinone *in vitro*

Lydiya N. Kapotina¹, Elena Yu. Morgunova¹, Stanislava A. Nelyubina¹, Elena D. Fedina¹, Ekaterina A. Koroleva¹, Bondareva N.E.¹, Tamara S. Perepanova², Naylia A. Zigangirova^{1✉}

¹National Research Center for Epidemiology and Microbiology named after Honorary Academician N.F. Gamaleya, Moscow, Russia;

²N. Lopatkin Scientific Research Institute of Urology and Interventional Radiology — the branch of the National Medical Research Radiological Center, Moscow, Russia

Abstract

The aim of this study was to investigate the effect of the innovative Russian antibacterial drug Fluorothiazinone, an inhibitor of the type III secretion system and flagellar function, on the virulence properties of urinary tract infection (UTI) pathogens.

Materials and methods. Clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* species were obtained from 4 patients with recurrent UTI. Cytotoxicity analysis was performed on HeLa cells using the CytoTox 96 kit. Bacterial flagellar motility was assessed in 0.25% agar by measuring the zone of motility. Biofilms were cultivated in 96-well plates and stained with crystal violet and Congo red. Biofilm structure was assessed by microscopy, and the bacterial biofilm and exopolysaccharide matrix were quantified by measuring the optical density of the dye bound to the biofilm. Intracellular development was studied in PC-3 cells by determining the number of intracellular bacteria using a cell lysate plating method and an immunochemical method involving staining the bacteria with specific antibodies.

Results. The Fluorothiazinone antibacterial specifically inhibited the cytotoxicity and motility of *E. coli* and *P. aeruginosa*. For *K. pneumoniae*, susceptibility to Fluorothiazinone was observed, associated with the suppression of cytotoxicity. For all the isolates studied, inhibition of biofilm formation on an abiotic surface was demonstrated. For *E. coli* and *K. pneumoniae*, a significant decrease in intracellular replication within human prostate adenocarcinoma cells and suppression of intracellular bacterial community formation were observed.

Conclusion. Fluorothiazinone disrupts mechanisms that contribute to pathogen persistence in the formation of chronic UTI, including biofilm formation on abiotic surfaces such as catheters, stents, and drains, and the formation of intracellular bacterial communities and dormant intracellular reservoirs.

Keywords: Fluorothiazinone, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella*, antibiotics, infectious diseases, intracellular bacterial communities, urinary tract infections, biofilms, flagellum, cytotoxicity

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol of a clinical trial No. 07-FT-2024, RCT No. 513 dated 10/30/2024.

Funding source. This study was not supported by any external sources of funding.

Conflict of interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

For citation: Kapotina L.N., Morgunova E.Yu., Nelyubina S.A., Fedina E.D., Koroleva E.A., Bondareva N.E., Perepanova T.S., Zigangirova N.A. Suppression of biofilm formation and survival of clinical isolates of uropathogens within epithelial cells under the effect of Fluorothiazinone *in vitro*. *Journal of microbiology, epidemiology and immunobiology*. 2025;102(5):547–559.

DOI: <https://doi.org/10.36233/0372-9311-723>

EDN: <https://www.elibrary.ru/KFAYZJ>

Оригинальное исследование
<https://doi.org/10.36233/0372-9311-723>

Подавление образования биоплёнок и выживания в эпителиальных клетках клинических изолятов уропатогенов при действии препарата Fluorothiazinone *in vitro*

Капотина Л.Н.¹, Моргунова Е.Ю.¹, Нелюбина С.А.¹, Федина Е.Д.¹,
Королёва Е.А.¹, Бондарева Н.Е.¹, Перепанова Т.С.², Зигангирова Н.А.^{1✉}

¹Национальный исследовательский центр эпидемиологии и микробиологии имени почетного академика Н.Ф. Гамалеи, Москва, Россия;

²Научно-исследовательский институт урологии и интервенционной радиологии им. Н.А. Лопаткина — филиал НМИЦ радиологии, Москва, Россия

Аннотация

Целью данного исследования было изучение влияния инновационного отечественного антибактериального препарата Fluorothiazinone, ингибитора системы секреции III типа и функционирования жгутика, на вирулентные свойства возбудителей инфекций мочевыводящих путей (ИМП).

Материалы и методы. Клинические изоляты видов *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* были получены от 4 пациентов с рецидивирующими инфекциями ИМП. Анализ цитотоксичности проводили на клетках HeLa с помощью набора «CytoTox 96». Жгутиковую подвижность бактерий оценивали в 0,25% агаре с измерением зоны подвижности. Биоплёнки культивировали в 96-луночных планшетах и окрашивали кристаллическим фиолетовым и Конго красным, оценивали структуру биоплёнок при микроскопировании и проводили количественную оценку бактериальной биоплёнки и экзополисахаридного матрикса при измерении оптической плотности связавшегося с биоплёнкой красителя. Внутриклеточное развитие изучали на клетках РС-3 при определении количества внутриклеточных бактерий методом высева лизатов клеток и иммунохимическим методом при окраске бактерий специфическими антителами.

Результаты. Препарат Fluorothiazinone специфически подавлял цитотоксичность и подвижность *E. coli* и *P. aeruginosa*. Для *K. pneumoniae* наблюдали чувствительность к препарату Fluorothiazinone, связанную с подавлением цитотоксичности. Для всех изученных изолятов было показано ингибирование образования биоплёнок на абиотической поверхности. Для *E. coli* и *K. pneumoniae* выявили достоверное снижение внутриклеточного размножения в клетках аденокарциномы простаты человека и подавление формирования внутриклеточных бактериальных сообществ.

Заключение. Препарат Fluorothiazinone нарушает механизмы, способствующие установлению персистенции патогенов при формировании хронических ИМП, среди которых образование биоплёнок на абиотических поверхностях, таких как катетеры, стенты, дренажи, и формирование внутриклеточных бактериальных сообществ и покоящихся внутриклеточных резервуаров.

Ключевые слова: Fluorothiazinone, кишечная палочка, синегнойная палочка, клебсиелла, антибиотики, инфекционные заболевания, внутриклеточные бактериальные сообщества, инфекции мочевыводящих путей, биоплёнки, жгутик, цитотоксичность

Этическое утверждение. Исследование проводилось при добровольном информированном письменном согласии пациентов. Протокол клинического исследования № 07-ФТ-2024, РКИ № 513 от 30.10.2024.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Для цитирования: Капотина Л.Н., Моргунова Е.Ю., Нелюбина С.А., Федина Е.Д., Королёва Е.А., Бондарева Н.Е., Перепанова Т.С., Зигангирова Н.А. Подавление образования биоплёнок и выживания в эпителиальных клетках клинических изолятов уропатогенов при действии препарата Fluorothiazinone *in vitro*. Журнал микробиологии, эпидемиологии и иммунобиологии. 2025;102(5):547–559.
DOI: <https://doi.org/10.36233/0372-9311-723>
EDN: <https://www.elibrary.ru/KFAYZJ>

Introduction

Recurrent urinary tract infections (UTIs) are characterized by the reappearance of an acute bacterial infection caused by the same pathogen. Recurrent UTIs (rUTIs) are typically defined as 2 or more episodes of uncomplicated UTIs in the past 6 months or 3 or more episodes in the past 12 months [1–4]. Every year, 5–10% of adult women suffer from UTIs [5], which affects treatment costs and reduces quality of life, especially considering that more than 60% of women are diagnosed with a UTI at least once in their lifetime [6, 7]. The pathogen of most UTIs is uropathogenic *Escherichia coli* (UPEC) [8–10]. However, *Klebsiella pneumoniae* is increasingly becoming the second most common etiological agent of rUTIs [11, 12]. This gram-negative bacterium has become a common pathogen responsible for both community-acquired and nosocomial UTIs, particularly in individuals with risk factors for infection, weakened immune systems, and chronic comorbidities [13–15].

The pathogens of UTIs are characterized by a rather complex mechanism of infection development in the bladder, where bacteria, after adhering to the bladder mucosa receptors, cause an inflammatory response and penetrate the urothelium, forming intracellular bacterial communities (IBC) [16, 17]. Intracellular UPEC undergo several stages in their development: invasion, IBC formation, IBC maturation and the formation of dormant intracellular reservoirs. IBC is a transient bacterial state that persists until the bacteria multiply and exit the host cells. Quiescent intracellular reservoirs are formed in the deeper layers of the urothelium. Bacteria, capable of forming biofilms and dormant intracellular reservoirs can evade phagocytosis, survive within host cells while protected from antibiotics and immune factors. They form reservoirs of persistent pathogens in the urinary tract and periodically cause UTIs.

One significant risk factor in the development of rUTIs is bladder catheterization. Catheter-associated infections account for up to 40% of all nosocomial infections [18]. The risk of infection with short-term catheterization (up to 7 days) is up to 10% and increases by 3–7% each day the catheterization continues [19]. With prolonged catheterization (over 30 days), bacteriuria develops in almost 100% of patients [18]. In patients with catheter-associated UTIs, the formation of biofilms on the surfaces of catheters, stents, drains, etc. is a factor in the development of urosepsis, leading to significant complications in the course of the infection and treatment inefficacy [20].

UTIs are also largely associated with the rise of antibiotic resistance in uropathogens. Antibiotics remain the cornerstone of treatment and prevention of UTIs—approximately 25% of antibiotic prescriptions are issued for UTIs [21]. Worldwide spread of antibiotics, prolonged antibiotics prophylaxis or frequent use of different antibiotic combinations in patients with rUTIs

can contribute to the development of further resistance. The accumulation of resistant forms of bacteria can develop within a few weeks. For example, *E. coli* resistant to at least 4 antibiotics was identified in fecal isolates from over 90% of patients who received prophylactic treatment with low-dose antibiotics for 2 weeks [22].

Identifying the characteristics of the complex interaction between uropathogens and the host organism is necessary for both a deeper understanding of the pathogenesis of UTIs and for selecting new treatment approaches for these widespread bacterial infections.

The aim of the study was to investigate the effect of the innovative Russian antibacterial drug Fluorothiazinone (FT; N.F. Gamaleya Research Institute of Epidemiology and Microbiology, RU LP-No. (005077)-(RG-RU)) on the virulence properties of isolates obtained from the urine of patients with chronic UTI.

Materials and methods

Bacterial isolates

Clinical isolates of *K. pneumoniae* 5811, 5672, *P. aeruginosa* 5595, and *E. coli* 5424/1 were isolated from the urine of 4 adult patients of both sexes from different age groups with clinical signs of UTI who were hospitalized at the N.A. Lopatkin Research Institute of Urology and Interventional Radiology, a branch of the National Medical Research Center of Radiology of the Ministry of Health of Russia. The study was conducted with the informed consent of the patients. Urine isolates were collected as part of a clinical trial according to Protocol 07-FT-2024, RCT No. 513 dated 10/30/2024.

Bacteria culturing

The bacterial species were confirmed by streak plate method on solid media: LB agar (BD Difco), cefrimide agar (“Himedia”), followed by analysis of the protein profile of single colonies using the Bruker MALDI Biotyper instrument (Beckman Colter Diagnostics).

Clinical uropathogenic isolates were grown for 18 hours at 37°C in Luria–Bertani broth (LB broth) with continuous aeration on a shaker (240 rpm) to a concentration of 10^9 microbial cells/mL, which was assessed using a McFarland standard on a Densi-La-Meter II densitometer (ERBA Lachema). The resulting bacterial suspension was diluted to the desired concentrations in fresh LB broth.

Study drug

The active pharmaceutical substance FT was used in the study.

To study the effect of FT on motility and biofilm formation by clinical isolates, a stock solution with a concentration of 2 mg/mL in 0.3 M CH_3COONa buffer, pH 7.0 ± 0.2 , was used. The solution was prepared as follows: 0.021 g of the substance was added to a solu-

tion of 0.120 g of sodium hydroxide in 10 mL of distilled water. The mixture was then stirred on a magnetic stirrer until the FT crystals were completely dissolved, and subsequently acidified with glacial acetic acid to a pH of 7–8. The resulting solution was filtered through a 0.22 µm syringe filter into a sterile tube. In the tests, the antibacterial drug was used at a final concentration of 100 µg/mL.

To study the cytotoxicity and intracellular survival of the bacteria, a solution of FT in dimethyl sulfoxide at a concentration of 10 mg/mL was used, with a final concentration of FT in the medium of 20 µg/mL.

Cytotoxicity analysis

Cytotoxicity analysis was performed according to the method [23] with some modifications. HeLa cells (cervical carcinoma cells, ATCC CCL-2™) grown in IMDM culture medium (BioloT) with 10% fetal bovine serum (HyClone) without the addition of antibiotics were infected with a bacterial culture at a multiplicity of infection (MOI) of 10 and 50, with 1×10^5 cells per well. FT at a concentration of 20 µg/mL was added immediately after the bacterial culture was introduced and incubated at 37°C and 5% CO₂ for 20 hours. The plates were centrifuged for 20 minutes at 1500 rpm.

The activity of released lactate dehydrogenase (LDH) was determined in the supernatants using the CytoTox 96 non-radioactive cytotoxicity assay kit (Promega). LDH is a cytosolic enzyme present in nucleated cells that is released into the extracellular environment after the cell membrane is damaged. The percentage of LDH release was calculated relative to an uninfected control (0% LDH release) and HeLa cells lysed with Triton X-100 (100% LDH release). LDH in bacterial cultures in the medium without a cell monolayer was not detected.

Swimming motility

To assess swimming motility, cultures were grown for 18 hours at 37°C with aeration on a shaker (240 rpm), diluted 100-fold in LB broth, and cultured for 3 hours under the conditions described above. 2 µL were inoculated into the depth of 0.25% agar containing 1% tryptone and 0.5% NaCl in 90 mm diameter Petri dishes. They were observed for 48 hours. Bacterial motility was assessed by measuring the diameter of the bacterial migration zone from the center of the plate. These values served as positive controls when assessing bacterial motility.

To assess the effect of FT on the motility of clinical isolates, an FT solution in acetate buffer at a concentration of 100 µg/mL was used. For this purpose, the solution of FT was added to the prepared and cooled to 45–50°C 0.25% agar, mixed thoroughly, and poured into Petri dishes. After the agar solidified, the bacterial culture was introduced into the depth of the agar in a volume of 2 µL. The samples were cultured at 30°C in a humid chamber for 48 hours. The effect of FT was

evaluated by measuring the diameter of the bacterial movement zone. Isolates with a zone of motility less than 10 mm after 48 hours were classified as non-motile, while those with a zone greater than 10 mm were classified as motile. Acetate buffer without FT was added to the control cups.

Biofilm formation

The ability of the isolates to form biofilms was studied using a modified method by G.A. O'Toole *et al.* [24]. Biofilm cultivation was performed in TC-treated 96-well polystyrene microplates (Corning 3599). Bacterial cultures were grown in LB broth at 37°C overnight on a shaker at 240 rpm. Overnight cultures were grown to $\approx 10^7$ CFU/mL in sterile LB broth supplemented with an FT solution in acetate buffer at a final concentration of 100 µg/mL, and 200 µL were added to each well of the plate. The drug was not added to the wells selected as a positive control. Sterile LB broth was added to the wells as a negative control.

The plates were incubated statically at 37°C for 48 hours. After incubation, the contents of the wells were aspirated and washed three times with 200 µL of sterile phosphate-buffered saline, air-dried, and fixed with 96% ethanol for 15 minutes. The ethanol was then removed and the wells were dried.

Staining was performed using two methods: for 15 minutes with 0.1% crystal violet (Merck) or for 30 minutes with 0.1% Congo red (Merck). After the specified time, the dye was aspirated, the wells were washed with distilled water and air-dried. For quantitative analysis, the dyes bound to the biofilm were extracted with 200 µL of 96% ethanol, and the extract was transferred to the wells of a clean plate.

Optical density was measured using a Multiskan EX spectrophotometer (Thermo Scientific) at a wavelength of 540 nm for crystal violet dye and 492 nm for Congo red. To study the biofilm structure, staining was performed similarly; the wells were washed and thoroughly air-dried, then the plates were examined under a light microscope (Nikon Eclipse 50i, Nikon) at 200× magnification. For each isolate, 3 parallel experiments were performed and each were repeated thrice.

Internalization and survival of bacteria in PC-3 cells

The study used the PC-3 cell line (human prostate adenocarcinoma cells, ATCC CRL-1435). The cells were incubated in RPMI-1640 cell culture medium (BioLot) supplemented with 10% fetal bovine serum (HyClone) without the addition of antibiotics, at 37°C with 5% CO₂ for 24 hours. Cell counts were determined using a Scepter 2.0 cell counter (Millipore).

Clinical isolates were grown for 18 hours at 37°C in LB broth with continuous aeration on a shaker (240 rpm) to a concentration of 10^9 microbial cells/mL.

A monolayer of PC-3 cells, grown in a 24-well culture plate with sterile 12 mm diameter round cov-

erslips, was infected with a bacterial culture. Infection doses for the intracellular model were selected based on the cytotoxicity results obtained for the clinical isolates.

To assess cell invasion, cells were incubated for 1 hour, then washed three times with medium to remove unattached bacteria, and incubated for 1 hour at 37°C and 0.5% CO₂ in medium containing gentamicin (Gibco) to kill extracellular bacteria. The concentration of gentamicin was determined by the minimum inhibitory concentration (MIC) for each isolate. The cells were washed three times to remove gentamicin. To determine the number of bacteria, PC-3 cells infected with bacterial cultures were lysed with 0.25% Triton X-100 (Sigma) for 10 minutes at room temperature. Uninfected PC-3 cells were used as a negative control. Next, the lysates were plated on cetrimide or LB agar (depending on the species of the isolate being studied) using the serial dilution method. The number of intracellular bacteria was expressed in CFU/mL. The average value was calculated from the bacterial population of 2 wells in 3 experiments.

To assess the onset of intracellular reproduction, the cell monolayer was cultured for 4 hours, and all procedures for removing extracellular bacteria and reseeded were performed as described above.

Intracellular survival of bacteria in PC-3 cells was assessed 24 hours after infection with the bacterial culture. After this, 4 hours after infection, the cells were incubated with gentamicin for 1 hour to eliminate extracellular bacteria. The plate was then washed three times to remove the gentamicin, and fresh nutrient medium with a maintenance concentration of gentamicin was added. All procedures for removing extracellular bacteria and culturing were performed similarly to those described above.

To study the effect of FT, a solution of FT in dimethyl sulfoxide was added to the culture medium at a concentration of 20 µg/mL at different stages of the experiment: 1 hour before infection, at the moment of cell infection, 2 and 5 hours after infection and washing off gentamicin, followed by incubation in the presence of FT for 24 hours from the moment of infection.

For microscopic analysis, cells were fixed with acetone and stained with mouse polyclonal antibodies (antibodies were obtained for each isolate) for 30 minutes at 37°C and 5% CO₂ and with anti-mouse IgG-FITC conjugate ("Merck") for 30 minutes at 37°C and 5% CO₂. Samples were analyzed using a Nikon Eclipse 50i fluorescence microscope (Nikon) at 1000× magnification.

Statistical analysis

Statistical processing and visualization of the analysis results for *in vitro* bacterial studies were performed using GraphPad Prism v. 8.4.3 (GraphPad Software). The data from the experimental and control groups

were compared using the Student's t-test. The criterion for statistical significance of the difference in the data obtained was considered to be $p < 0.05$. All experiments were conducted in triplicate.

Results

Characteristics of clinical isolates

Antibiotic susceptibility analysis of the studied clinical isolates was performed in the clinical diagnostic laboratory of the N.A. Lopatkin Research Institute of Urology and Interventional Radiology, a branch of the National Medical Research Center of Radiology of the Ministry of Health of Russia, and the results are presented in the **Table**.

FT does not affect the viability of clinical isolates

The effect of FT on bacterial viability was assessed by culturing in nutrient LB broth in the presence of FT at concentrations of 60, 80 and 100 µg/mL for 24 hours with continuous aeration on a shaker (240 rpm) and determining CFU by the streak/spread plate method. According to the mechanism of action, and as previously shown for bacteria of different species, FT did not affect the viability of the studied clinical isolates.

FT suppresses the cytotoxicity of clinical isolates

Both *K. pneumoniae* isolates were characterized by significant cytotoxicity. For *K. pneumoniae* 5811 isolate, cytotoxicity at MOI₁₀ was 65%, and at MOI₅₀, it was 78%. For *K. pneumoniae* 5672 isolate, cytotoxicity at an MOI of 10 was 70%, and at an MOI of 50, it was 76%.

The cytotoxicity of *P. aeruginosa* 5595 and *E. coli* 5424/1 on the HeLa cell line was less pronounced. At MOI₁₀, the cytotoxicity of the *P. aeruginosa* 5595 isolate was 50%, and at MOI₅₀, it was 61%. For *E. coli* isolate 5424/1, 38 and 60% at MOI₁₀ and MOI₅₀, respectively.

In the presence of FT at a concentration of 20 µg/mL, cytotoxicity after 20 hours of culturing was significantly reduced, by 1.5–2.0 times at MOI₁₀ and MOI₅₀ for all 4 clinical isolates.

FT suppresses swimming motility

In a semi-liquid medium, bacterial cells are capable of swimming motility, moving by means of flagella. *K. pneumoniae* belongs to bacteria that do not have swimming motility via flagella. In light of this, the effect of FT on swimming activity was evaluated for 2 uropathogenic isolates: *P. aeruginosa* 5595 and *E. coli* 5424/1.

It has been established that both clinical isolates exhibit swimming motility. In the presence of FT at a concentration of 100 µg/mL in the culture medium, a pronounced suppression of motility associated with flagellar activity was observed (**Fig. 1**).

MIC values of antibiotics in relation to the tested clinical isolates

Antibiotic susceptibility testing for isolated microorganisms	<i>E. coli</i> 5424/1		<i>P. aeruginosa</i> 5595		<i>K. pneumoniae</i> 5811		<i>K. pneumoniae</i> 5672	
	quality result	MIC, mg/L	quality result	MIC, mg/L	quality result	MIC, mg/L	quality result	MIC, mg/L
Amikacin	S	≤ 8	S		S	≤ 8	S	≤ 8
Amoxicillin/clavulanic acid	R		R		R			
Ampicillin	R	> 16	R		R	> 16	R	> 16
Ampicillin/sulbactam					R			
Gentamicin	R	128	R	16	R	512	S	≤ 2
Imipenem	S	≤ 0.25	S		R	> 8	S	≤ 0.5
Colistin					R			
Levofloxacin			S		R	> 2	R	> 4
Meropenem	S	≤ 0.125	S		R	> 8	I	8
Nitrofurantoin			R					
Piperacillin/tazobactam	S	≤ 4/4	S		R		S	≤ 4/4
Tigecycline	S	≤ 1	R					
Tobramycin	S	≤ 2	S				S	≤ 2
Trimethoprim/sulfamethoxazole	S	≤ 2/38			I		S	≤ 2/38
Fosfomycin with glucose-6-phosphate	S	≤ 16					S	≤ 16
Cefazolin	R	> 32	R		R	> 32	R	> 32
Cefepime			S		R	> 8		
Ceftazidime			R		R	> 8	R	> 16
Ceftriaxone	S	≤ 1	R		R	> 4	R	> 4
Cefuroxime					R	> 16		
Ciprofloxacin	S	≤ 0.25			R	> 1	R	> 1
Ertapenem	S	≤ 0.25	R		R	> 1	R	> 2

Note: S — susceptible; R — resistant; I — intermediate.

The diameter of the bacterial growth zone in semi-solid agar for *P. aeruginosa* 5595 after 48 hours was 65–67 mm, and in the presence of FT, it was 25–27 mm. For *E. coli* 5424/1 isolate, the diameter of the zone of motility after 48 hours was 35–37 mm; in the presence of FT, the zone decreased to 12–14 mm. In control dishes with acetate buffer without the addition of FT, motility was not inhibited. This demonstrated the suppression of flagellar motility by FT.

FT suppresses biofilm formation

The ability to form biofilms and the effect of FT on this process were evaluated for 4 isolates. The biofilm density was quantified by staining the bacteria with crystal violet. After 48 hours, *P. aeruginosa* 5595 formed an intense multi-layered mature biofilm, *K. pneumoniae* 5672 and *E. coli* 5424/1 isolates formed a dense monolayer with areas of multi-layered structure, and *K. pneumoniae* 5811 isolate showed the slowest biofilm formation rate: by 48 hours, a monolayer with the formation of microcolonies was observed.

Adding FT simultaneously with the introduction of the bacterial culture led to a significant reduction in

biofilm density and disruption of its structure. All isolates formed a sparse monolayer with a cellular structure on an abiotic surface under the influence of FT, but microcolony formation and biofilm maturation did not occur (Fig. 2).

When quantifying bacterial density in biofilms under the influence of phototherapy, a significant reduction in the number of bacteria within the biofilm was observed: the maximum suppression (by 85%) was for *K. pneumoniae* 5672, 79% for *E. coli* 5424/1, 75% for *P. aeruginosa* 5595, and 71% for *K. pneumoniae* 5811 (Fig. 2).

The formation of the biofilm exopolysaccharide matrix (EPM) was assessed by Congo Red staining. It was found that all the isolates studied are capable of forming EPM, but the *K. pneumoniae* 5811 biofilm was characterized by the lowest EPM intensity. Under the effect of FT, all isolates lost their ability to synthesize EPM (Fig. 3).

Quantitative assessment showed a significant decrease in biofilm formation for all isolates: *K. pneumoniae* 5811 — 76%, *K. pneumoniae* 5672 — 74%, *P. aeruginosa* 5595 — 70%, *E. coli* 5424/1 — 67%

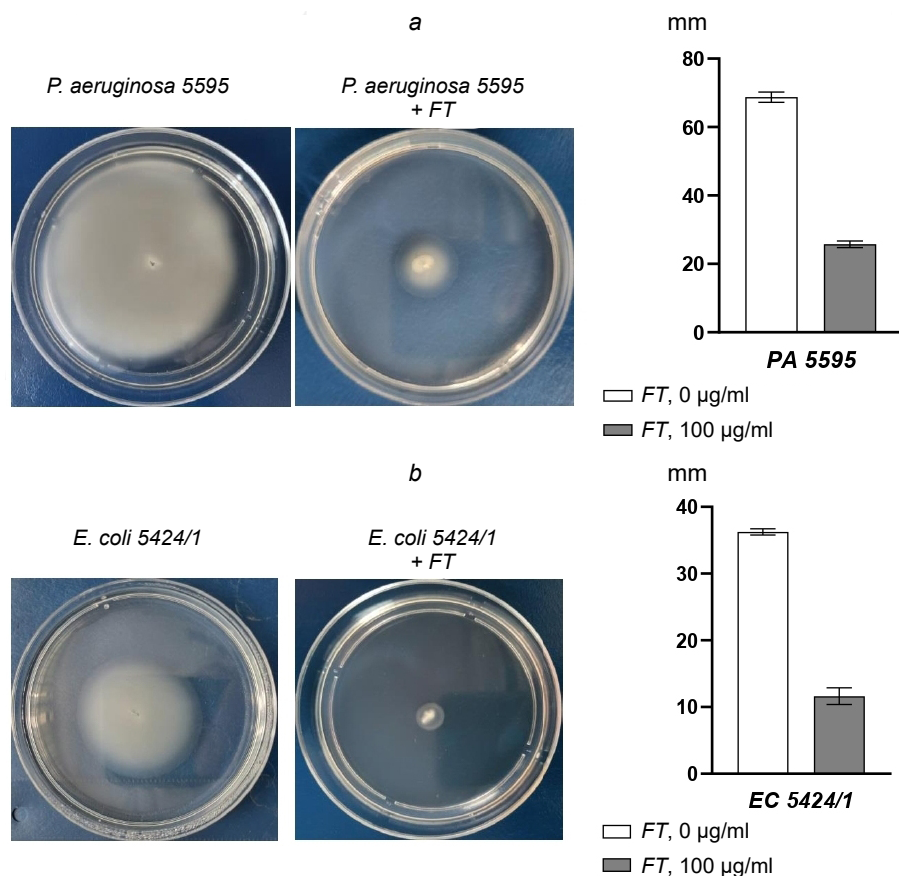


Fig. 1. Suppression of swimming motility of isolates after 48 hours of incubation in the presence of FT. Representative images of swimming motility on Petri dishes and assessment of the change in the diameter of the zone of motility of *P. aeruginosa* 5595 (a) and *E. coli* 5424/1 (b) in the presence of FT at a concentration of 100 µg/mL. Error bars represent the SD of 2 replicates.

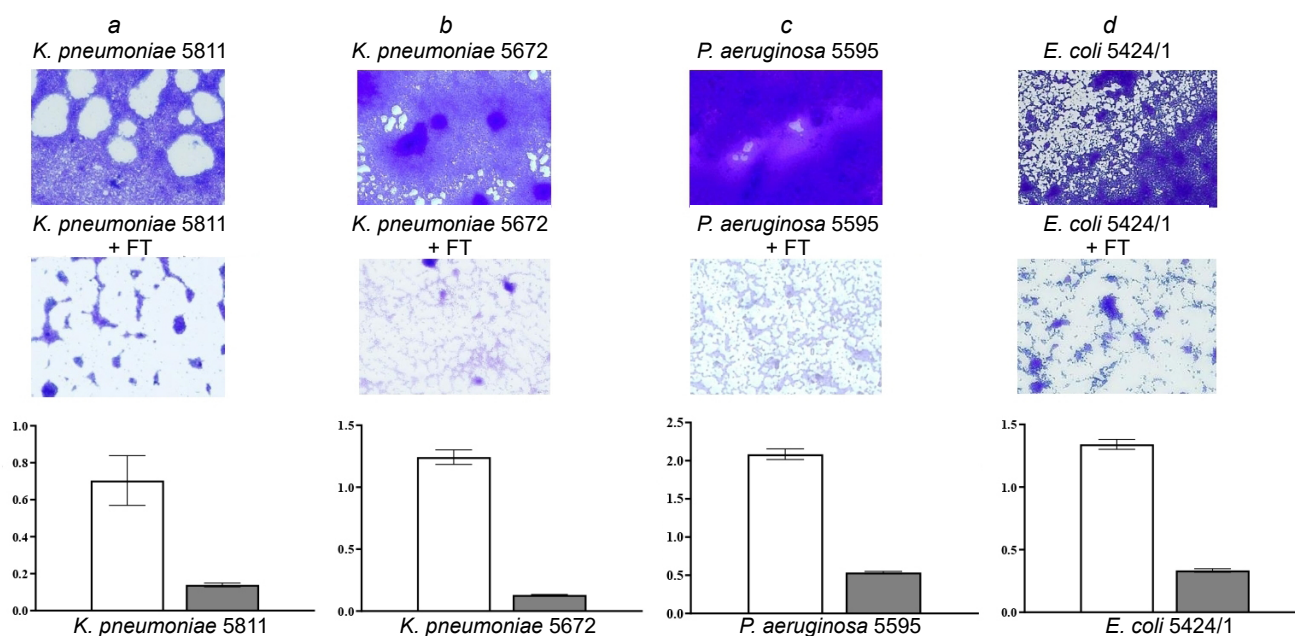


Fig. 2. FT alters the structure and biomass of biofilms formed by isolates *K. pneumoniae* 5811 (a), *K. pneumoniae* 5672 (b), *P. aeruginosa* 5595 (c) and *E. coli* 5424/1 (d).

Microphotographs of formed biofilm fragments from control and experimental samples with the addition of FT at a concentration of 100 µg/mL after 48 hours of incubation are presented. Biofilm biomass accumulation was assessed by staining with crystal violet at an optical density of 540 nm.

(Fig. 3). The solvent without FT had no effect on biofilm formation.

Thus, all the isolates studied are capable of formation of a biofilm on an abiotic surface, and FT inhibited the formation of a mature biofilm.

FT suppresses the invasion and intracellular replication of uropathogens

The ability of the studied uropathogenic isolates to form biofilms was assessed using the PC-3 cell line model (human prostate adenocarcinoma cells) by infecting the cells with the isolates and observing them during the invasion and intracellular replication stages at 4 and 24 hours. To assess the effect of FT on these processes, the drug was introduced 1 hour before infection, simultaneously with infection, and also after the medium was changed (at 2 and 5 hours).

The number of viable intracellular bacteria was assessed by culturing dilutions of the cell lysate after removing extracellular bacteria by gentamicin treatment for 1 hour. Simultaneously, the cell monolayer was analyzed by microscopy after staining with specific antibacterial antibodies.

For the *E. coli* 5424/1 isolate, an inoculum of 1.5×10^6 CFU/mL was used for infection, which resulted in MOI_{55} . After 1 hour, the invasion rate was 0.47%, while under the effect of FT, it was 0.053%. After 4 hours, the number of intracellular bacteria in the control group was 2.1×10^5 CFU/mL, while in the presence of FT, it

was an order of magnitude lower, at 1.1×10^4 CFU/mL. After 24 hours, an increase in the number of *E. coli* 5424/1 was observed to 2.4×10^6 CFU/mL in the control samples, while bacterial growth was significantly reduced in the presence of FT to 7.5×10^2 CFU/mL.

In the micrographs of the infected cell monolayer in the control samples, intracellular cell conglomerates were detected as early as 4 hours, which formed typical IBC by 24 hours. In the presence of FT, IBC were practically not identified (Fig. 4).

For the *K. pneumoniae* 5811 isolate, IBC formation was also observed, and it was suppressed in the presence of FT. This isolate showed lower rate of invasion, which was 0.005% for the control, while almost similar rate of invasion was observed under FT treatment. This isolate showed lower rate of invasion, which was 0.005% for the control and similar for the FT treatment group. (бы я исправила) This isolate showed to have effective intracellular replication: after 4 hours, the number of intracellular bacteria was 5×10^5 CFU/mL, and after 24 hours, it was 2.0×10^6 CFU/mL. Under the effect of FT, bacterial replication? was suppressed — 5.9×10^2 CFU/mL after 24 hours. The inhibition of IBC formation in the presence of FT is clearly demonstrated in the microphotographs (Fig. 5).

Discussion

The diagnosis of UTIs, which can mean both infectious disease and asymptomatic bacteriuria, is some-

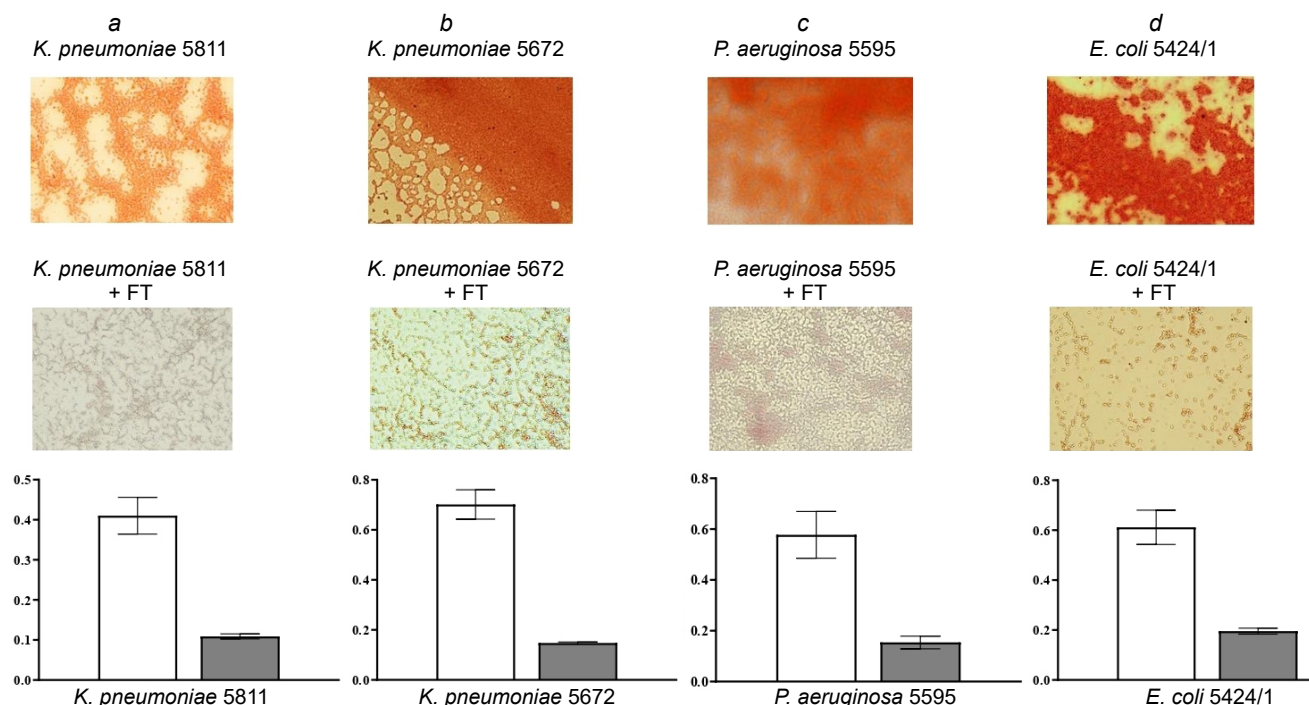


Fig. 3. FT inhibits the production of EPM biofilm by *K. pneumoniae* 5811 (a), *K. pneumoniae* 5672 (b), *P. aeruginosa* 5595 (c), and *E. coli* 5424/1 (d).

Microphotographs of biofilm fragments from control and experimental samples with the addition of FT at a concentration of 100 µg/mL are presented after 48 hours of incubation. EPM biofilm products were evaluated by the degree of Congo Red staining of the matrix at an optical density of 492 nm.

times difficult to establish, especially in recurrent cases, due to complex mechanism of interaction between uropathogens and tissues of the urinary tract and the body's immune system. International guidelines recommend relying solely on clinical symptoms of the disease [1]. Although UTIs are caused by pathogens from various taxonomic groups, and most of these bacteria are extracellular, they nevertheless utilize a common strategy for developing an infectious process: effective adhesion, invasion of epithelial cells, and the formation of pathogen reservoirs in the form of biofilms, dormant intracellular reservoirs, or surface biofilms (on catheters, foreign bodies). This strategy allows them to evade the action of antibiotics and host immunity factors, which determines clinical and epidemiological relevance of recurrent UTIs.

New approaches in treating such chronic infections should be based on understanding the pathogenesis mechanisms and on blocking of specific links of these mechanisms. In the current study, we evalu-

ated the effect of the active pharmaceutical substance, antibacterial drug FT, on the expression of virulent properties of clinical isolates of 3 different species: *K. pneumoniae*, *P. aeruginosa* and *E. coli* under *in vitro* conditions.

FT is an original domestic antibacterial drug that differs from antibiotics in its mechanism of action, as it does not kill the bacteria but suppresses their virulence. The specific inhibition of the type III secretion system (T3SS), which is responsible for the production of toxins that destroy immune cells and promote intracellular bacterial survival, has been demonstrated for this antibacterial drug. FT also inhibits bacterial flagellar motility and biofilm formation. Suppression of the virulence factors leads to the halting of infectious process in the host organism, as demonstrated in animal experiments [25–27]. FT therapy is effective against bacteria resistant to different classes of antibiotics because the target of action is present regardless of the presence of resistance determinants [28].

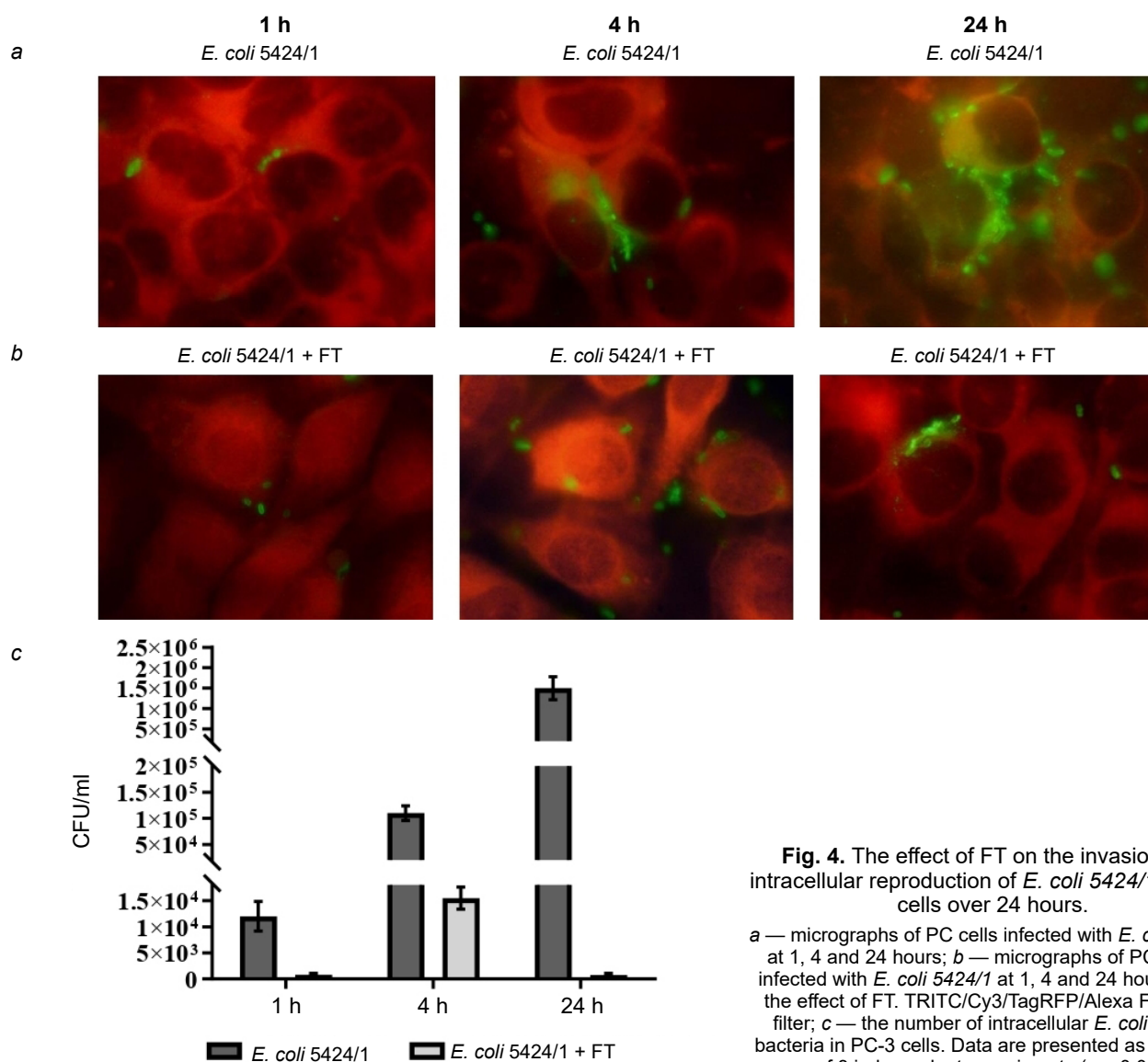


Fig. 4. The effect of FT on the invasion and intracellular reproduction of *E. coli* 5424/1 in PC-3 cells over 24 hours.

a — micrographs of PC cells infected with *E. coli* 5424/1 at 1, 4 and 24 hours; b — micrographs of PC-3 cells infected with *E. coli* 5424/1 at 1, 4 and 24 hours under the effect of FT. TRITC/Cy3/TagRFP/Alexa Fluor 546 filter; c — the number of intracellular *E. coli* 5424/1 bacteria in PC-3 cells. Data are presented as M ± SEM of 3 independent experiments ($p < 0.05$).

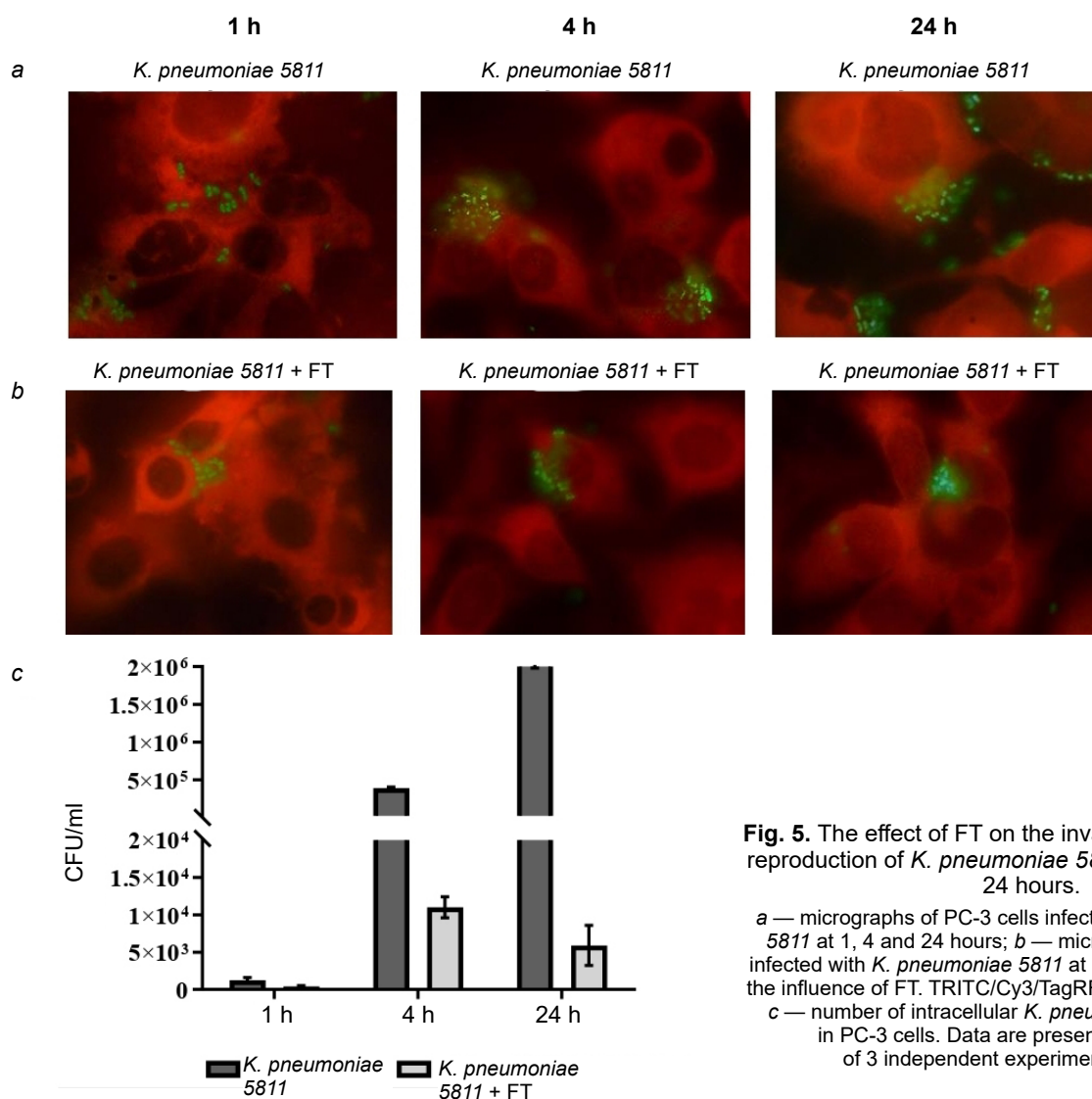


Fig. 5. The effect of FT on the invasion and intracellular reproduction of *K. pneumoniae* 5811 in PC-3 cells over 24 hours.

a — micrographs of PC-3 cells infected with *K. pneumoniae* 5811 at 1, 4 and 24 hours; b — micrographs of PC-3 cells infected with *K. pneumoniae* 5811 at 1, 4 and 24 hours under the influence of FT. TRITC/Cy3/TagRFP/Alexa Fluor 546 filter. c — number of intracellular *K. pneumoniae* 5811 bacteria in PC-3 cells. Data are presented as M ± SEM of 3 independent experiments ($p < 0.05$).

In clinical trials on patients with acute UTI, it was found that FT reduced the development of recurrences by 10 times at 2 and 3 months after the end of therapy compared to cefepime, which can be explained by the effective eradication of uropathogens from their reservoirs, including the intracellular ones [29].

It is known that *P. aeruginosa* and *E. coli* secrete cytotoxins directly into eukaryotic cells via the T3SS. In this regard, we evaluated the effect of FT on the cytotoxicity of these isolates. The presence of the T3SS is not demonstrated for *K. pneumoniae*, but a cytotoxic effect associated with the presence of the capsular polysaccharide has been described [30].

For all analyzed isolates that were characterized by significant toxicity toward epithelial cells, a statistically significant suppression of cell death was demonstrated in the presence of FT.

Along with the flagellum, the target of FT is the cilium, which has a similar structure at the base of the organelle. For motile isolates of *P. aeruginosa* and *E. coli*, almost complete blockage of flagellar function was observed, which was expressed by the absence of

a spreading zone in semi-solid agar. It should be noted that bacterial growth was not inhibited in the presence of FT, as shown in preliminary experiments.

The presence of surface structures in uropathogens, such as pili, fimbriae and flagella, facilitates adhesion to urothelial receptors as the first stage of bladder colonization, and thus they are an important target for suppression of infection development. One example of confirmation of the efficacy of this adhesion-blocking approach is the use of D-mannose in a clinical study, which showed a statistically significant reduction in the likelihood of recurrent UTIs [31].

Thus, the suppression of bacterial motility observed under the effect of FT, which is related to the function of flagella, is aimed at blocking of the first stage of infection development — the stage of urothelial colonization.

The key link in the development of a chronic infectious process with a recurrent course, caused by the long-term persistence of pathogens, is the bacteria's ability to actively invade urothelial cells and form intracellular biofilm-like communities.

The effect of FT on the 24-hour development cycle of *K. pneumoniae* and *E. coli* isolates in human prostate adenocarcinoma cells was evaluated. It was previously established that FT has high tissue availability due to its pharmacological properties, it penetrates cells, and retains activity in eukaryotic cells [32]. The introduction of FT into the culture medium significantly reduced the number of bacteria forming intracellular bacterial communities, as demonstrated by determining viable microorganisms and identifying intracellular structures under microscopy 24 hours after the pathogens came into contact with the cells.

Currently, the mechanisms by which FT suppresses intracellular bacterial replication are being studied. Given that FT does not have a direct antibacterial effect, the proposed mechanism involves blocking bacterial virulence factors responsible for interaction with the host cell during bacterial coexistence and persistence.

The ability of FT to suppress biofilms has been previously demonstrated for various pathogens, including carbapenem-resistant hospital-acquired infection agents. Studying the dynamics of biofilm formation *in vitro* and the effect of FT on this process showed that the FT blocks biofilm formation at the stage of microcolony aggregation and multilayer structure formation. Cultivation in the presence of FT has an inhibitory effect on the ability of isolates to form biofilms and, consequently, reduces the production of exopolysaccharides [26, 27]. The obtained results are of significant practical importance, as the EPM is a key factor in biofilm resistance to antibiotics and protection against the immune response.

Conclusion

In experiments conducted to study the effect of FT, the inhibitor of the type III secretion system and flagella, on the virulence properties of clinical isolates of three species of uropathogens under *in vitro* conditions, it was shown that FT suppresses key stages of bacterial persistence development in chronic infections, such as invasion of epithelial cells and the formation of pathogen reservoirs in the form of intracellular bacterial communities and surface biofilms. Properties of FT, such as biofilm suppression and intracellular replication inhibition, are a significant advantage of the innovative Russian antibacterial drug over currently used antibiotics.

СПИСОК ИСТОЧНИКОВ | REFERENCES

1. Bonkat G., Wagenlehner F., Kranz J. Keep it simple: a proposal for a new definition of uncomplicated and complicated urinary tract infections from the EAU urological infections guidelines panel. *Eur. Urol.* 2024;86(3):195–7.
DOI: <https://doi.org/10.1016/j.eururo.2024.05.007>
2. Bell B.G., Schellevis F., Stobberingh E., et al. A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance. *BMC Infect. Dis.* 2014;14:13.
DOI: <https://doi.org/10.1186/1471-2334-14-13>
3. Pigrau C., Escolà-Vergé L. Recurrent urinary tract infections: from pathogenesis to prevention. *Med. Clin. (Barc.)*. 2020; 155(4):171–7.
DOI: <https://doi.org/10.1016/j.medcli.2020.04.026>
4. WHO. Antimicrobial resistance: global report on surveillance;2014. <https://who.int/publications/i/item/9789241564748>
5. Flores-Mireles A.L., Walker J.N., Caparon M., Hultgren S.J. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat. Rev. Microbiol.* 2015;13(5):269–84.
DOI: <https://doi.org/10.1038/nrmicro3432>
6. Sabih A., Leslie S.W. *Complicated Urinary Tract Infections*. Treasure Island;2025.
7. Tandogdu Z., Wagenlehner F. Global epidemiology of urinary tract infections. *Curr. Opin. Infect. Dis.* 2016;29(1):73–9.
DOI: <https://doi.org/10.1097/QCO.0000000000000228>
8. Klein R.D., Hultgren S.J. Urinary tract infections: microbial pathogenesis, host–pathogen interactions and new treatment strategies. *Nat. Rev. Microbiol.* 2020;18(4):211–26.
DOI: <https://doi.org/10.1038/s41579-020-0324-0>
9. Палагин И.С., Сухорукова М.В., Дехнич А.В. и др. Антибиотикорезистентность возбудителей внебольничных инфекций мочевых путей в России: результаты многоцентрового исследования «ДАРМИС-2018». *Клиническая микробиология и антимикробная химиотерапия*. 2019;21(2):134–46. Palagin I.S., Sukhorukova M.V., Dekhnich A.V., et al. Antimicrobial resistance of pathogens causing community-acquired urinary tract infections in Russia: results of multicenter study «DARMIS-2018». *Clinical Microbiology and Antimicrobial Chemotherapy*. 2019;21(2):134–46.
DOI: <https://doi.org/10.36488/cmacc.2019.2.134-146>
EDN: <https://elibrary.ru/vdyqtb>
10. Mittal S., Sharma M., Chaudhary U. Biofilm and multidrug resistance in uropathogenic *Escherichia coli*. *Pathog. Glob. Health.* 2015;109(1):26–9.
DOI: <https://doi.org/10.1179/2047773215Y.0000000001>
11. Paczosa M.K., Mecsas J. *Klebsiella pneumoniae*: going on the offense with a strong defense. *Microbiol. Mol. Biol. Rev.* 2016;80(3):629–61.
DOI: <https://doi.org/10.1128/MMBR.00078-15>
12. Jalil M.B., Al Atbee M.Y.N. The prevalence of multiple drug resistance *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients with urinary tract infections. *J. Clin. Lab. Anal.* 2022;36(9):e24619. DOI: <https://doi.org/10.1002/jcla.24619>
13. Ashurst J.V., Dawson A. *Klebsiella Pneumonia*. Treasure Island;2023.
14. Clegg S., Murphy C.N. Epidemiology and virulence of *Klebsiella pneumoniae*. *Microbiol. Spectr.* 2016;4(1).
DOI: <https://doi.org/10.1128/microbiolspec.UTI-0005-2012>
15. Filev R., Lyubomirova M., Bogov B., et al. Urinary tract infections caused by *Klebsiella pneumoniae* and prolonged treatment with trimethoprim/sulfamethoxazole. *Microorganisms*. 2025;13(2):422.
DOI: <https://doi.org/10.3390/microorganisms13020422>
16. Andersen T.E., 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(5):1858–67.
DOI: <https://doi.org/10.1128/iai.06075-11>
17. Anderson G.G., Palermo J.J., Schilling J.D., et al. Intracellular bacterial biofilm-like pods in urinary tract infections. *Science*. 2003;301(5629):105–7.
DOI: <https://doi.org/10.1126/science.1084550>
18. Spaulding C.N., Hultgren S.J. Adhesive pili in UTI pathogenesis and drug development. *Pathogens*. 2016;5(1):30.
DOI: <https://doi.org/10.3390/pathogens5010030>
19. Oh W.S., Hur J.A., Kim E.S., et al. Factors associated with specific uropathogens in catheter-associated urinary tract infection: developing a clinical prediction model. *J. Int. Med. Res.*

- 2014;42(6):1335–47.
DOI: <https://doi.org/10.1177/0300060514543035>
20. Jiménez-Alcaide E., Medina-Polo J., García-González L., et al. Healthcare-associated urinary tract infections in patients with a urinary catheter: Risk factors, microbiological characteristics and patterns of antibiotic resistance. *Arch. Esp. Urol.* 2015;68(6):541–50. (in Spanish)
 21. Akkerman A.E., Kuyvenhoven M.M., Verheij T.J.M., van Dijk L. Antibiotics in Dutch general practice: nationwide electronic GP database and national reimbursement rates. *Pharmacoepidemiol. Drug Saf.* 2008;17(4):378–83.
DOI: <https://doi.org/10.1002/pds.1501>
 22. Maharjan G., Khadka P., Siddhi S.G., et al. Catheter-associated urinary tract infection and obstinate biofilm producers. *Can. J. Infect. Dis. Med. Microbiol.* 2018;2018:7624857.
DOI: <https://doi.org/10.1155/2018/7624857>
 23. Specian A.F.L., Serpeloni J.M., Tuttis K. LDH, proliferation curves and cell cycle analysis are the most suitable assays to identify and characterize new phytotherapeutic compounds. *Cytotechnology.* 2016;68(6):2729–44.
DOI: <https://doi.org/10.1007/s10616-016-9998-6>
 24. O'Toole G., Kaplan H.B., Kolter R. Biofilm formation as microbial development. *Annu. Rev. Microbiol.* 2000;54:49–79.
DOI: <https://doi.org/10.1146/annurev.micro.54.1.49>
 25. Zigangirova N.A., Nesterenko L.N., Sheremet A.B., et al. Fluorothiazinone, a small-molecular inhibitor of T3SS, suppresses salmonella oral infection in mice. *J. Antibiot.* 2021;74(4):244–54. DOI: <https://doi.org/10.1038/s41429-020-00396-w>
 26. Koroleva E.A., Soloveva A.V., Morgunova E.Y., et al. Fluorothiazinone inhibits the virulence factors of uropathogenic *Escherichia coli* involved in the development of urinary tract infection. *J. Antibiot. (Tokyo).* 2023;76(5):279–90.
DOI: <https://doi.org/10.1038/s41429-023-00602-5>
 27. Bondareva N.E., Soloveva A.V., Sheremet A.B., et al. Preventative treatment with Fluorothiazinone suppressed *Acinetobacter baumannii*-associated septicemia in mice. *J. Antibiot. (Tokyo).* 2022;75(3):155–63.
DOI: <https://doi.org/10.1038/s41429-022-00504-y>
 28. Savitskii M.V., Moskaleva N.E., Brito A., et al. Pharmacokinetics, tissue distribution, bioavailability and excretion of the anti-virulence drug Fluorothiazinone in rats and rabbits. *J. Antibiot. (Tokyo).* 2024;77(6):382–8.
DOI: <https://doi.org/10.1038/s41429-024-00719-1>
 29. Белобородов В.Б., Зигангирова Н.А., Лубенец Н.Л. и др. Влияние цефепима/фтортиазинона в сравнении с цефепимом/плацебо на клиническое излечение и микробиологическую эрадикацию у пациентов с осложнёнными инфекциями мочевыводящих путей: проспективное рандомизированное клиническое исследование. *Антибиотики и Химиотерапия.* 2024;(9-10):31–9. Beloborodov V.B., Zigangirova N.A., Lubenec N.L., et al. The effect of cefepime/fluorothiazinone compared with cefepime/placebo on clinical cure and microbiological eradication in patients with complicated urinary tract infections: a prospective randomized clinical trial. *Antibiotics and Chemotherapy.* 2024;(9-10):31–9.
DOI: <https://doi.org/10.37489/0235-2990-2024-69-9-10-31-39>
EDN: <https://elibrary.ru/AAHDQM>
 30. Cano V., Moranta D., Llobet-Brossa E., et al. *Klebsiella pneumoniae* triggers a cytotoxic effect on airway epithelial cells. *BMC Microbiol.* 2009;9:156.
DOI: <https://doi.org/10.1186/1471-2180-9-156>
 31. Parazzini F., Ricci E., Fedele F., et al. Systematic review of the effect of D-mannose with or without other drugs in the treatment of symptoms of urinary tract infections/cystitis (review). *Biomed. Rep.* 2022;17(2):69.
DOI: <https://doi.org/10.3892/br.2022.1552>
 32. Zigangirova N.A., Lubenec N.L., Beloborodov V.B., et al. A new «non-traditional» antibacterial drug fluorothiazinone – clinical research in patients with complicated urinary tract infections. *Antibiotics (Basel).* 2024;13(6):476.
DOI: <https://doi.org/10.3390/antibiotics13060476>

Information about the authors

Lydiya N. Kapotina — Cand. Sci. (Technics), senior researcher, Chlamydia department, National Research Center for Epidemiology and Microbiology named after N.F. Gamaleya, Moscow, Russia, lidiya.kapotina57@mail.ru, <https://orcid.org/0000-0003-0159-5053>

Elena Yu. Morgunova — researcher, Chlamydia department, National Research Center for Epidemiology and Microbiology named after N.F. Gamaleya, Moscow, Russia, lena.morgunova.1968@mail.ru, <https://orcid.org/0000-0003-3142-0983>

Stanislava A. Nelyubina — junior researcher, Chlamydia department, National Research Center for Epidemiology and Microbiology named after N.F. Gamaleya, Moscow, Russia, 19ctasy94@mail.ru, <https://orcid.org/0000-0002-5157-1415>

Elena D. Fedina — Cand. Sci. (Med.), senior researcher, Chlamydia department, National Research Center for Epidemiology and Microbiology named after N.F. Gamaleya, Moscow, Russia, e.fedina@mail.ru, <https://orcid.org/0000-0002-7205-4310>

Ekaterina A. Koroleva — Cand. Sci. (Biol.), senior researcher, Chlamydia department, National Research Center for Epidemiology and Microbiology named after N.F. Gamaleya, Moscow, Russia, korolevakate@yandex.ru, <https://orcid.org/0000-0001-9702-2940>

Nataliya E. Bondareva — Cand. Sci. (Med.), senior researcher, Chlamydia department, National Research Center for Epidemiology and Microbiology named after N.F. Gamaleya, Moscow, Russia, nataliia.d@mail.ru, <https://orcid.org/0000-0002-5244-2965>

Tamara S. Perepanova — Dr. Sci. (Med.), Professor, Head, Infectious and inflammatory diseases and clinical pharmacology group, N. Lopatkin Scientific Research Institute of Urology and Interventional Radiology — Branch of the National Medical Research Radiological Center, Moscow, Russia, perepanova2003@mail.ru, <https://orcid.org/0000-0002-2877-0029>

Naylia A. Zigangirova — Dr. Sci. (Biol.), Professor, RAS Corresponding member, Head, Department of medical microbiology, National Research Center for Epidemiology and Microbiology named after N.F. Gamaleya, Moscow, Russia, zigangirova@mail.ru, <https://orcid.org/0000-0003-3188-1608>

Author contribution: *Kapotina L.N.* — cytotoxicity analysis, assessment of swimming motility, biofilm formation, processing of results, text writing; *Morgunova E.Yu.* — study of invasion and intracellular development of pathogens, processing of results; *Nelyubina S.A.* — cultivation of isolates, processing of results, editing of the article; *Fedina E.D.*, *Koroleva E.A.*, *Bondareva N.I.*, *Perepanova T.S.* — editing the article; *Zigangirova N.A.* — concept and design of the study, text writing. All authors confirm that they meet the International Committee of Medical Journal Editors criteria for authorship, made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published.

The article was submitted 05.08.2025;
accepted for publication 14.10.2025;
published 31.10.2025

Информация об авторах

Капотина Лидия Николаевна — канд. техн. наук, с. н. с. лаб. хламидиозов НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, lidiya.kapotina57@mail.ru, <https://orcid.org/0000-0003-0159-5053>

Моргунова Елена Юрьевна — н. с. лаб. хламидиозов НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, lena.morgunova.1968@mail.ru, <https://orcid.org/0000-0003-3142-0983>

Нелюбина Станислава Андреевна — м. н. с. лаб. хламидиозов НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, 19ctasy94@mail.ru, <https://orcid.org/0000-0002-5157-1415>

Федина Елена Дмитриевна — канд. мед. наук, с. н. с. лаб. хламидиозов НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, e.fedina@mail.ru, <https://orcid.org/0000-0002-7205-4310>

Королёва Екатерина Андреевна — канд. биол. наук, с. н. с. лаб. хламидиозов НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, korolevakate@yandex.ru, <https://orcid.org/0000-0001-9702-2940>

Бондарева Наталия Евгеньевна — канд. мед. наук, с. н. с. лаб. хламидиозов НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, nataliia.d@mail.ru, <https://orcid.org/0000-0002-5244-2965>

Перепанова Тамара Сергеевна — д-р мед. наук, профессор, рук. группы инфекционно-воспалительных заболеваний и клинической фармакологии НИИ урологии и интервенционной радиологии им. Н.А. Лопаткина — филиала НМИЦ радиологии, Москва, Россия, perepanova2003@mail.ru, <https://orcid.org/0000-0002-2877-0029>

Зигангирова Наиля Ахатовна — д-р биол. наук, профессор, член-корр. РАН, зав. отделом медицинской микробиологии НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, zigangirova@mail.ru, <https://orcid.org/0000-0003-3188-1608>

Участие авторов: *Капотина Л.Н.* — анализ цитотоксичности, оценка плавательной подвижности, биоплёнкообразование, обработка результатов, написание текста; *Моргунова Е.Ю.* — изучение инвазии и внутриклеточного развития патогенов, обработка результатов; *Нелюбина С.А.* — культивирование изолятов, обработка результатов, редактирование статьи; *Федина Е.Д.*, *Королёва Е.А.*, *Бондарева Н.Е.*, *Перепанова Т.С.* — редактирование статьи; *Зигангирова Н.А.* — концепция и дизайн исследования, написание текста. Все авторы подтверждают соответствие своего авторства критериям Международного комитета редакторов медицинских журналов, внесли существенный вклад в проведение поисково-аналитической работы и подготовку статьи, прочли и одобрили финальную версию до публикации.

Статья поступила в редакцию 05.08.2025;
принята к публикации 14.10.2025;
опубликована 31.10.2025