Hindawi
Cellular Microbiology
Volume 2023, Article ID 3201540, 9 pages
https://doi.org/10.1155/2023/3201540

*Research Article*

# Shionone Relieves Urinary Tract Infections by Removing Bacteria from Bladder Epithelial Cells

**Hao Yin, Jiaoli Zhu, Yi Jiang, Yijing Mao, Chenquan Tang, Hui Cao, Yufang Huang, Huijun Zhu, Jianping Luo, Qingjiang Jin, Qinglei Jin, Yi Xue, and Xin Wang**

*Department of Nephrology, Suzhou Hospital of Integrated Traditional Chinese and Western Medicine, Suzhou, China*

Correspondence should be addressed to Yi Xue; xybft2012@163.com and Xin Wang; frogprince417@sina.com

Received 16 July 2022; Revised 18 December 2022; Accepted 23 January 2023; Published 3 February 2023

Academic Editor: Jayaprakash Kolla

Copyright © 2023 Hao Yin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

In clinical practice, urinary tract infections (UTIs) are second only to respiratory infections in terms of infectious diseases. In recent years, drug resistance of *Escherichia coli* (*E. coli*) has increased significantly. The therapeutic effects of Shionone on UTI were assessed by modelling UTI in SD rats and SV-HUC-1 cells with *E. coli* solution. After treatment of Shionone, the UTI rat model showed a decrease in wet weight/body weight of bladder, as well as a reduction in cellular inflammatory infiltration of bladder tissue and a decrease in urinary levels of IL-6, IL-1$\beta$, and TNF-$\alpha$. In addition, the levels of proinflammatory factors were significantly reduced in a dose-dependent manner in UTI cell model treated with different doses of Shionone (5, 10, and 20 $\mu$g/kg). The results of immunofluorescence analysis in both *in vivo* and *in vitro* experiments revealed that Shionone reduced bacterial load and the number of *E. coli* colonies growing on the plates was greatly reduced. These results suggested that Shionone has a good therapeutic effect on UTI, achieved by reducing bacterial load in bladder epithelial cells. The data presented here provide a basis for further research into the treatment of UTI.

## 1. Introduction

Urinary tract infection (UTI) is one of the most common infectious diseases and is an inflammatory response caused by bacterial invasion of the urinary tract epithelium [1]. It is significantly more common in women than men due to the anatomy of the urinary tract and other factors [2]. Recurrent episodes are a difficult part of the management of UTI, and studies showed that 30% of women will contract UTI and with a 20% recurrence rate within one year [3–5]. Invasion of bladder epithelial cells (BECs) by uropathogenic *Escherichia coli* (UPEC) is one of the main causes of recurrent UTI [6].

With a stressed bladder, the culprit of UTI, *E. coli*, may have retrograde infection to the kidneys, which can lead to irreversible kidney injury and cause physical and psychological harm to the patient [7]. UPEC is a Gram-negative bacterium, and routine clinical control is usually with intermittent or long-term antibiotic therapy [8]. However, the proportion of antibiotic-resistant strains is increasing and is necessary to develop new nonantibiotic alternative therapies.

Shionone is the major triterpenoid isolated from *Aster tataricus* and possesses a unique six-membered tetracyclic skeleton and 3-oxo-4-monomethyl structure [9]. Previous studies have reported that Shionone has antitussive, anti-inflammatory activities and could alleviate interstitial cystitis by inhibiting pyroptosis [9–11]. Compared with antibiotics, the natural ingredient has fewer side effects, does not cause microbial disorders, and has a lower economic cost [12]. Considering that UPEC is the main cause of UTI, we constructed an *in vivo* UTI model of SD rats and an *in vitro* UTI model of SV-HUC-1 cells all induced by UPEC [13] and demonstrated the effect of Shionone on UTI by examining pathological sections of bladders, bacterial detection in bladders, storage of *E. coli* in bladder tissues and cells, and detection of proinflammatory factors in urine and cell supernatants.

## 2. Materials and Methods

*2.1. Reagents.* Shionone was purchased from Chengdu Plant Standardization Pure Biotechnology Co. Rat IL-1$\beta$ ELISA Kit (PI303), Rat IL-6 ELISA Kit (PI328), and Rat TNF-$\alpha$ ELISA Kit (PT516) were purchased from Beyotime Biotechnology. Phalloidin Actin-Tracker Red-Rhodamine (C2207S) was obtained from Beyotime Biotechnology. Anti-*E. coli* antibody (ab137967) was purchased from Abcam.

*2.2. Preparation of the Bacterial Solution.* The preserved *E. coli* UT189 strain was inoculated into LB liquid medium and incubated on a constant shaker at 37°C for 18 h. The bacterial broth was then adjusted to a concentration of 1.5 $\times 10^7$ CFU/mL with sterile PBS.

*2.3. Animals and Experimental Protocol.* Healthy female SD rats (220 ± 10 g) were purchased from Jiangsu ALF Biotechnology Co. Ltd. Rats were housed at 25°C, received a 12 h/12 h-light/dark cycle under pathogen free conditions, and were allowed to eat and drink freely. Female rats were water fasted for 24 h and randomly divided into five groups of nine rats each: Saline group, UPEC group, 100-Shi (Shionone) group, 200-Shi group, and 100-LVX (levofloxacin hydrochloride, positive control) group. Levofloxacin is a quinolone antibiotic that is relatively effective against the common pathogens of urinary tract infections, such as Gram-negative rods and Gram-positive cocci [14]. After the rats were anesthetized and their perineum disinfected, the F4 ureteral catheter was passed through the urethra for about 3 cm to reach the bladder, the bladder was emptied of residual fluid, and the prepared bacterial solution (300 $\mu$L, $1 \times 10^7$ CFU/each rat) was pushed into the bladder, after which the urethra was clamped with a paper clip for 2-3 h and then released. The Saline group was injected with phosphate-buffered solution (PBS) in the same way. The 100-Shi and 200-Shi groups were gavaged with 100 mg/kg and 200 mg/kg of Shionone, respectively, the 100-LVX group was gavaged with 100 mg/kg of levofloxacin hydrochloride, and the Saline and UPEC groups were gavaged with an equal volume of saline. Gavage was administered 2 h before surgery, 2 h after surgery, and 24 h after surgery, respectively. Two hours after the last dose, urine was collected from the rats and bladder tissue was removed. All animal procedures were carried out in accordance with the Ethics Committee of Suzhou Integrated Traditional Chinese and Western Medicine Hospital (No. 2022DW010).

*2.4. Hematoxylin-Eosin (HE) Staining.* Bladder tissues were fixed in Bouin's fixative and rinsed under water for 30 min after successful fixation. The tissues were then dehydrated in various concentrations of alcohol, placed in xylene, and then embedded in wax and cut into 5 $\mu$m slices. After dewaxing, the nuclei were stained with hematoxylin for 2 min and the cytoplasm was stained with eosin for 1 min and finally dehydrated and placed under a microscope.

*2.5. Tissue Transmission Electron Microscopy Experiments.* The bladder tissue was cut into small pieces of about 1 mm$^3$ and fixed in a special 2.5% glutaraldehyde fixative for electron microscopy. After rinsing with phosphate buffer, the tissue was fixed in osmium acid for 2 hours and then dehydrated in an ethanol gradient, the sample was embedded by immersion and sectioned, and the sections were retrieved from the copper mesh, stained with uranyl acetate and washed, dried overnight, and placed under a transmission microscope for observation.

*2.6. Counting of Bacteria in Bladder Homogenates and Urine.* 0.1 g of bladder tissue was homogenized in 600 $\mu$L PBS; 10 $\mu$L was aspirated onto LB agar plates, incubated at 37°C for 18 h, and then counted [15]. Based on the actual growth of the bacteria, it was found that the bacteria grew too densely to be distinguished at that dilution, so another 10-fold dilution was made on top of that. Bacterial cultures in urine were counted according to the previous literature [16]. In brief, a 96-well plate was prepared by adding 180 $\mu$L of PBS to each well. 20 $\mu$L of raw urine was added to the first well and mixed; 20 $\mu$L of liquid from the first well was pipetted into the second well and diluted six times; then, 10 $\mu$L from the sixth well was applied to an LB agar plate and incubated at 37°C for 18 h.

*2.7. ELISA Assay for IL-1β, IL-6, and TNF-α.* According to the manufacturer's instructions, kits were kept at room temperature for 20 min after removal from the refrigerator to equilibrate. 50 $\mu$L of gradient diluted standards was added to the standard wells and 50 $\mu$L of sample to the sample wells; then, 100 $\mu$L of horseradish peroxidase-labelled antibody was added and incubated for 1 h at 37°C. The liquid was removed, and the plate was washed 5 times with 350 $\mu$L of washing solution. After adding the substrate and incubating for 15 min at 37°C protected from light, the stop solution was added and the absorbance was measured at $OD_{450}$. The content of the corresponding sample was calculated from the content of the standard.

*2.8. SV-HUC-1 Cell Culture.* Human bladder epithelial immortalized cells (SV-HUC-1) were used for the cell experiments. The cells were administered by Shionone at concentrations of 0.1, 1, 2.5, 5, 10, 20, 40, and 80 $\mu$g/mL for 24 h. The maximum nontoxic concentration of the drug was screened by MTT assay for cell viability, and three Shionone concentrations of 5, 10, and 20 $\mu$g/mL were set for subsequent experiments. SV-HUC-1 cells were inoculated into 24-well plates and grown to confluence in Ham's F-12 K containing 10% FBS and 1% penicillin/streptomycin, then infected with UTI89 at a multiplicity of infection of 100, and incubated at 37°C for 1 h [13].

*2.9. Bacterial Infection in SV-HUC-1 Cells. E. coli* UTI89 and SV-HUC-1 cells were mixed in a 10 : 1 ratio, washed 3 times with PBS after 2 h, added 100 $\mu$g/mL of gentamicin for 2 h and then replaced with medium of each concentration of drug (containing 10 $\mu$g/mL of gentamicin), and incubated for 24 h. The cells were fixed, and the bacterial reservoirs were detected by immunofluorescence. After the cells were fixed in immunostaining solution (P0098, Beyotime), they could be washed twice with immunostaining washing solution (P0106) for 5 min each time. After blocking with

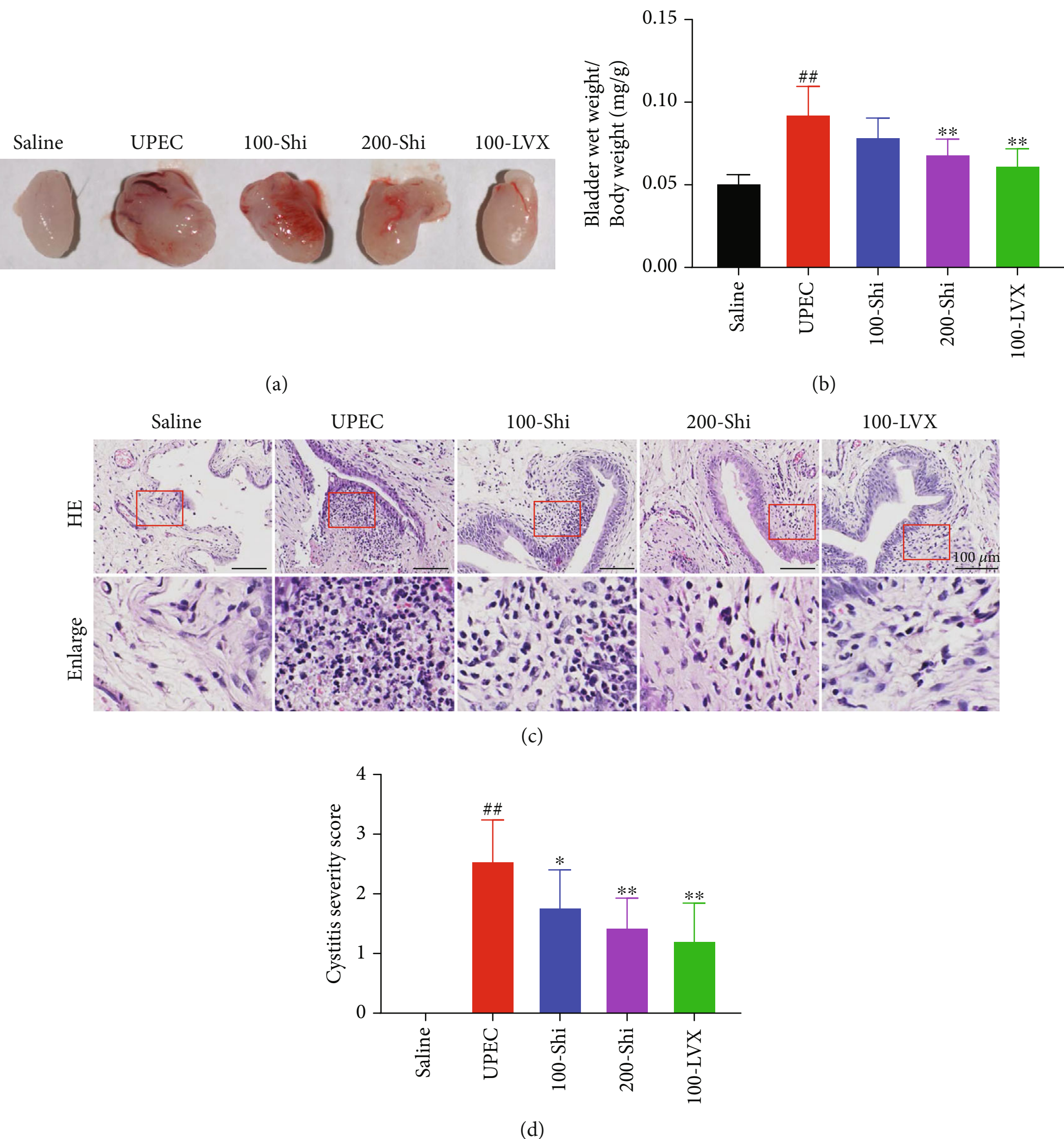


Figure 1: Shionone improves edema and congestion in the bladder of rats with acute urinary tract infection. (a) Status of the bladder in each group of rats. (b) Bladder wet weight/body weight in each group of rats. (c) Pathological analysis of HE staining of bladder tissues in each group. (d) Cystitis severity of bladder tissues in each group. Compared with the saline group, # means $p < 0.05$ and ## means $p < 0.01$. Compared with the UPEC group, * means $p < 0.05$ and ** means $p < 0.01$.

immunostaining blocking solution (P0102, Solarbio) for 60 min, the cells were incubated with E. coli antibody (E3500-26, United States Biological) at 37°C for 1 h at room temperature, and then, 1: 400 dilutions of HRP-conjugated antibodies were incubated for 20 min at room temperature. After reblocking, the cells were stained with phalloidin (AB176755, Abcam) and DAPI (AB104139, Abcam), respectively. The stained sections were observed under a fluorescence microscope, and the fluorescence intensity was quantified using ImageJ software.

*2.10. Testing the Effect of Shionone on the Ability of SV-HUC-1 Cells to Kill Bacteria.* After the cells were seeded into plate, UTI89 bacteria were added at 10 : 1 (bacteria : cells) and engulfed for 2 h. The cells were washed 3 times with PBS, and 100 $\mu$g/mL of gentamicin was added for 2 h to kill the bacteria, and then, the medium was replaced with various concentrations of Shionone (containing 10 $\mu$g/mL of gentamicin). The cells were then lysed by adding 1 mL of PBS containing 1 g/L Triton X-100 to each well, the lysate was diluted 1 : 1000, and 100 $\mu$L was spread on LB plates, incubated overnight at 37°C, and the number of colonies ($n$) was counted.

*2.11. Statistical Analysis.* GraphPad Prism software was used for statistical analysis of the experimental data. The results of the three independent replicate experiments were expressed as mean ± SD, and one-way ANOVA was used for analysis between groups, and differences were considered statistically significant at $p < 0.05$.

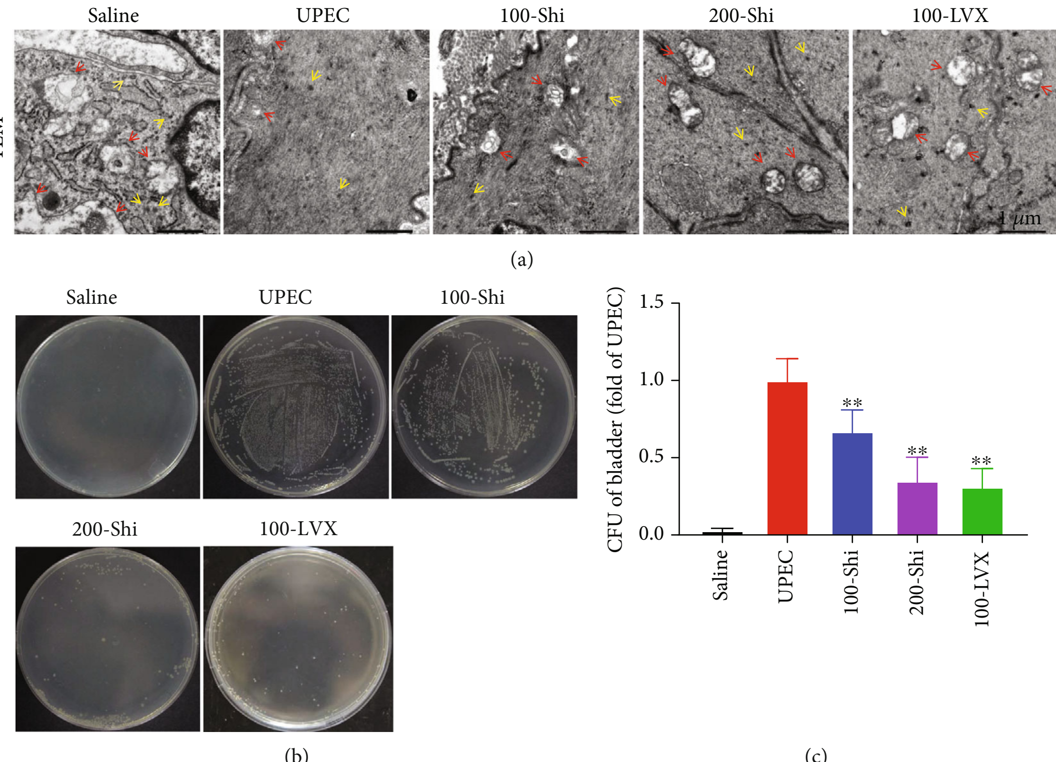


Figure 2: Shionone decreases the number of bacteria. (a) Electron micrograph of bladder tissue, with red arrows pointing to vesicles and yellow arrows to lysosomes. The magnification scale is 8000x, and the scale added to the images is 1 $\mu$m. (b) Bacterial colony counts in bladder homogenates of rats in each group. The magnification scale is 400x, and the scale added to the images is 100 $\mu$m. (c) Histogram statistics of bacterial CFU in bladder homogenates of rats in each group. Compared with the saline group, # means $p < 0.05$ and ## means $p < 0.01$. Compared with the UPEC group, $^*$ means $p < 0.05$ and $^{**}$ means $p < 0.01$.

## 3. Results

*3.1. Shionone Improves Edema and Congestion in the Bladder of UTI Rats.* The rats in UPEC group showed signs of bladder edema and congestion compared with the rats in Saline group, which were improved in the Shionone- and Levofloxacin Hydrochloride-administered groups (Figures 1(a) and 1(b)). The HE results showed that the rats in Saline group had normal bladder tissue structure without significant inflammatory cell infiltration, while the rats in UPEC group showed bladder mucosa thickening and large submucosal inflammatory cell infiltration, and bladder injury was significantly improved in the Shionone- and Levofloxacin Hydrochloride-administered groups (Figures 1(c) and 1(d)).

*3.2. Shionone Decreases the Number of Bacteria.* Transmission electron microscopy experiments were performed on superficial cystic tissue cells to detect vesicles and lysosomes, reflecting the vesicular haemolysis phenotype. As shown in Figure 2(a), red arrows point to vesicles and yellow arrows point to lysosomes. It can be seen that vesicles and lysosomes were significantly reduced in the UPEC group compared with the Saline group, whereas vesicles and lysosomes were increased in the Shionone-administered group compared with the UPEC group, indicating that Shionone can reduce the increase in bacteria. Bladder homogenate dilutions applied overnight to MacConkey plates revealed a significant presence of bacteria in the UPEC group, which was alleviated after Shionone administration (Figures 2(b) and 2(c)).

*3.3. Shionone Reduces E. coli and Inflammation in the Bladder of UTI Rats.* To further investigate the bacteria in bladder tissue, we performed paraffin sections of bladder tissue from each group, followed by immunofluorescence assays for phalloidin and *E. coli*. The green+red-positive rate indicates bacterial storage, and as seen in Figures 3(a) and 3(b), the treatment of Shionone greatly reduced bacteria in the bladders of UEPC rats, in agreement with previous results.

In patients with urinary tract infection, the levels of IL-1$\beta$, IL-6, and TNF-$\alpha$, which are inflammatory factors, are much higher than those in normal patients, so we examined these inflammatory factors in the urine of rats. In the present study, the urinary levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ were increased in the UPEC group compared with the Saline group, and the urinary levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ were decreased significantly in the Shionone-treated groups compared with the UPEC group (Figures 3(c)–3(e), $p < 0.05$).

*3.4. Shionone Reduces Inflammation and E. coli in SV-HUC-1 Cells Infected with Urinary Pathogenic E. coli.* We treated SV-HUC-1 cells with different concentrations of Shionone and found that cell viability decreased significantly at a

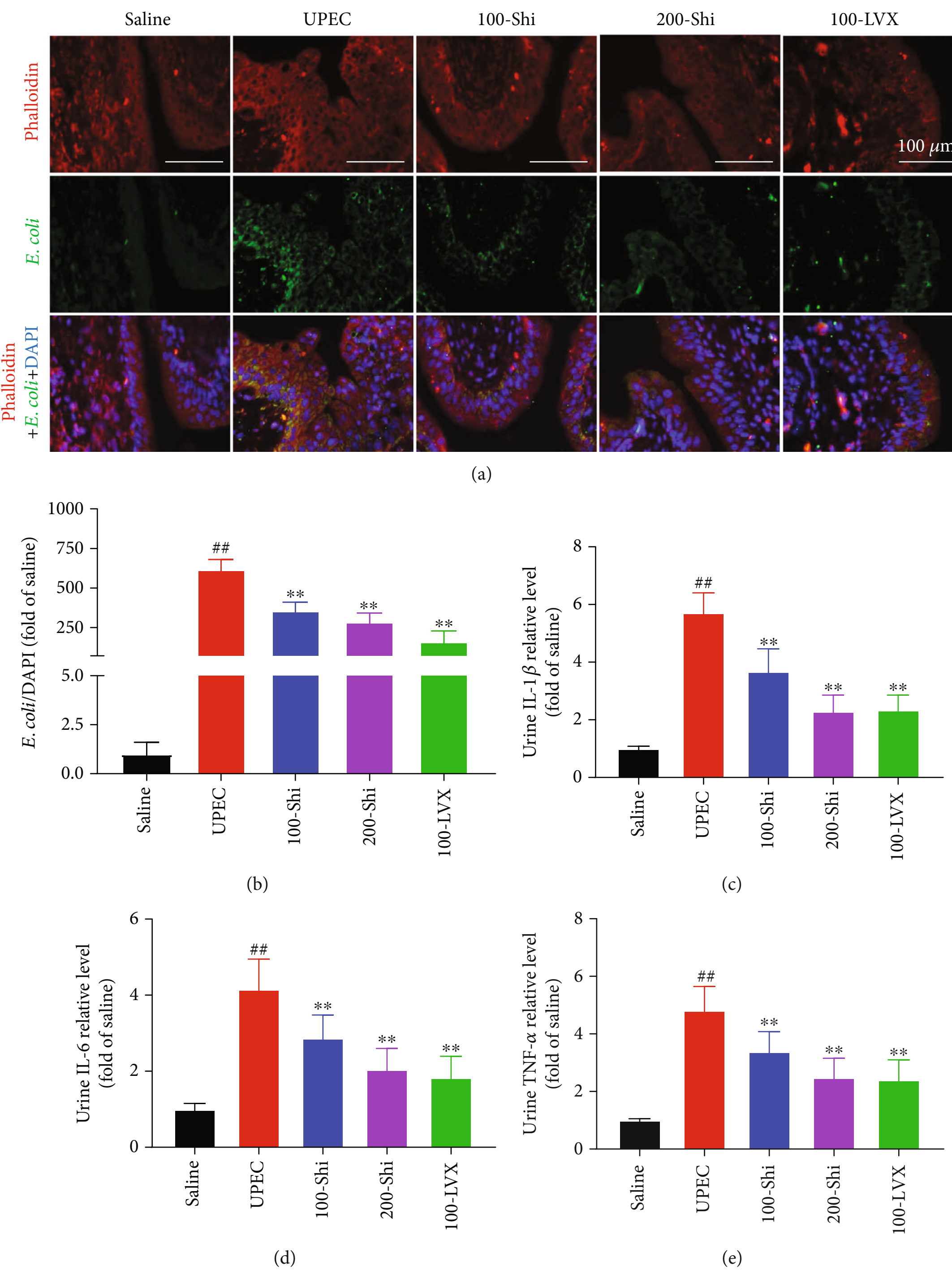


FIGURE 3: Shionone reduces *E. coli* and inflammation in the bladder of UTI rats. (a) Staining of cytoskeleton, *E. coli*, and nuclei in bladder tissue of each group of rats, with the cytoskeleton stained for phalloidin in red, *E. coli* in green, and nuclei stained for DAPI in blue. (b) Immunofluorescence intensity ratio of *E. coli* and DAPI. (c) The level of the inflammatory factor IL-1$\beta$ in the urine of rats in each group. (d) The level of the inflammatory factor IL-6 in the urine of rats in each group. (e) The level of the inflammatory factor TNF-$\alpha$ in the urine of rats in each group. Compared with the saline group, # means $p < 0.05$ and ## means $p < 0.01$. Compared with the UPEC group, * means $p < 0.05$ and ** means $p < 0.01$.

concentration of 40 $\mu$g/mL, and 5, 10, and 20 $\mu$g/mL were chosen as the experimental concentrations (Figure 4(a)). In the *in vitro* experiments, the levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ were increased in the UPEC group compared with the control group, and the levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ were decreased significantly in the Shionone-treated groups compared with the UPEC group (Figures 4(b)–4(d), $p < 0.05$).

Fluorescent staining of the cells showed that there was no *E. coli* present in the control group and a large amount of *E. coli* fluorescence in the UEPC group, which was greatly

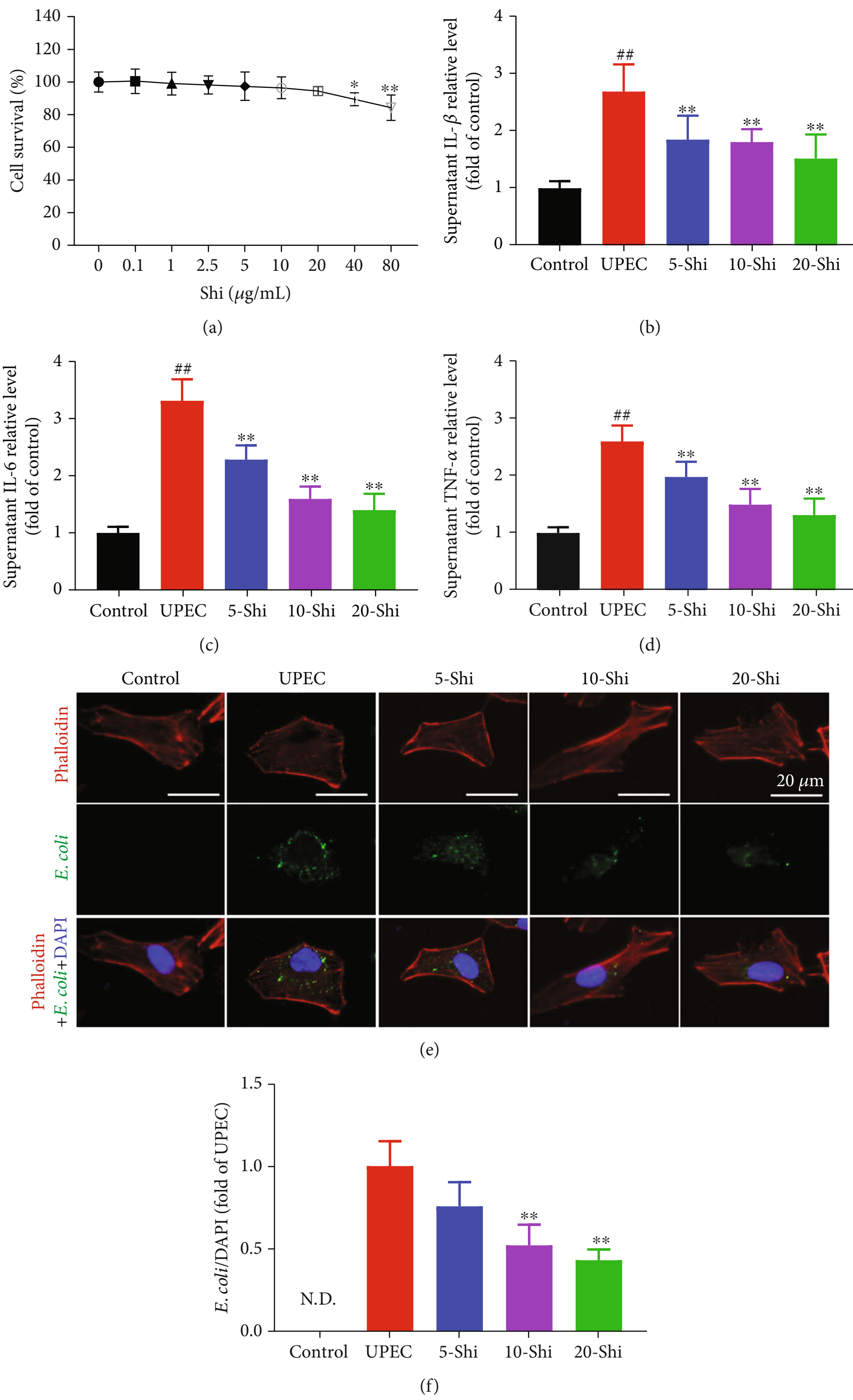


Figure 4: Continued.

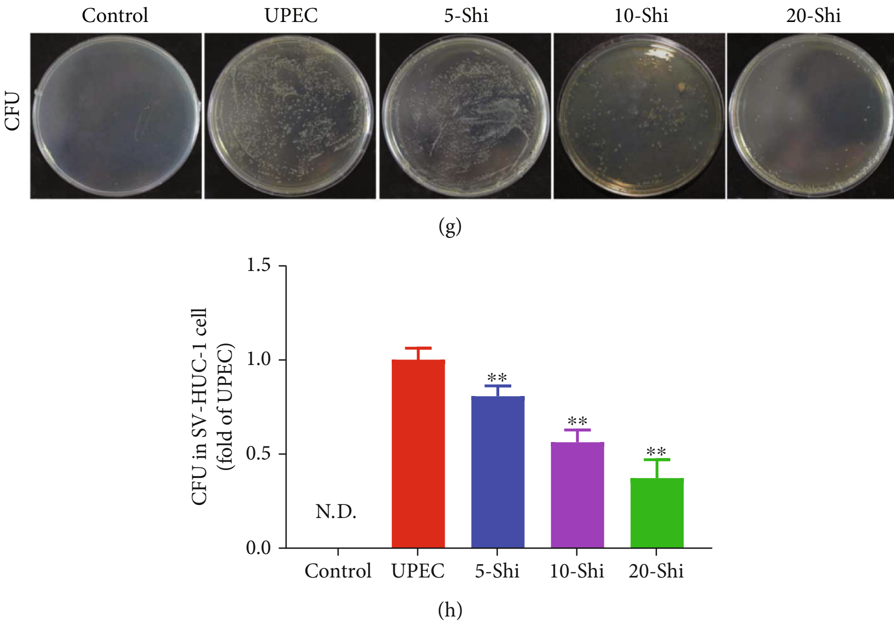


Figure 4: Shionone reduces inflammation and *E. coli* in SV-HUC-1 cells infected with urinary pathogenic *E. coli*. (a) The cell viability of each group. (b) The level of the inflammatory factor IL-1$\beta$ in supernatant of cell in each group. (c) The level of the inflammatory factor IL-6 in the supernatant of cell in each group. (d) The level of the inflammatory factor TNF-$\alpha$ in the supernatant of cell in each group. (e) Staining of cytoskeleton, *E. coli*, and nuclei in SV-HUC-1 cells of each group of cell, with the cytoskeleton stained for phalloidin in red, *E. coli* in green, and nuclei stained for DAPI in blue. (f) Immunofluorescence intensity ratio of *E. coli* and DAPI. (g) Bacterial colony counts of each group. (h) Histogram statistics of bacterial CFU of each group. Compared with the control group, # means $p < 0.05$ and ## means $p < 0.01$. Compared with the UPEC group, $^*$ means $p < 0.05$ and $^{**}$ means $p < 0.01$.

reduced in comparison to the UEPC group after Shionone use and showed a dose dependence (Figures 4(e) and 4(f)). Similarly, cell smear plate experiments demonstrated that there was a significant amount of *E. coli* bacteria in the cells of the UPEC group and that the CFU of bacteria in the cells of the groups that had treatment of Shionone was negatively correlated with the concentration of Shionone (Figures 4(g) and 4(h)).

## 4. Discussion

UTI is an inflammatory disease caused by the growth and multiplication of various pathogenic microorganisms in the urinary tract. Women have a higher incidence of UTI due to their short urethra, which seriously affects their health. Modern medical research on UTI has shown that *E. coli*, a Gram-negative bacterium of the intestinal tract, is the common causative agent of UTI, accounting for approximately 70%-90% of cases, and that patients experience urinary frequency and urgency, which seriously affects the quality of life [17, 18]. The use of drugs often leads to the development of drug resistance in E. coli, which affects the effectiveness of treatment. Therefore, the development of effective therapeutic drugs is a matter of urgency, but in order to study and screen drugs, it is necessary to establish suitable experimental animal models in order to better "target" the right drug. In this study, a model of UTI was developed by injecting bacteria from the urethra into female SD rats for retrograde infection based on the characteristics of UTI (high prevalence in females, *E. coli* as the main pathogen and upstream infection as the common type of infection). Shionone, a triterpene compound with antibacterial activity, also has a palliative effect on interstitial cystitis, but the specific effect on urinary tract infections is not yet known.

Inflammation is a common consequence observed during UTI [19]. Studies have shown that when bacteria infect the body, macrophages rapidly secrete large amounts of cytokines such as IL-1$\beta$, IL-6, and TNF-$\alpha$ [20]. These cytokines often amplify inflammation in a cascade through autocrine and paracrine actions, increasing the probability of urinary tract infections and causing damage to the kidneys [21]. In comparison with the Saline group, serum levels of IL-6, IL-1$\beta$, and TNF-$\alpha$ were reduced in the Shionone group, and the reduction in IL-6 levels slowed the increase in the number of morphologically altered cells and enhanced viscosity. It was suggested that Shionone had significant anti-inflammatory effects and HE staining of bladder tissue also showed that Shionone reduced inflammatory cell infiltration in rats.

Previous studies have shown that a key pathogenic mechanism of UTI in mice is the ability of UPEC to form bacterial communities within the surface cells of the bladder epithelium [22, 23]. Bacterial communities are one of the main factors contributing to recurrent UTI [6]. UPEC can interfere with the signal transduction mechanisms of the host cells by using a variety of virulence factors carried by the UPEC themselves, and the signal transduction mechanisms of the host cell help the bacteria to internalise into the cell, thus evading the flushing of the urinary stream and the immune effect of the host cell attack. The entry of UPEC into the host cell results in intracellular proliferation and the formation of intracellular colonies

that become the cause of reignition or recurrence of urinary tract infection [24, 25]. When the invaded host cell is shed, large amounts of UPEC are released, invading neighbouring cells and starting a new cycle of infection, thus causing a recurrence [25, 26]. The immunofluorescence results of the present study showed a significant amount of *E. coli* fluorescence in the UEPC model group both in rat bladder and SV-HUC-1 cells and a significant reduction in *E. coli* fluorescence after treatment with Shionone, indicating that Shionone has the ability to reduce *E. coli*. Combining Shionone reduces the levels of proinflammatory cytokines.

In summary, UTI is a common and easily recurring disease, and current antibiotic treatment has reached a plateau. In this study, we established a female SD rat UTI model and a SV-HUC-1 cell UTI model to evaluate the effects of Shionone on UTI. The results showed that Shionone had a good therapeutic effect on UTI, which was achieved by reducing bacteria. The data provided in this study provide new ideas and methods for the treatment of UTI and lay the foundation for further research on the treatment of UTI.

## Data Availability

The raw data supporting the conclusions of the present manuscript will be made available by the authors, without undue reservation.

## Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Authors' Contributions

Hao Yin was responsible for the data curation (equal) and wrote the original draft (lead). Jiaoli Zhu was responsible for the formal analysis (equal). Yi Jiang was responsible for the investigation (supporting). Yijing Mao was responsible for the methodology (supporting). Chenquan Tang was responsible for the methodology (equal). Hui Cao was responsible for the project administration (lead). Yufang Huang was responsible for the project administration (supporting). Huijun Zhu was responsible for the resources (supporting). Yi Xue was responsible for the software (supporting). Jianping Luo was responsible for the validation (equal). Xin Wang wrote, reviewed, and edited the manuscript (equal). Qinglei Jin wrote, reviewed, and edited the manuscript (supporting). Qingjiang Jin was responsible for the conceptualization (lead). Hao Yin and Jiaoli Zhu contributed equally to this work.

## Acknowledgments

The project was supported by the Basic Research on Medical and Health Application of Suzhou Science and Technology Program (Grant/Award Numbers: SKJY2021019 and SYSD2020056). The work was also supported by the Science and Technology Project of Jiangsu Bureau of Traditional Chinese Medicine (Grant/Award Number: YB2020059).

## References

[1] A. S. Naik, V. R. Dharnidharka, M. A. Schnitzler et al., "Clinical and economic consequences of first-year urinary tract infections, sepsis, and pneumonia in contemporary kidney transplantation practice," *Transplant International*, vol. 29, no. 2, pp. 241–252, 2016.

[2] J. Peck and J. P. Shepherd, "Recurrent urinary tract infections: diagnosis, treatment, and prevention," *Obstetrics and Gynecology Clinics of North America*, vol. 48, no. 3, pp. 501–513, 2021.

[3] A. Aydin, K. Ahmed, I. Zaman, M. S. Khan, and P. Dasgupta, "Recurrent urinary tract infections in women," *International Urogynecology Journal*, vol. 26, no. 6, pp. 795–804, 2015.

[4] T. M. Hooton, "Recurrent urinary tract infection in women," *International Journal of Antimicrobial Agents*, vol. 17, no. 4, pp. 259–268, 2001.

[5] S. Salvatore, E. Cattoni, G. Siesto, M. Serati, and P. Sorice, "Urinary tract infections in women," *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, vol. 156, no. 2, pp. 131–136, 2011.

[6] M. Totsika, D. G. Moriel, and A. Idris, "Uropathogenic *Escherichia coli* mediated urinary tract infection," *Current Drug Targets*, vol. 13, no. 11, pp. 1386–1399, 2012.

[7] O. Ciani, D. Grassi, and R. Tarricone, "An economic perspective on urinary tract infection: the "costs of resignation"," *Clinical Drug Investigation*, vol. 33, no. 4, pp. 255–261, 2013.

[8] D. G. Lee, J. J. Cho, H. K. Park et al., "Preventive effects of hyaluronic acid on *Escherichia coli*-induced urinary tract infection in rat," *Urology*, vol. 75, no. 4, pp. 949–954, 2010.

[9] S. Sawai, H. Uchiyama, S. Mizuno et al., "Molecular characterization of an oxidosqualene cyclase that yields shionone, a unique tetracyclic triterpene ketone of Aster tataricus," *FEBS Letters*, vol. 585, no. 7, pp. 1031–1036, 2011.

[10] X. Wang, H. Yin, L. Fan et al., "Shionone alleviates NLRP3 inflammasome mediated pyroptosis in interstitial cystitis injury," *International Immunopharmacology*, vol. 90, article 107132, 2021.

[11] P. Yu, S. Cheng, J. Xiang et al., "Expectorant, antitussive, anti-inflammatory activities and compositional analysis of *Aster tataricus*," *Journal of Ethnopharmacology*, vol. 164, pp. 328–333, 2015.

[12] F. Han, R. H. Xing, L. Q. Chen et al., "Research progress of anti-drug resistance in traditional Chinese medicine," *Zhongguo Zhong Yao Za Zhi*, vol. 41, no. 5, pp. 813–817, 2016.

[13] C. Wang, K. A. Bauckman, A. S. B. Ross et al., "A non-canonical autophagy-dependent role of the ATG16L1$^{T300A}$ variant in urothelial vesicular trafficking and uropathogenic *Escherichia coli* persistence," *Autophagy*, vol. 15, no. 3, pp. 527–542, 2019.

[14] X. Q. Li, J. Y. Zhu, R. R. Pan et al., "Therapeutic effect of Dong-bai-Tonglin-Fang, a Chinese herbal formula, on urinary tract infection in rat model," *Journal of Ethnopharmacology*, vol. 241, article 112028, 2019.

[15] F. Emamghorashi, S. M. Owji, and M. Motamedifar, "Evaluation of effectiveness of vitamins C and E on prevention of renal scar due to pyelonephritis in rat," *Advances in Urology*, vol. 2011, Article ID 489496, 6 pages, 2011.

[16] T. J. Hannan and D. A. Hunstad, "A murine model for *Escherichia coli* urinary tract infection," *Methods in Molecular Biology*, vol. 1333, pp. 159–175, 2016.

[17] B. Foxman, "The epidemiology of urinary tract infection," *Nature Reviews Urology*, vol. 7, no. 12, pp. 653–660, 2010.

[18] K. L. Nielsen, P. Dynesen, P. Larsen, and N. Frimodt-Møller, "Faecal *Escherichia coli* from patients with E. coli urinary tract infection and healthy controls who have never had a urinary tract infection," *Journal of Medical Microbiology*, vol. 63, no. 4, pp. 582–589, 2014.

[19] C. N. Rudick, B. K. Billips, V. I. Pavlov, R. E. Yaggie, A. J. Schaeffer, and D. J. Klumpp, "Host-pathogen interactions mediating pain of urinary tract infection," *The Journal of Infectious Diseases*, vol. 201, no. 8, pp. 1240–1249, 2010.

[20] S. T. Chou, H. Y. Lo, C. C. Li et al., "Exploring the effect and mechanism of _Hibiscus sabdariffa_ on urinary tract infection and experimental renal inflammation," *Journal of Ethnopharmacology*, vol. 194, pp. 617–625, 2016.

[21] S. Hedges and C. Svanborg, "The mucosal cytokine response to urinary tract infections," *International Journal of Antimicrobial Agents*, vol. 4, no. 2, pp. 89–93, 1994.

[22] G. G. Anderson, J. J. Palermo, J. D. Schilling, R. Roth, J. Heuser, and S. J. Hultgren, "Intracellular bacterial biofilm-like pods in urinary tract infections," *Science*, vol. 301, no. 5629, pp. 105–107, 2003.

[23] S. S. Justice, C. Hung, J. A. Theriot et al., "Differentiation and developmental pathways of uropathogenic *Escherichia coli* in urinary tract pathogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 5, pp. 1333–1338, 2004.

[24] M. A. Mulvey, J. D. Schilling, and S. J. Hultgren, "Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection," *Infection and Immunity*, vol. 69, no. 7, pp. 4572–4579, 2001.

[25] I. U. Mysorekar and S. J. Hultgren, "Mechanisms of uropathogenic *Escherichia coli* persistence and eradication from the urinary tract," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 38, pp. 14170–14175, 2006.

[26] T. J. Hannan, M. Totsika, K. J. Mansfield, K. H. Moore, M. A. Schembri, and S. J. Hultgren, "Host-pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic *Escherichia coli* bladder infection," *FEMS Microbiology Reviews*, vol. 36, no. 3, pp. 616–648, 2012.