

A Rat Model of Ascending Uncomplicated Urinary Tract Infection for Anti-Infective Efficacy Studies

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Abstract

Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide and contribute substantially to antimicrobial resistance, highlighting the need for robust preclinical models to support development of novel anti-infective therapies. Although mouse models dominate UTI research, anatomical and technical constraints can limit translational pharmacokinetic and pharmacodynamic (PK/PD) studies, particularly for intravenously administered agents. Here, we describe and validate an ascending model of uncomplicated UTI in adult female Wistar rats that enables reproducible bladder and kidney infection and supports comprehensive treatment evaluation.

Transurethral catheterization was used to inoculate the bladder with the well-characterized uropathogenic *Escherichia coli* strain CFT073. Compared with Sprague Dawley rats, Wistar rats exhibited more stable infection kinetics and lower intra-group variability and were therefore selected for model optimization. Enrichment of type 1 fimbriae expression significantly enhanced bladder colonization, urinary bacterial shedding, and ascending infection of the kidneys. Using this optimized model, a single intravenous dose of ciprofloxacin produced rapid bactericidal activity within two hours of treatment and sustained efficacy, with three to four log reductions in bacterial burdens in urine, bladder, and kidneys at twenty-four hours post-treatment. Histopathological analysis demonstrated infection-associated immune cell infiltration and tissue edema in the bladder, both of which were markedly reduced following antibiotic treatment. Multiplex cytokine profiling of bladder tissue revealed a localized inflammatory response characterized by increased IL-1 α and MIP-3 α during infection, with broader pro-inflammatory cytokine induction observed following ciprofloxacin administration.

Together, these findings establish a reproducible, physiologically relevant rat model for integrated preclinical evaluation of intravenous antimicrobials targeting UTIs.

1. Introduction

Urinary tract infections (UTIs) are one of the most common types of bacterial infections in the world with more than 150 million cases diagnosed every year¹. *Escherichia coli* (*E. coli*) is the predominant bacterial pathogen responsible for UTIs². It accounts for approximately 70% to 95% of community-acquired UTIs and 30% to 50% of hospital-acquired cases³⁻⁷. UTIs are more likely to occur in women, children and the elderly⁸. It is estimated that one in two women will experience at least one UTI episode during their lifetime⁹.

The human gut is a critical reservoir of uropathogenic *E. coli* (UPEC) with the host's fecal flora being a major source of the infecting strain^{10,11}. Additionally, contaminated food and water sources have been proposed as potential vehicles of community breaks of UTIs¹²⁻¹⁴. Thus, the pathogenesis of community acquired UTI typically begins with colonization of the periurethral and vaginal areas by bacteria from the gastrointestinal tract¹⁵. From there, the bacteria ascend the urethra into the bladder, causing cystitis, and sometimes further into the ureters and kidneys, causing pyelonephritis¹⁶. Bacterial entry into the

bloodstream results in sepsis which can prove fatal without timely and appropriate treatment^{17,18}. Thus, UTIs pose a significant burden on global healthcare systems, and the emergence of several multidrug resistant *E. coli* clonal lineages has made treatments more prolonged, complicated and expensive. It is not uncommon for drug resistant UTIs to turn fatal due to treatment failure¹⁹.

Treatment generally involves a course of oral antibiotics. First-line agents for uncomplicated UTIs include nitrofurantoin, fosfomycin, cefalexin, and trimethoprim-sulfamethoxazole, while fluoroquinolones and other beta-lactams may be used in more complicated cases^{20–22}. However, the increasing rates of antibiotic-resistance in clinical *E. coli* isolates and the associated increase in extended-spectrum beta-lactamase (ESBL) and carbapenemase producing strains, pose a significant therapeutic challenge, particularly in cases of recurrent or healthcare-associated UTIs^{23–29}. In fact, cephalosporin and carbapenem resistant *E. coli* (*Enterobacterales*) constitute two out of three critical pathogen groups highlighted by the World Health Organization for urgent drug development³⁰. According to the 2023 WHO report on antibacterials under clinical development, only 16 new antibacterials have been approved for clinical use since 2017³¹. Unfortunately, ~ 77% of the traditional agents approved since 2017 belong to existing antibiotic classes for which resistance mechanisms are well known³¹. Furthermore, most of the antibacterial agents in the pipeline are designed for intravenous use, with few oral options. Therefore, appropriate *in vivo* infection models that allow detailed pharmacokinetic and pharmacodynamic evaluations of novel preclinical candidates are invaluable in the quest for better, more effective therapies.

Both mouse and rat models are commonly used in UTI research and drug development, each offering unique advantages and limitations. Mice, particularly females, are favored for their relatively simple anatomy and ability to mimic human UTI pathogenesis and remain the model of choice for early-stage, *in vivo* investigations³². They are relatively easy to handle and manipulate for experimental purposes and benefit from a large array of molecular resources for further post-mortem investigations. However, mice have very limited bladder volumes, which can affect the experimental design and interpretation of results^{32,33}. Moreover, certain strains (such as the C3H HeN, HeJ, HeOuJ strains) are predisposed to vesicoureteral reflux (VUR disease), which can deliver bacteria directly into the kidneys during experimental transurethral inoculation^{34,35}. While useful for studying VUR disease, this can be a hindrance to the interpretation of pathogenesis of a natural ascending mode of UTI. Additionally, intravenous (IV) drug dosing is technically challenging in mouse models, particularly the instillation of IV catheter lines for prolonged infusion studies to mimic routinely used clinical trial parameters in human subjects. Urine sample collection can be difficult as well considering the limited bladder volume and samples can often be inadvertently 'lost' by natural micturition during anxiogenic handling procedures or during any instance of perceived stress by the animal³⁶.

Rats, on the other hand, are sometimes preferred for longitudinal studies or when studying specific aspects of the urinary tract, like bladder function or response to therapeutic interventions³⁷. They are often the model of choice for pharmacokinetic and toxicology studies of novel drug candidates. Rats

typically have larger body sizes, allowing for longitudinal sampling and more complex surgical interventions³⁸. The urinary tract structure and bladder voiding patterns in rats resemble human physiology more closely, compared to mice. Additionally, both rat and human bladders contain high concentrations of desmin intermediate filaments that affect bladder wall morphology and function, both critical parameters for the study of severe or chronic infections^{39, 40}.

Here we describe an ascending model of acute urinary tract infection in female rats with a well characterized UPEC strain, *E. coli* CFT073 (a pyelonephritis isolate). Infection was established via temporary transurethral catheterization into the bladder. Utilization of type 1 fimbriae enriched *E. coli* CFT073 enabled the establishment of an ascending mode of infection with significant bacterial shedding in urine, accompanied with bladder and kidney colonization within a day of infection initiation. Ciprofloxacin was administered intravenously as a treatment demonstration for future anti-infective trials. We have also documented bladder histopathology showing considerable immune cell infiltration and localized tissue edema in infected animals and a cytokine profile underpinning infection-mediated inflammation.

2. Methods

2.1. Ethics Statement

All animal procedures were approved by the Animal Ethics Committee, Murdoch Children's Research Institute (Application ID A967).

2.2. Bacterial culture

E. coli CFT073 (sourced from the American Type Culture Collection 7009™) is a well-characterized UPEC blood-culture isolate, originally collected from a woman with acute pyelonephritis⁴¹. Bacteria were cultured at 37°C in lysogeny broth (LB) under aerated or static conditions. The initial pilot experiment (described below) was performed with overnight, aerobic cultures of CFT073 (37°C, 180 rpm).

For type 1 fimbriae enrichment (used in latter UTI treatment model experiments), bacteria were grown overnight on LB agar, and single colonies were picked to inoculate 5 ml LB culture for overnight aerobic (shaking, 180 rpm) growth. Subsequently, 1 µl of overnight culture was inoculated into a new tube containing 5 ml LB and cultured for 24 hrs under static conditions. The strain was further sub-cultured from the air–liquid interface in a similar manner for an additional 24 hrs of static growth. Type 1 fimbriae expression was determined at each stage of subculture using a mannose-sensitive yeast cell agglutination assay as previously described^{42, 43}. Type 1 fimbriae enriched CFT073 (CFT073Fim+) with strongly positive yeast agglutination phenotypes were utilized for bladder inoculations in Wistar rats.

2.3. Inoculum preparation

Overnight cultures of *E. coli* CFT073 (shaking or static) were centrifuged at 3700 g for 10 min. Bacterial pellets were washed once with sterile PBS with final resuspensions made in 1 ml of PBS. Bacteria were

visually counted in a hemocytometer (center grids) and concentration adjusted to 2×10^8 bacterial cells/ml in PBS, for bladder inoculations. Serial dilutions were plated on LB agar plates for retrospective colony counts of inoculum used in each infection experiment.

2.4. Study design

All animal experimentation was performed in adherence to the Animal research: reporting of *in vivo* experiments (ARRIVE 2.0) guidelines⁴⁴. All experiments were performed at the Murdoch Children's Research Institute (Australia). Adult female rats (8–10 weeks old) were sourced from the Ozgene Animal Resources Centre (Australia) and housed in filter-top cages (5 rats per cage) with *ad libitum* access to feed and sterile water. Randomization was carried out as follows: upon arrival, rats were arbitrarily assigned to cages. After one week of acclimatization, rat tails were marked with a permanent marker and assigned a number within the cage. Cages were then randomly taken from the rack and assigned a group number ($n = 3$ for uninfected controls, $n = 5$ for all other groups). Each rat (or tissues/samples collected from each rat, such as bladders or kidneys, as indicated in Results and graphs) is considered an experimental unit for the studies reported here. Additional details are provided in the Pilot study and Treatment model sections below.

2.5. Pilot study

Different rat strains have been used in previous UTI studies with no clear consensus on ideal strain of choice^{45–48}. An initial pilot study was performed to identify the optimal rat strain for subsequent experiments. 8–10 weeks old female Wistar and Sprague Dawley rats (180 g – 200 g; $n = 5$ per group) were placed under sedation via isoflurane anesthesia. Animals were turned around with the abdomen exposed and anterior end facing the operator (Figure S1). The urethral opening was swabbed twice with 10% povidone-iodine swab-stick (Clifford Hallam Healthcare, Australia) for local disinfection and then with sterile saline to remove any excess. A drop of 2% lidocaine jelly (Xylocaine, Australia) was applied to the opening for analgesia and lubrication. A 1-ml Terumo Tuberculin syringe (Interpath Services, Australia) was pre-loaded with the bacterial inoculum (CFT073) and fitted with a 24 g (19.05 mm) intravenous catheter (tubing only, needle removed; Becton Dickinson, Australia). The catheter tip was dipped in additional lidocaine jelly and inserted into the urethral opening at a 45° angle till the tip reached the pelvic bone (Figure S1 A). Then the catheter was turned perpendicularly to the abdomen and slid all the way into the bladder while lowering it on the opposite side (Figure S1 B, C). Once inside, the syringe plunger was pushed gently over 30–60 sec to instill 100 μ l of bacterial inoculum directly into the bladder. The animal was then placed in a clean cage for recovery and monitoring. At 24 hrs post infection (hpi), all animals were humanely euthanized and tissues (urine, bladder, kidneys) collected for analysis.

2.6. Analysis of bacterial burden

Urine was collected post-mortem directly from the bladder (with a 25 g needle) and 10-fold serial dilutions made in sterile phosphate buffered saline (PBS). LB agar plates were spotted with 5 μ l of each dilution series and incubated at 37°C overnight for enumeration of colony forming units (CFUs). Bladders

and kidneys were collected in PBS and homogenized using a PolyTron PT2100 (Kinematica AG) tissue homogenizer. Serial dilutions of tissue lysates were plated on LB agar as before for CFU enumeration and unused samples were flash frozen in a dry ice bath and stored at -80°C for downstream analyses.

2.7. UTI Treatment model

In this model, female Wistar rats were randomly assigned to experimental groups and transurethrally inoculated with 2×10^7 CFU (in 100 µl saline) of type 1 fimbriae enriched CFT073 (CFT073Fim+) or saline (uninfected controls). At 4 hpi, rats were gently sedated under isoflurane anesthesia for intravenous (IV) ciprofloxacin (Merck, Australia) administration (as a 0.5 ml bolus dose, 50 mg/kg). Untreated control groups received saline. Animals were euthanized at 6 hpi and 28 hpi (24 hours post treatment administration) for analysis of bacterial burden.

2.8. Bladder histopathology

Rat bladders were harvested post-mortem under aseptic conditions. Surrounding adipose and connective tissues were carefully removed and each bladder was incised longitudinally along the anterior wall to expose the lumen. The tissue bisected into two equal halves and immersion-fixed in 4% paraformaldehyde for 24 hrs at 4°C. Following fixation, tissues were processed through graded ethanol dehydration, cleared in xylene, and infused with paraffin in an Excelsior AS tissue processor (Thermo Scientific). Processed tissue halves were embedded in paraffin blocks which were then oriented to obtain transverse sections encompassing the bladder urothelium, lamina propria, and detrusor muscle layers. For histopathological assessment, sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E). Slides were examined by light microscopy to evaluate inflammatory infiltrates, tissue edema, and structural changes associated with urinary tract infection. Histopathology was graded as follows^{49, 50}: Grade 0 (Normal): no edema or infiltrating immune cells; Grade 1 (Mild): mild interstitial edema and a few infiltrating inflammatory cells in the lamina propria; Grade 2 (Moderate): moderate edema and moderate numbers of inflammatory cells; Grade 3 (Severe): frank/severe edema, vascular congestion, and high numbers of inflammatory cells.

2.9. Cytokine analysis

The Bio-Plex Pro™ Rat Cytokine 23-Plex Assay kit (catalogue no. 171K1001M; Bio-Rad Laboratories) was used for multiplexed cytokine analysis in the Bio-Plex® 200 instrument with an automated Bio-Plex Pro™ Wash station. Bladder homogenates were thawed on ice and samples processed as per manufacturer instructions for the quantification of various cytokines (23-plex kit included G-CSF, GM-CSF, GRO/KC, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 (p70), IL-13, IL-17A, IL-18, M-CSF, MCP-1, MIP-1α, MIP-3α, RANTES, TNF-α and VEGF). The total protein content in each sample was quantified by the Pierce™ BCA Protein Assay kit (catalogue no: 23225; Thermo Scientific) for data normalization.

2.10. Statistical analysis

Datasets were plotted in GraphPad Prism (v10.6) for graphical visualization and analysis of bacterial burden, histopathological scores and cytokine levels by Mann-Whitney U non-parametric tests between

relevant groups. P values < 0.05 were considered statistically significant in all cases. Data is depicted as individual markers (each marker corresponds to an individual rat) where possible with group medians and interquartile ranges or 95% confidence intervals (as noted in figure legends).

3. Results

3.1. Optimizing the rat ascending UTI model

In the absence of a clear consensus on an ideal rat strain for the establishment of ascending UTI, both Wistar and Sprague Dawley rats were examined in a pilot study utilizing aerobically grown *E. coli* CFT073 (n = 5 per group). At 24 hours post infection (hpi), both rat strains showed significant bladder colonization (10^2 CFU/mg bladder tissue) but bacterial shedding was higher in Wistar females (Fig. 1). While differences in bladder colonization and urine shedding were not statistically different in the pilot study, colonization appeared to be less stable in the Sprague-Dawley rats which demonstrated a higher degree of variability within the infected group. Moreover, stable kidney colonization was not achieved in either of the rat strains with bacterial colonization being detected only in 40% (2 out of 5) of animals in each group (Fig. 1C).

Considering the greater intra-group variability in bacterial colonization in Sprague-Dawley rats and the documented evidence of congenital, spontaneous vesicoureteral reflux (VUR) in this strain⁵¹, Wistar rats were selected for subsequent model optimization. *E. coli* CFT073 was subjected to type 1 fimbrial enrichment via two rounds of static culture which yielded a strong positive mannose-sensitive yeast agglutination phenotype (< 10 seconds for a positive reaction). This strain was designated as CFT074Fim+. On the other hand, CFT073 cultured under shaking/aerobic conditions shows a weakly positive agglutination phenotype (> 30 seconds for a positive reaction), denoting a relatively lower level of type 1 fimbriae expression. Females Wistar rats were infected with the CFT073Fim+ strain as before and bacterial shedding in the urine and colonization levels in bladders and kidneys were assessed at 6 hpi and 28 hpi (Fig. 2). Analysis of bacterial numbers at both timepoints show stable bladder colonization and bacterial shedding in the urine (Fig. 2A). Kidney colonization is variable at 6 hpi but significantly increased at 28 hpi (P < 0.05). Bacterial colonization was significantly improved upon type 1 fimbriae enrichment with CFT073Fim+ showing higher bacterial shedding (P < 0.05) and bladder colonization (P < 0.01) at both timepoints, compared to infection with shaking cultures of CFT073 with low type 1 fimbriae expression levels (Fig. 2B, C). This difference in colonization between the two strain variants is particularly striking in the context of kidney colonization, with the CFT073Fim+ strain demonstrating an ascending mode of infection with significantly higher colonization at the later timepoint (Fig. 2C). Additionally, type 1 fimbrial enrichment led to lower intra-group variability in this model, thus making it suitable for subsequent treatment studies.

3.2. Efficacy of intravenous ciprofloxacin in the rat ascending UTI model

Patients treated for UTIs in hospital settings or those with complicated UTI cases (like pyelonephritis), often receive intravenous (IV) ciprofloxacin before de-escalation to oral therapy once the patient stabilizes or is discharged^{52, 53}. We tested the administration of a single dose of IV ciprofloxacin (at 4hpi) in our model as a validation step, with parallel evaluation of antibiotic efficacy in different compartments of the urinary tract. IV ciprofloxacin showed > 5 log reduction in bacterial shedding in the urine at 6hpi with efficacy being maintained at 28hpi (> 4 log reduction, $P < 0.01$; Fig. 3A, B). Similar trends were observed in bladder bacterial loads, with ciprofloxacin treatment resulting in > 4-log reduction in CFT073 colonization at 6hpi and ~ 3-log reduction at 28 hpi (24 hours post treatment), compared to infected but untreated controls ($P < 0.01$; Fig. 3C, D). The bactericidal effect of ciprofloxacin was most profound in the kidneys with initial reduction of CFT073 at 6 hpi (Fig. 3E) having a significant impact on reducing kidney colonization at 28 hpi, resulting in > 4.5-log difference in bacterial numbers compared to the untreated group at the later timepoint ($P < 0.01$; Fig. 3F).

3.3. Histopathological analysis of rat bladders

In experimental UTI models, infection can cause significant localized inflammation and measurable changes to urinary organs, specifically increases in bladder weights due to localized tissue edema, leukocyte infiltration and ensuing epithelial damage⁵⁴. Analysis of organ weights at 28 hpi in our age-matched cohort showed a significant increase only in the bladders of ciprofloxacin treated rats (compared to infected but untreated controls, $P < 0.05$; Fig. 4A). No differences were observed in kidney weights at this timepoint (Fig. 4B).

Transverse sections of rat bladders were stained with routine H&E and encompassed the urothelium with underlying lamina propria and the detrusor muscle layers. Histopathological analysis of these bladder tissues (Fig. 5) revealed significant immune cell infiltration in the lamina propria (Fig. 5C), especially in the infected group which also had higher scores for tissue edema, however this trend did not reach statistical significance (Fig. 5D). Assessment of total histology scores showed significantly increased pathology markers in infected animals (compared to uninfected controls, Fig. 5E). Furthermore, ciprofloxacin treatment was successful in greatly reducing bladder pathology in this model ($P < 0.05$).

3.4. Inflammatory signature associated with cystitis

Rat bladder homogenates were subjected to multiplex cytokine analysis to identify the inflammatory signature associated with infection in this model of ascending UTI. Figure 6 includes cytokines that were differentially expressed or secreted in the infected and/or ciprofloxacin treated groups. With serum and bladder homogenates from infection-naïve animals as baseline controls, the infectious challenge with CFT073Fim + led to significantly higher levels of Interleukin 1 alpha (IL-1 α ; $P < 0.01$) and Macrophage Inflammatory Protein 3 alpha (MIP-3 α ; $P < 0.001$). Additionally, there was a trend towards increased IL-1 β , MIP-1 α , Monocyte Chemoattractant Protein 1 (MCP-1) and Growth Regulated Oncogene/Keratinocyte Chemoattractant (GRO/KC) in the infected group but this did not reach

statistical significance. Interestingly, IV ciprofloxacin treatment resulted in a more pronounced inflammatory signature with highly elevated IL-1 α , IL-1 β , MIP-1 α , MIP-3 α , MCP-1 and GRO/KC compared to uninfected controls. While there was considerable overlap in data ranges between infected/untreated and ciprofloxacin treated groups, the levels of IL-1 α , IL-1 β and MIP-3 α were significantly higher in the latter group, thus indicating an increase in proinflammatory signaling as a result of ciprofloxacin treatment.

Other UTI relevant cytokines, namely IL-6, Tumor Necrosis Factor alpha (TNF- α), Interferon gamma (IFN- γ), IL-10, IL-17A and IL-2 were not meaningfully elevated in our model at this timepoint (28 hpi; Fig. 7). There was a trend towards higher IL-6 levels in the infected/untreated and ciprofloxacin treated groups, but this difference was not statistically significant (Fig. 7A). The multiplex cytokine panel further included IL-4, IL-5, IL-7, IL12, IL-13, IL-18, Vascular Endothelial Growth Factor (VEGF), RANTES (Regulated upon Activation, Normal T-cell Expressed and presumably Secreted), Granulocyte Colony-stimulating Factor (G-CSF), Macrophage Colony-stimulating Factor (M-CSF), and Granulocyte-Macrophage Colony-stimulating Factor (GM-CSF), none of which were remarkably different between infected groups and uninfected controls (Supplementary Figures S2, S3).

4. Discussion

As one of the most common bacterial infections, UTIs have been a significant driver of the rise of antimicrobial resistance (AMR) across the world⁵⁵. The World Health Organization's Bacterial Priority Pathogen List acts as a guide for prioritizing research and investment by diverse stakeholders in AMR and includes 3rd generation cephalosporin and carbapenem resistant *E. coli* in the critical priority group for urgent drug development³⁰. Incidentally, most of the traditional antimicrobials in the pre-clinical and clinical pipelines targeted towards UTIs, are intravenous drugs⁵⁶. This highlights the importance of effective preclinical UTI models that allow for efficacy testing of intravenous agents alongside PK/PD (pharmacokinetic/pharmacodynamic) and toxicology studies.

Mouse models of UTI are perhaps the most commonly used in the field and have been invaluable in the dissection of critical host-pathogen interactions underpinning bacterial pathogenesis³². Aside from obvious anatomical differences, key limitations of mouse UTI models include, i) a higher urinary voiding pressure leading to more frequent, smaller volume voiding compared to humans; and ii) a pronounced vesicoureteral reflux leading to urine backflow into the kidneys which is not typical in healthy humans. On the other hand, rat bladders can offer several functional advantages over their mouse counterparts. The larger bladder capacity of rats supports more complex surgical procedures, and their urodynamic features, including coordinated sphincter control and bladder contraction dynamics, closely resemble those of humans^{57, 58}. From a technical standpoint, rats enable reliable longitudinal urine sampling and provide a more practical platform for intravenous drug administration compared to smaller rodent models. This can be an added advantage considering the extensive PK/PD modelling and toxicology

studies that are routinely conducted in this species for drug candidates under advanced preclinical development^{59, 60}.

In this study, we describe an ascending model of UTI in adult female Wistar rats with a well characterized pyelonephritis isolate, UPEC CFT073⁴¹. While there are several examples of catheter-associated⁶¹ and foreign body-associated UTI in rats⁶², it is challenging to establish kidney infection via the traditional route of transurethral catheterization, without triggering the vesicoureteral reflux (VUR) of bacteria directly into the kidneys.

As Sprague Dawley and Wistar rats represent the two most extensively used strains in urogenital research, we performed a preliminary comparative study to determine which strain provided greater reliability in establishing bacterial colonization. Transurethral catheterization was chosen as the preferred method of infection initiation. This is a minimally invasive, pain-free technique that enables bacterial inoculation directly into the bladder and is more representative of natural UTI acquisition compared to surgical bladder instillation⁵⁷. Aerobically cultured CFT073 (under shaking conditions) yielded low bladder colonization in both strains within 24 hours of infection, with only 40% of the cohort showing any significant kidney colonization. However, bacterial shedding in the urine was markedly higher in the Wistar rats, although this difference was not statistically significant. Considering that Sprague Dawley rats have a strong VUR which can confound the assessment of ascending UTI dynamics⁵¹, and the high infection variability that we observed within this strain, Wistar rats were selected for further model optimization.

The UPEC CFT073 strain has been widely studied for its virulence potential and host pathogen interactions, with type 1 fimbriae being identified as a major virulence factor contributing to biofilm formation and bladder colonization⁶³. We found that enriching for type 1 fimbriae expression in CFT073, via consecutive rounds of static subculture, significantly enhanced the bladder colonization and detectable bacterial shedding in the urine at both early (6 hpi) and late (28 hpi) timepoints. While previous models have employed a large volume inoculation (~ 0.5 ml) with high bacterial loads (~ 10e9 bacteria) to manually push the bacteria up to the renal epithelium⁶⁴, we found that a relatively small number of fimbriae enriched CFT073 (~ 100-fold reduction in inoculation load) was successful in establishing pyelonephritis through an ascending mode of infection. This was evident in our cohort where kidney colonization was detectable in 60% of animals at 6 hpi and in all animals by 28 hpi, with bacterial burdens at the later timepoint reaching levels similar to those in the bladder. During model optimization, we did not find it necessary to impose water deprivation or physical manipulation of the bladder to induce micturition prior to bacterial inoculation. In fact, both of these can further trigger VUR and are best avoided, if possible.

While the need for effective oral antibiotics against UTIs is stronger than ever, most of the anti-infective agents in the development pipeline are intravenous compounds that are compared against existing antibiotics during preclinical testing⁵⁹. Intravenous (IV) ciprofloxacin is often used in hospital settings for

the empirical management of serious UTIs, including pyelonephritis⁶⁵. We tested a single dose treatment with IV ciprofloxacin in our UTI model to establish a baseline for future benchmarking studies for novel antimicrobials. A single dose of IV ciprofloxacin (50 mg/kg) was successful in achieving $> 4\text{-log}_{10}$ reduction in bacterial shedding in the urine and within the bladder within 2 hours of treatment, an effect that was maintained up to 24 hours post-treatment. The initial reduction in bacteria also had a significant effect on preventing further kidney infection thus supporting the clinical use of IV ciprofloxacin for the treatment of acute pyelonephritis⁶⁶. A recent study describing the *in vivo* efficacy of gepotidacin, a novel antibacterial agent developed by GlaxoSmithKline plc for UTIs, employed a pyelonephritis model where the bacterial suspension was injected through the skin directly into each kidney⁵⁹. This model included log-phase cultures of several multidrug resistant (MDR) UPEC strains from predominant clonal lineages for the establishment of UTI. While CFT073 is relatively sensitive to most of the commonly used antibiotics⁶⁷, similar enrichment of type 1 fimbrial expression in MDR strains may enhance the colonization potential and allow for the establishment of UTI via the natural route of infection, as previously reported in mice⁴².

Although IV ciprofloxacin administration was associated with significantly reduced bacterial loads, we observed an increase in bladder tissue weights in this group. Further histopathological analysis revealed an increase in immune cell infiltration into the bladder tissue upon infection, alongside localized tissue edema which decreased upon ciprofloxacin treatment. This is similar to observations in mouse models of UTI where immune responses associated with acute UTI are better characterized^{68, 69}. While the increase in bladder weights in the ciprofloxacin treated group was not correlated with histopathology, cytokine profiling showed a more pronounced pro-inflammatory signature in the treated group compared to infected but untreated controls.

The serum and urinary cytokine milieu prevalent during acute UTIs have been extensively documented in humans and within experimental models⁷⁰⁻⁷², whereas infection/treatment associated changes in the immediate tissue microenvironment are often unexplored. In the current study, infection was associated with elevated IL-1 α and MIP-3 α in the bladder. We further observed trends towards increased IL-1 β , IL-6, MIP-1 α , MCP-1 and GRO/KC in the infected group albeit these differences were not statistically significant. Both IL-1 α and IL-1 β are clinically relevant cytokines, elevated during symptomatic and asymptomatic bacteriuria^{72, 73}. On the other hand, increased MIP-3 α (also known as CCL20) can be indicative of renal distress and may mediate innate antimicrobial activity against various uropathogens, including *E. coli*^{74, 75}.

Interestingly, IV ciprofloxacin treatment resulted in significantly increased MCP-1, GRO/KC, MIP-1 α , MIP-3 α , IL-1 α and IL-1 β , compared to infection naïve animals with the latter three cytokines being even higher than the infected animals. This shows an additional inflammatory burden arising out of bactericidal antibiotic treatment, likely due to an increased release of bacterial DNA and associated proinflammatory signaling mediated by TLR9⁷⁶. Ciprofloxacin is often the empirical treatment of choice for acute uncomplicated pyelonephritis and is generally well tolerated. However, there have been instances of

urinary tissue injury⁷⁷ which highlights the importance of studying the inflammatory sequelae of resolving infections.

5. Conclusions

Overall, this study describes a readily available resource for establishing an *in vivo* model of ascending urinary tract infection (cystitis and pyelonephritis) with technical refinements that eliminate the need for invasive procedures. We have further documented the histopathological features and inflammatory biomarkers associated with infection and intravenous ciprofloxacin treatment. However, there are several limitations that have not been addressed and remain outside the scope of the current investigations. These include i) lack of longitudinal evaluation of cytokine responses and histopathology, ii) testing of a single, relatively antibiotic sensitive, UPEC strain, and iii) lack of additional timepoints after the establishment of pyelonephritis. Furthermore, compared to mice, rats are generally more expensive to maintain and study, and may require more specialized handling and care⁷⁸.

There is an urgent and unmet need for innovative antimicrobials targeting common bacterial infections, such as UTIs, that have a potential for significant morbidity and mortality due to the emergence of resistance. Considering that majority of antimicrobials in the development pipeline are intravenous formulations, this model can be readily adapted for pharmacokinetic/pharmacodynamic (PK/PD) studies for test agents. Our team has administered antimicrobials both as intravenous bolus and infusion doses via the tail vein (under anesthesia, 30–60 minutes; data not shown) and found it significantly less invasive with a lower technical threshold than the typical catheterization surgeries required for repeated intravenous dosing.

In summary, this work establishes a robust ascending UTI model in female Wistar rats that supports reliable bladder and kidney colonization, enabling meaningful evaluation of anti-infective therapies. By integrating microbiological, histopathological and cytokine analyses, the model captures both infection dynamics and treatment-associated inflammatory responses. Despite limitations, including the use of a single UPEC strain and restricted timepoints, this platform provides a valuable foundation for PK/PD and efficacy testing of intravenous antimicrobials. As the burden of AMR-driven UTIs continues to grow, such refined preclinical models will be essential for accelerating the development of urgently needed therapeutics.

Abbreviations

UTI- urinary tract infections; AMR- antimicrobial resistance; UPEC- uropathogenic *Escherichia coli*; PK/PD- pharmacokinetic/pharmacodynamic; IV- intravenous; VUR- vesicoureteral reflux.

Declarations

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Author Contributions: SY: methodology, investigation, writing (original draft) SS: conceptualization, methodology, investigation, formal analysis, data curation, writing (review & editing), visualization, supervision & funding acquisition.

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Figures

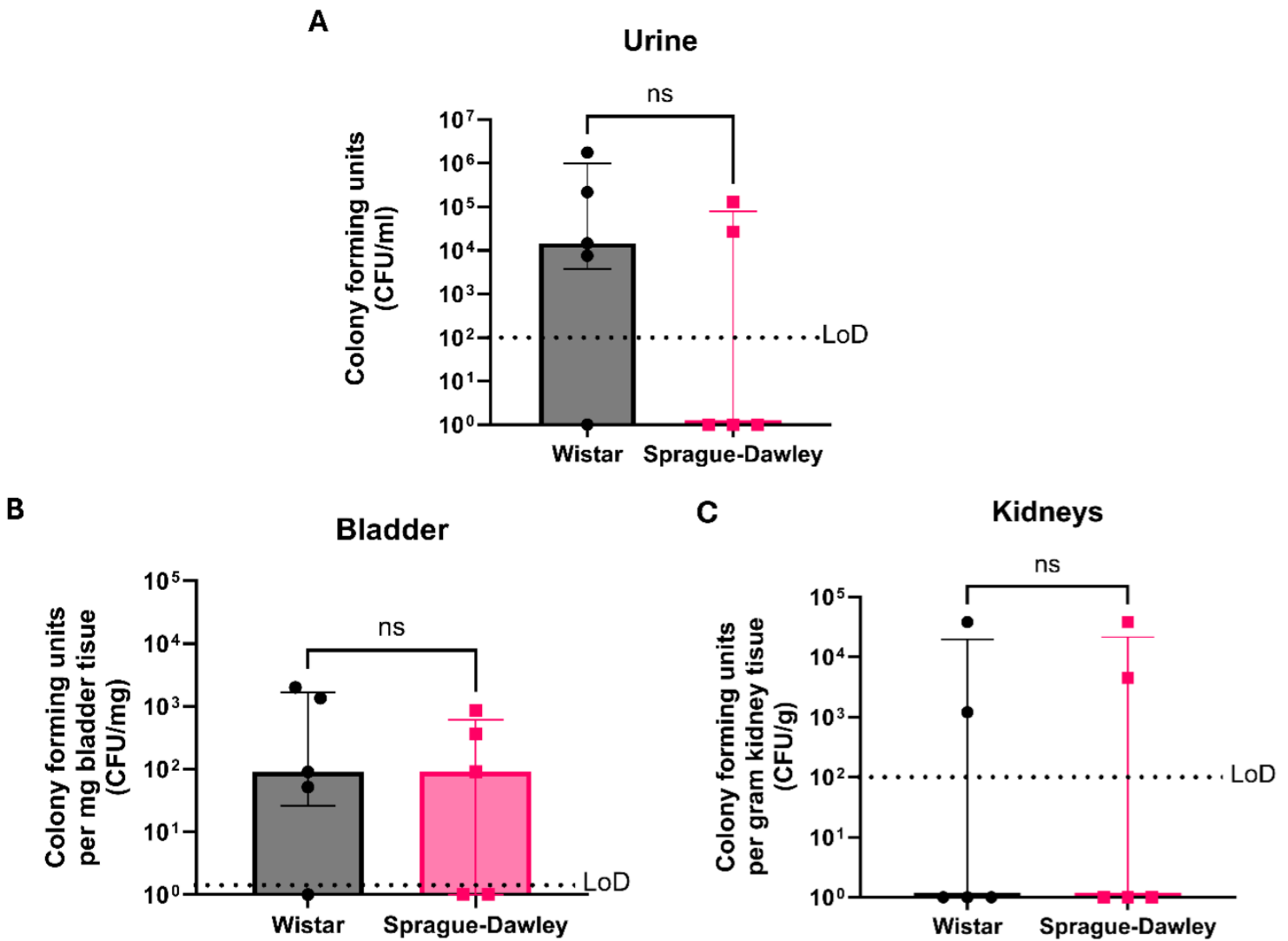


Figure 1

Bacterial colonization at in Wistar and Sprague-Dawley rats at 24 hours post infection (hpi). 8-10 weeks old female rats (n = 5 per group) were transurethrally inoculated with 2 x 10⁷ colony forming units (CFU) of CFT073. Bacterial burden in urine (A), bladders (B) and kidneys (C), were determined at 24 hpi by

colony forming assays. Graphs show medians with interquartile ranges. Each marker is an individual rat. LoD: limit of detection; ns: not significant, $P>0.05$, as determined by Mann-Whitney U test.

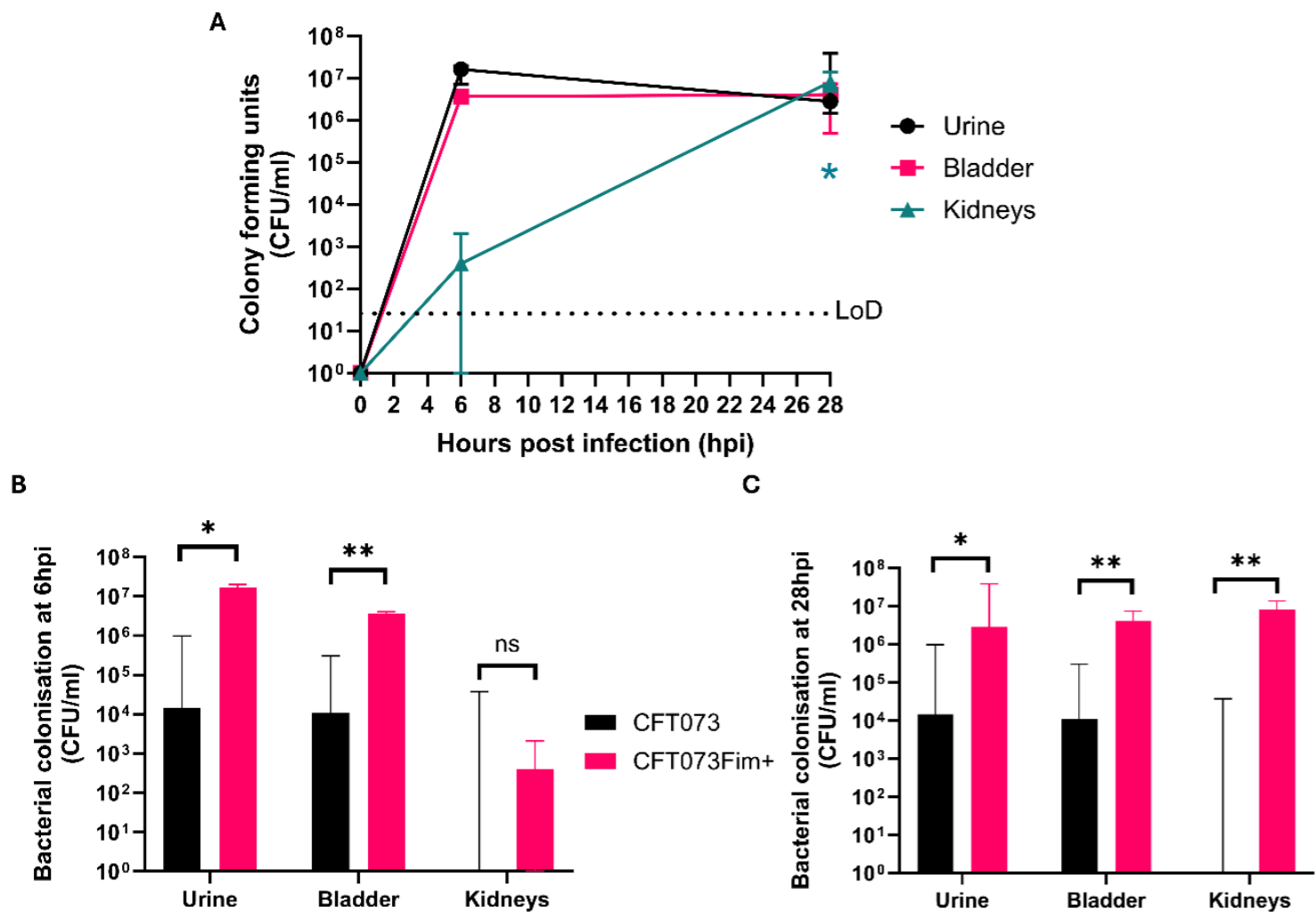


Figure 2

Enhanced urinary tract colonization by type 1 fimbriae enriched *E. coli* CFT073. A) Infection timecourse: 8-10 weeks old female Wistar rats (n = 5 per group) were transurethrally inoculated with 2 x 10⁷ CFU of type 1 fimbriae enriched CFT073 (CFT073Fim+). Total bacterial burden in urine, bladders and kidneys was determined at 6- and 28-hours post infection (hpi) by colony forming assays (expressed as CFU/ml). Line markers show medians with 95% confidence intervals. LoD: limit of detection; * $P<0.05$, as determined by a two-way ANOVA. B, C) Comparison of bacterial burden in urine, bladders and kidneys between CFT073 and CFT073Fim+ at 6 hpi (B) and 28 hpi (C). Bars show group medians with interquartile ranges. * $P<0.05$, ** $P<0.01$, ns- not significant ($P>0.05$) as determined by Mann-Whitney U tests between groups as indicated.

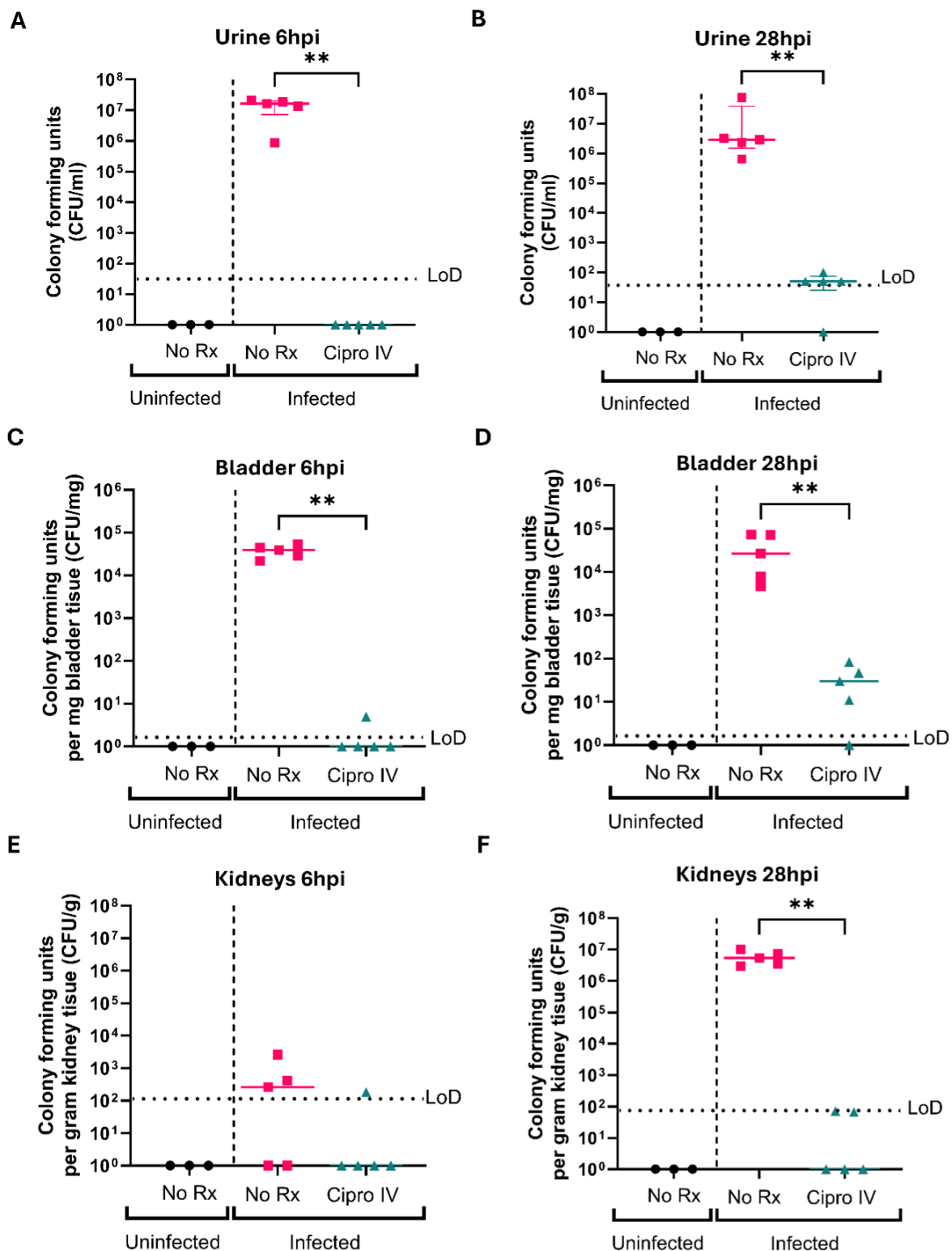


Figure 3

Effect of intravenous ciprofloxacin on bacterial colonization and shedding. 8-10 weeks old female Wistar rats (n = 5 per group) were transurethrally inoculated with 2×10^7 CFU of type 1 fimbriae enriched CFT073 (CFT073Fim+). At 4 hours post infection (hpi), rats were treated with ciprofloxacin (50 mg/kg; administered as an intravenous bolus dose; Cipro IV). Total bacterial burden in urine, bladders and kidneys was determined at 6hpi and 28hpi by colony forming assays. No Rx: Untreated controls; LoD:

(Cipro IV) compared to infected-untreated controls (No Rx). C-E) Analysis of bladder histopathological markers: immune cell infiltrates (C), localized tissue edema (D) and total histopathology score (E). ns: not-significant ($P>0.05$), * $P<0.05$, as determined by Mann-Whitney U tests between groups shown.

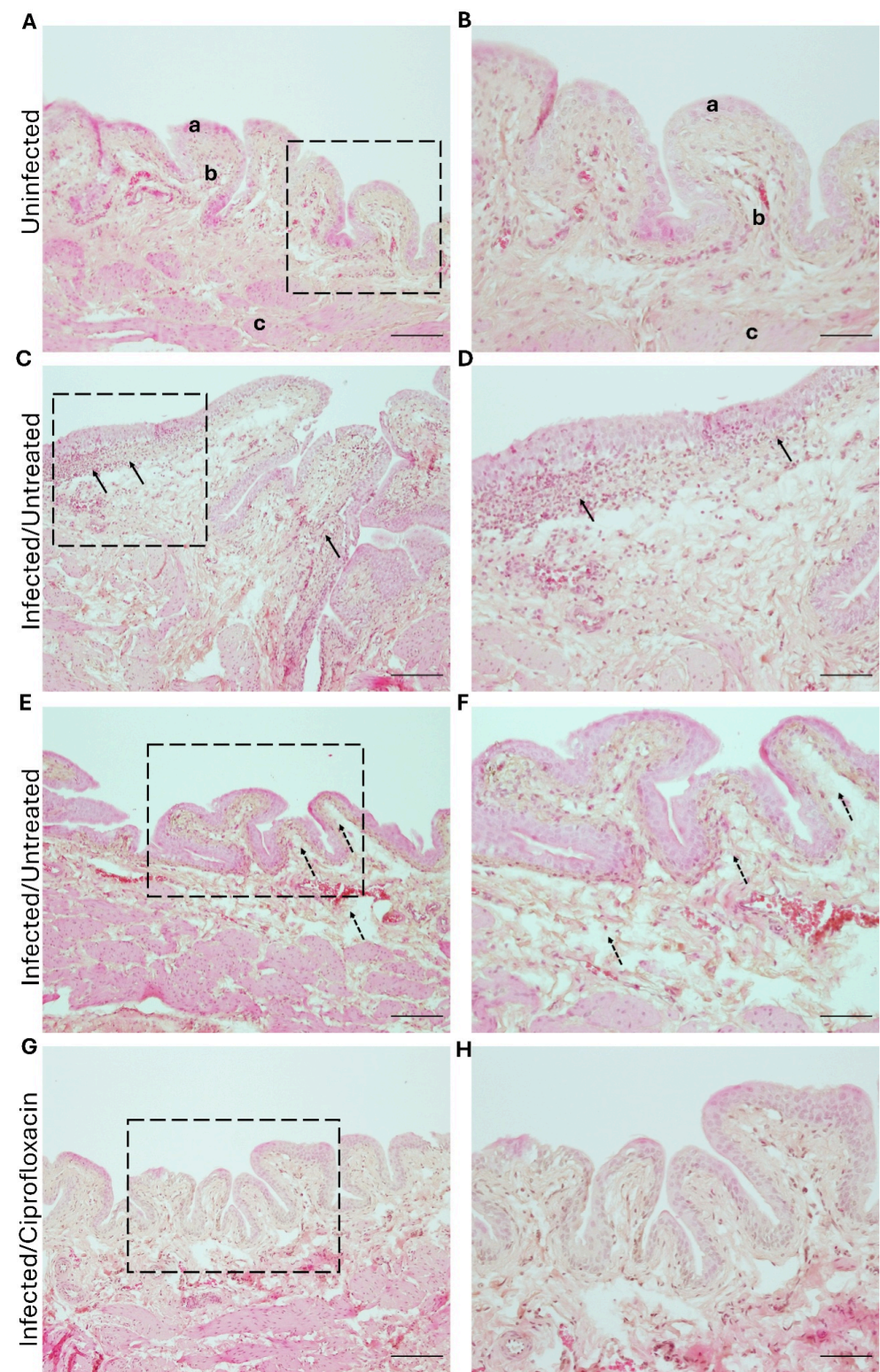


Figure 5

Bladder histopathology images of female Wistar rats. Hematoxylin and eosin-stained transverse sections of bladder halves encompassing the urothelium (a), lamina propria (b) and detrusor muscle (c) layers. A-B) Uninfected controls (n = 3); C-F) Infected-untreated group (n = 5); G-H) Infected- ciprofloxacin treated group (n = 5). Left panels at 100X (scale bar 50 μ m) and right panels at 200X (scale bar 100 μ m) with dashed boxes showing region of magnification for right panels. Solid arrows (C, D) show immune cell infiltrates and dashed arrows (E, F) show localized tissue edema.

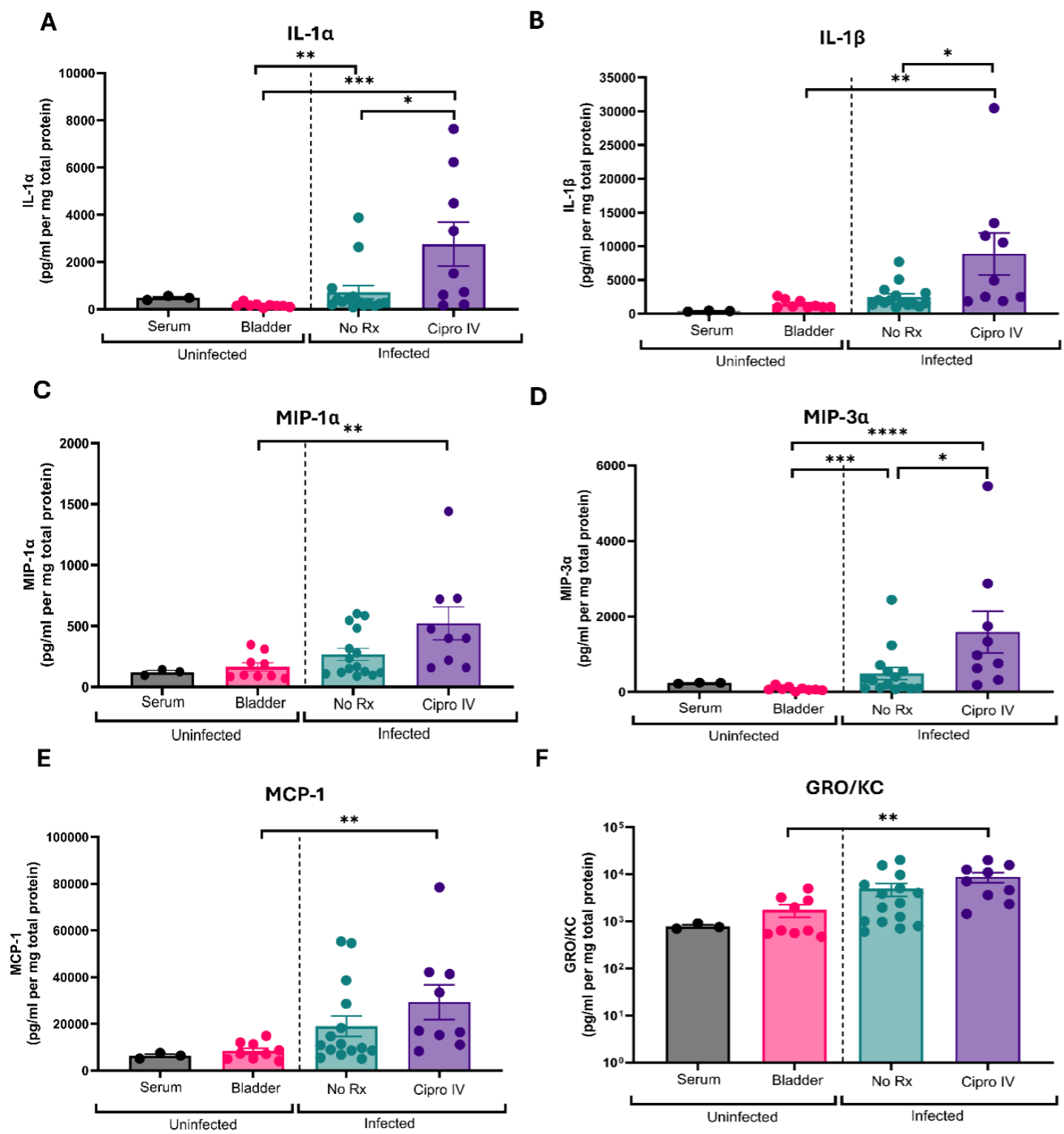


Figure 6

Multiplex cytokine analysis of bladder homogenates. Levels of bladder cytokines (as labelled) at 28 hpi, normalized to total protein content in samples. Rat sera from age-matched infection-naïve animals are included as baseline controls (shown in grey). The samples included are from at least two separate experimental repeats. Each marker represents an individual animal. Bars show group means with standard errors. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; as determined by Mann-Whitney tests between indicated groups.

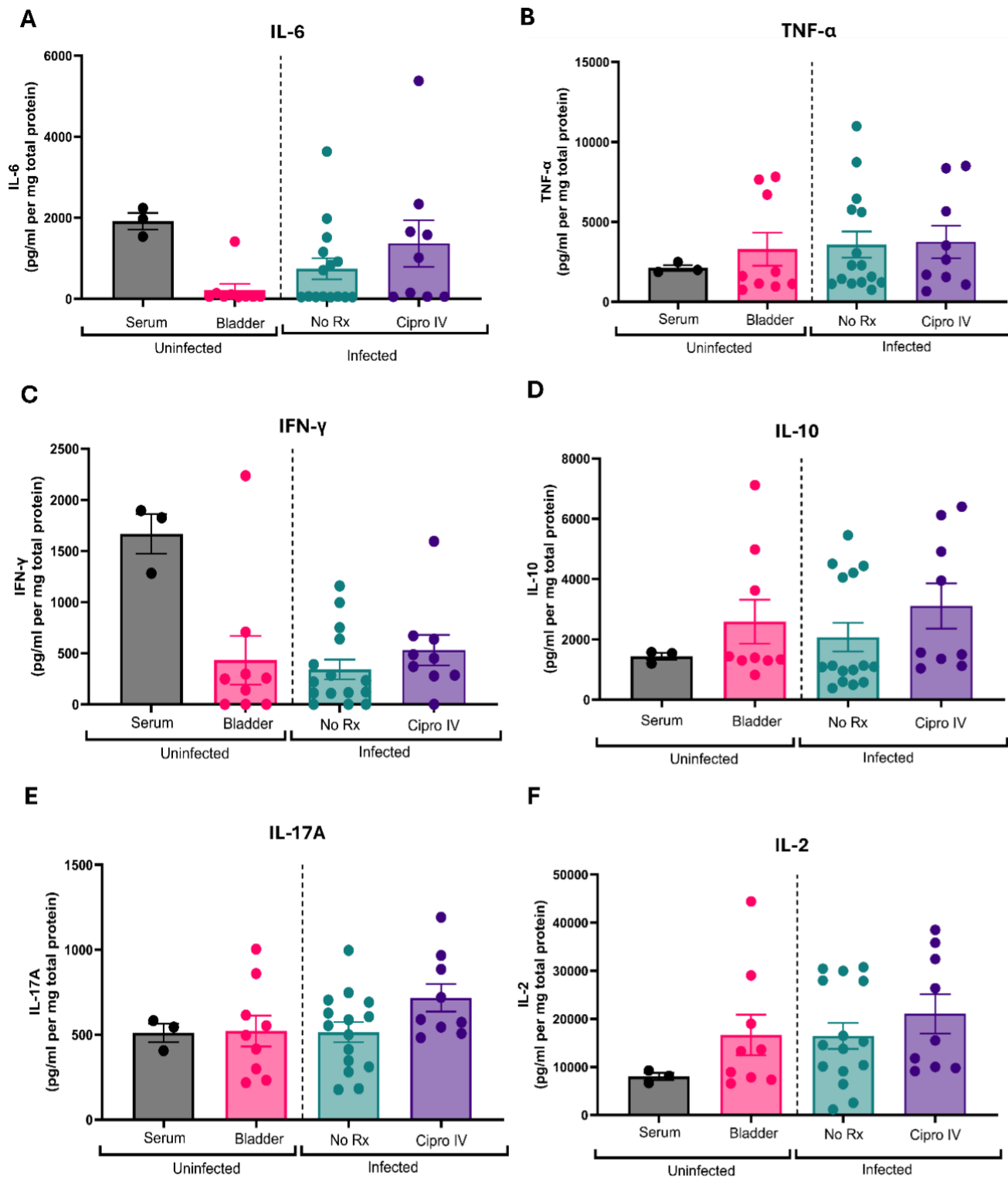


Figure 7

Multiplex cytokine analysis of bladder homogenates. Levels of additional UTI-relevant bladder cytokines (as labelled) at 28 hpi, normalized to total protein content in samples. Rat sera from age-matched infection-naïve animals are included as baseline controls (shown in grey). The samples included are from at least two separate experimental repeats. Each marker represents an individual animal. Bars

show group means with standard errors. No significant differences were observed between the experimental groups (Mann-Whitney tests).

Supplementary Files

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