Video Article

Establishment and Characterization of UTI and CAUTI in a Mouse Model

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Abstract

Urinary tract infections (UTI) are highly prevalent, a significant cause of morbidity and are increasingly resistant to treatment with antibiotics. Females are disproportionately afflicted by UTI: 50% of all women will have a UTI in their lifetime. Additionally, 20-40% of these women who have an initial UTI will suffer a recurrence with some suffering frequent recurrences with serious deterioration in the quality of life, pain and discomfort, disruption of daily activities, increased healthcare costs, and few treatment options other than long-term antibiotic prophylaxis. Uropathogenic *Escherichia coli* (UPEC) is the primary causative agent of community acquired UTI. Catheter-associated UTI (CAUTI) is the most common hospital acquired infection accounting for a million occurrences in the US annually and dramatic healthcare costs. While UPEC is also the primary cause of CAUTI, other causative agents are of increased significance including *Enterococcus faecalis*. Here we utilize two well-established mouse models that recapitulate many of the clinical characteristics of these human diseases. For UTI, a C3H/HeN model recapitulates many of the features of UPEC virulence observed in humans including host responses, IBC formation and filamentation. For CAUTI, a model using C57BL/6 mice, which retain catheter bladder implants, has been shown to be susceptible to *E. faecalis* bladder infection. These representative models are being used to gain striking new insights into the pathogenesis of UTI disease, which is leading to the development of novel therapeutics and management or prevention strategies.

Video Link

The video component of this article can be found at https://www.jove.com/video/52892/

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections and can be divided into two categories based on the mechanism of acquisition, community and nosocomial acquired UTI. Community-acquired UTIs often occur in otherwise healthy women and studies have shown that approximately 50% of women will have at least one UTI in their lifetime [1]. Additionally, recurrence is a major problem. A woman who has an initial acute infection has a 25-40% chance of having a second infection within six months despite appropriate antibiotic treatment and many women continue to have frequent recurrences [2]. The bacteria that cause these infections are also becoming increasingly antibiotic resistant further confounding treatment protocols [3-6]. UTI affect millions of individuals every year costing approximately 2.5 billion dollars in health care related expenses in the US, underscoring the impact and prevalence of the disease [1,7].Nosocomial acquired UTIs are mainly associated with the presence of foreign bodies such as indwelling catheters. Catheter-associated UTIs (CAUTI) remain the most common nosocomial acquired UTI, accounting for ~70-80% of such infections [8]. Furthermore, CAUTI is associated with increased morbidity and mortality and it is the most common cause of secondary bloodstream infections [9].

UPEC associated community acquired UTIs are thought to be caused by the introduction of bacteria into the bladder from reservoirs in the gastrointestinal tract via mechanical manipulation during sexual intercourse, poor hygiene or other microbial population dynamics between different host niches [10]. Once inside the bladder, UPEC employ numerous virulence factors, including capsule, iron acquisition systems, toxins, a virulence plasmid, tRNAs, pathogenicity islands and colonization factors that have been shown to play a role in pathogenesis [11-14]. Critical to the establishment of UPEC colonization, UPEC also encode multiple types of adhesive chaperone usher pathway (CUP) pili that recognize receptors with stereochemical specificity [15]. Type 1 pili, tipped with the FimH adhesin, are expressed by UPEC and bind mannosylated uroplakins [16] and α-1, β-3 integrins [17], which are expressed on the luminal surface of both human and mouse bladders [18]. These FimH-mediated interactions facilitate bacterial colonization and invasion of the superficial epithelial cells[19,20]. Once inside the cell, UPEC can escape into the cytoplasm where a single bacterium can rapidly divide to form an intracellular bacterial community (IBC), which upon maturation, can contain ~10^4 bacteria [21]. IBC formation has been demonstrated in at least six different mouse strains, C3H/HeN, C3H/HeJ, C57Bl/6, CBA, FVB/NJ and BALB/c, and with a wide variety of different UPEC strains and other Enterobacteriaceae [22-24]. However temporal and spatial differences of IBC formation can vary depending upon the mouse background and the infecting UPEC strain. In C3H/HeN mice infected with the prototypical UPEC strains UTI89 or CFT073, IBC formation can be visualized as small biomasses of bacteria as early as 3 hpi (hr post infection). This community continues to expand and reaches a “midpoint” of development approximately 6 hpi when the rod shaped bacteria occupy a large percentage of the cytoplasmic space of terminally differentiated superficial umbrella cells These early IBCs form in a relatively synchronous manner with the majority displaying similar dimensions and morphologies. ~8 hpi the bacteria in the IBC change from a bacilli to cocci morphology. IBCs are transient in nature. Thus, IBC maturation from 12-18 hpi results in continued expansion of the bacterial population, followed by their filamentation and dispersal out of the cell with subsequent spread to neighboring cells [23]. Thus, the IBC niche allows for rapid bacterial growth in

an environment protected from host immune responses and antibiotics [25]. The distinct stages of UPEC infection that are seen in mice are also observed in humans, such as IBCs and filamentation, supporting the mouse model of UTI as a beneficial tool that can be used to model UTI in humans [22,26-28].

While a majority of women experience a UTI in their lifetime, the outcome of these infections can range from acute self-limiting infection with no recurrence, to frequent recurrent cystitis. Further, studies have shown a strong familial occurrence of UTI, suggesting a genetic component contributes to UTI susceptibility [29]. We have found that the differing UTI outcomes seen in clinics can be mirrored by the differing outcomes of experimental UPEC infection among inbred mouse strains [30]. For example, C3H/HeN, CBA, DBA, and C3H/HeOuJ mice are susceptible, in an infectious dose-dependent manner, to long-lasting, chronic cystitis characterized by persistent, high titer bacteria (>10^4 colony forming units (CFU)/ml), high titer bacterial bladder burdens at sacrifice >4 weeks post-infection (wpi), chronic inflammation, and urothelial necrosis . These mice also display elevated serum levels of IL-6, G-CSF, KC, and IL-5 within the first 24 hpi that serve as biomarkers for the development of chronic cystitis. This may accurately represent the natural course of UTI in some women, as placebo studies have shown that a large percentage of women experiencing UTI will retain high levels of bacteria in their urine for several weeks after the first symptoms of cystitis if not given antibiotic treatment [31,32]. Further, using C3H/HeN mice, we found that a history of chronic cystitis is a significant risk factor for subsequent severe recurrent infections. Recurrent UTI is the most significant clinical manifestation of UTI and the C3H/HeN mouse is currently the only studied model that recapitulates an increased predisposition after previous exposure. A second UTI outcome is recapitulated in C57Bl/6 mice where acute UPEC infection is self-limiting, with resolution of bladder inflammation and bacteriuria within approximately a week. Interestingly, in this model, UPEC readily form quiescent intracellular reservoirs within the bladder tissue from which UPEC are capable of emerging from a dormant state to reinitiate an active UTI, potentially explaining one mechanism for same strain recurrent UTI in humans [33,34].

In addition to genetic influences on UTI susceptibility, introduction of a catheter into the bladder greatly increases the likelihood of having an infection as well as increasing the range of bacteria able to cause an infection. It has been demonstrated that human urinary catheterization causes histological and immunological changes in the bladder due to mechanical stress that results in a robust inflammatory response, exfoliation, edema of the lamina propria and submucusa, urothelial thinning, and mucosal lesion of the urothelium and kidney [35,36]. Additionally, the catheter provides a surface for bacterial attachment thereby creating an environment utilized by several species to cause CAUTI. While UPEC are still a major contributor, *Enterococcus faecalis* accounts for 15% of these CAUTI [37]. *E. faecalis* is becoming increasingly resistant to antibiotics with vancomycin resistance emergence, posing a serious health concern [38]. *E. faecalis* possess numerous virulence factors including toxins and adhesins necessary for attachment to both the catheter and epithelium [38]. During urinary catheterization, the host is vulnerable to microbial adhesion, multiplication and dissemination in the urinary tract [39,40]. *E. faecalis* forms a biofilm on the catheter as part of a mechanism to persist in the bladder and disseminate to the kidneys, which is reproduced in a mouse CAUTI model[41]. Recently, it has been shown during urinary catheterization, fibrinogen (Fg) is released into the bladder as part of the inflammatory response. Fg accumulates in the bladder, coats the catheter and is essential for *E. faecalis* biofilm formation, functioning as an attachment scaffold. In a C57BL/6 mouse model of CAUTI, we discovered that *E. faecalis* biofilm formation on the catheter, and thus persistence in the bladder, was dependent on the Ebp pilus, specifically its tip adhesin EbpA. We found that the N-terminal domain of EbpA specifically binds to Fg coating the catheter. Additionally, it was found that *E. faecalis* utilizes Fg as metabolite source during infection, thus enhancing biofilm formation [42].

Mouse models have proven critical to understanding as well as predicting clinical manifestations of UTI and CAUTI [41]. In this article we demonstrate inoculum preparation of the cystitis UPEC isolate UTI89 and transurethral inoculation of C3H/HeN mice. Additionally, we demonstrate a protocol for catheter insertion in C57BL/6 mice and inoculation of the *E. faecalis* OG1RF strain. Both of these techniques lead to consistent and reliable UTI or CAUTI in mice. We also display techniques used to observe IBC formation during acute cystitis and urine collection for the analysis of chronic or recurrent cystitis. C3H/HeN mice have been used to study aspects of UPEC pathogenesis including initial bacterial invasion, IBC formation, filamentation and the development of chronic cystitis [23,33,43]. These virulence parameters have also been studied in a variety of other mouse backgrounds [22,33]. For CAUTI, the C57BL/6 model allows for foreign body implantation into the bladder with subsequent bacterial colonization, which can be maintained for 7 days post infection [41]. These models have been useful for assessing bacterial virulence mechanisms, host responses to UTI and mechanisms to subvert host responses, much of which has been subsequently recapitulated or observed in clinical human populations.

Protocol

Ethics statement: The Washington University Animal Studies Committee approved all mouse infections and procedures as part of protocol number 20150226, which expires 12/10/2018. Overall care of the animals was consistent with The Guide for the Care and Use of Laboratory Animals from the National Research Council and the USDA Animal Care Resource Guide. Euthanasia procedures are consistent with the “AVMA guidelines for the Euthanasia of Animals 2013 edition.”

1. UPEC UTI Protocol, Inoculation Needle Preparation (Figure S1)

1. Remove the cap of the 30 G needle. Thread approximately 1 inch of PE10 tubing onto the shaft of the needle. UV sterilize the needle assemblies overnight. Catheterized needles can be stored indefinitely in sterile petri plates.

2. UPEC Bacterial Inoculum Preparation

1. Prepare UTI89 inoculum (start 72 hr prior to the inoculation day)
    1. Streak UTI89 onto an LB (Luria-Bertani) agar plate from a frozen stock. Incubate overnight at 37 °C. Pick a colony and inoculate it into 10 ml of LB in a 125 ml flask. Incubate at 37 °C for 24 hr, statically.
    2. Inoculate 10 µl of overnight culture into 10 ml of LB in a new 125 ml flask for an additional 24 hr at 37 °C, statically.
    3. Transfer 3 ml (will vary based on strain and desired inoculum size) to sterile 1.5 ml tubes and centrifuge at 7,000 x g for 3 min and resuspend the pellet in 1x PBS and centrifuge a second time. Resuspend the bacterial pellet in 1 ml 1x PBS.

2. Measure bacterial optical density and adjust concentration to the desired inoculum density. The standard concentration of the inoculum used is 10^7 bacteria; however, this may vary due to the design of the study.

3. Bacterial Inoculation

1. Clean the workstation with 70% ethanol and cover area with absorbent paper (or use sterile flow hood).
2. Draw up to 0.9 ml of the prepared bacterial inoculum into a 1 ml (TB) syringe (remove air bubbles). Attach a prepared sterile inoculation needle with PE10 tubing, onto the syringe containing the inoculum, then sterilely trim the polyethylene tubing.
   NOTE: Leave 1 mm of tubing above the tip of the needle to avoid puncturing the bladder.
3. Cut a 1 inch square piece of parafilm and put a dab of surgical lubricant (approximately the size of a dime) on top.
4. Anesthetize female C3H/HeN mice by putting them in a vaporizer chamber (following manufactures protocol) until unconscious but still breathing normally (1 breath/sec).
   NOTE: Some IACUC committees do not approve the use of a vaporizer. Please follow the indication of the IACUC committee of your institution.
   CAUTION: Isoflurane is an inhalation anesthetic. Use in a well-ventilated area and minimize inhalation.
5. Remove the mouse from the vaporizer and place it on its back on a paper towel and spread the legs.
6. Cover the nose of the mouse with a nose cone (a tube connected to the vaporizer that provides a controlled isoflurance dose that comes equipped on some vaporizer units) to maintain anesthetization.
7. Gently palpitate the bladder to induce urination and ensure a voided bladder. Wipe the periurethral area with 100% ethanol wipe. Dab the inoculation needle/syringe, point first, into the surgical lubricant.
8. Inoculate each mouse with 50 µl of the bacteria solution by inserting the inoculation needle transurethrally, approximately 12 mm, and pressing down on the syringe plunger gently to dispense the inoculum into the bladder gently (10 µl/sec). Remove the inoculation needle from the mouse.
   NOTE: Immediate return of inoculum at the urethral opening when beginning to inoculate indicates improper or incomplete insertion of the needle.
9. Remove the mouse from nose cone and return it to its cage. Repeat steps 3.3-3.8 for each mouse. When/if switching inoculum conditions/ strains, dispose of the syringe and inoculation needle in an approved sharps container and start again step 3.2.
   NOTE**:** Inoculation with uropathogenic organisms generally does not cause severe pain symptoms. However, in rare instances, administering large doses of pathogens may cause fever, reduction in food and water intake and abnormal behavior. Animal health should be monitored throughout the experiment. If overt pain symptoms are notice, an analgesic, such as Buprenorphine (0.05−0.1 mg/kg given subcutaneously), can be applied. Procedures should be in accordance with each institution's IACUC.

4. Determination of Bacterial Burdens

1. Prepare separate 5 ml tubes for each bladder and kidneys pair to be harvested. Add 1ml of 1x PBS/tube/mouse bladder to be harvested. Add 0.8 ml of 1x PBS/tube/pair of mouse kidneys to be harvested. Label each tube to correspond to a given mouse. Prepare a 96-well plate containing 180 µl of 1x PBS in each well for 1:10 serial dilutions.
2. Clean the work area with 70% ethanol and cover the area with an absorbent cover or paper towel.
3. Anesthetize the mouse with isoflurane (see step 3.4) until the mouse stops breathing for approximately 1 min, then place it on the absorbent cover or paper towel. Other methods of euthanasia are also accepted by IACUC committees, such as carbon dioxide, and can be substituted for isoflurane overdose.
4. Sacrifice the mouse by rapid cervical dislocation that consists of restraining the neck at the base of the head and pulling the tail horizontally away from the body until dislocation occurs.
   NOTE: Most IACUC committees require a secondary means of euthanasia and cervical dislocation is a common method. However, other methods can be used if approved by the IACUC committee.
5. Place the dead mouse on its back and spray 70% ethanol on the abdomen. Dissect the mouse, harvest the bladder and kidneys, in that order to minimize potential contamination from the blood following the kidney dissection. Place the organs independently into the prepared 5 ml tubes containing 1x PBS (see step 4.1).
   NOTE: Use different scissors for the external dissection and for organ harvesting to minimize contamination.
6. Tap the tube to ensure the organs are in the 1x PBS. Repeat step 4.3-4.5 for each mouse. Clean scissors and forceps in 70% ethanol after each mouse.

5. Bacterial Recovery

1. Homogenize harvested bladder and kidneys in the 5 ml tubes with a tissue homogenizer, 15 sec per kidney and 30 sec per bladder. Rinse homogenizer sequentially in 1x PBS, 70% ethanol and 1x PBS between samples.
2. Make serial 1:10 dilutions out to 10^{-8} and plate each dilution for CFU (colony forming units) on LB agar plates. Incubate the plates at 37 °C overnight and count colonies the next day.

6. IBC Enumeration

1. Aseptically remove the bladder and place it in 1x PBS in a 6-well plate with a silicone bottom. Plates can be prepared using a silicone elastomer kit and pouring approximately 1 cm of silicone in each well, see manufactures instructions. Cut the bladder in half using scissors.
2. Using small metal pins gently splay the bladder so that the bladder lumen is facing upward. Be sure to place the pins at the outermost edges of the bladder sections to ensure the maximal amount of urothelium is exposed.

3. After splaying, gently wash the bladder once with 1x PBS. Remove the 1x PBS by pipet. Fix the bladder by adding enough 3% paraformaldehyde to cover the entire splayed bladder. CAUTION: Paraformaldehyde is carcinogenic. Proper protective equipment, including gloves, should be worn at all times. Disposal should follow institutional guidelines.
4. Incubate at room temperature for 1 hr. Remove paraformaldehyde solution. Wash once with 1x PBS.
5. Wash with LacZ wash solution (2 mM $MgCl_2$, 0.01% sodium deoxycholate and 0.02% nonidet-p40in 1x PBS) three times for 5 min per wash.
6. Remove wash solution and add enough LacZ stain (9.5 ml LacZ wash solution, 0.4 ml 25 mg/ml X-gal and 0.1 ml of 100 mM K-ferrOcyanide/ K-ferrIcyanide) to cover the bladders. Incubate at 30 °C overnight protected from light.
7. Remove splayed bladders from incubator and observe IBCs, which appear as blue puncta as visualized with a dissecting scope, 40-60X magnification.
   NOTE: Sometimes a mouse may urinate prior to placement of the tube under the urethra. In this case, wait 15-20 min before attempting to collect urine again.

7. Urine Collection for Bacteriuria CFU Enumeration (Not Applicable for CAUTI)

1. To collect urine pre or post infection gently restrain the mouse by holding the tail and placing it on a flat elevated surface (top of mouse cage).
2. Hold a sterile 1.5 ml tube under the mouse urethra. Gently press down on the back of the mouse near the tail to apply pressure to the bladder. Catch the urine in the 1.5 ml tube.
3. Make serial 1:10 dilutions out to 10^{-7} and plate each dilution on appropriate LB. Incubate the plates at 37 °C overnight and count colonies the next day.

8. CAUTI Model Protocol, Catheter Needle Preparation for CAUTI Model (Figure S2)

1. Remove the cap of the 30 G needle. Cut a 7 mm piece of PE10 tubing and 5 mm piece of silicone tubing such as RenaSIL. Thread the needle with PE10 tubing until the tubing touches the base of the needle.
2. Then feed the 5 mm piece onto the PE10-containing needle. UV sterilize the needles assemblies overnight.
   NOTE: When loading the silicone tubing, leave approximately 1 mm of the tubing extending past the needle tip.

9. *E. faecalis* OG1RF Bacterial Inoculum Preparation

1. Prepare OG1RF inoculum starting 48 hr prior to the inoculation day. Streak OG1RF onto a BHI (brain heart infusion) plate from a frozen stock.
2. Pick a colony and inoculate it into 10 ml of BHI and grow it statically for 18 hr at 37 °C. Centrifuge the culture and wash the pellet (3 times) in 1 ml volumes of sterile 1x PBS.
3. Resuspend the bacterial pellet into 1x PBS. Measure bacterial optical density and adjust the concentration to the desired inoculum size. The standard concentration of the inoculum used is 10^7; however, this may vary due to the design of the study

10. Catheter Implantation

1. Clean the workstation with 70% ethanol and cover the area with an absorbent cover (or use sterile flow hood).
2. Attach catheter-needle to an empty 1 ml (TB) syringe. Cut a 1 inch square piece of parafilm and put a dab of surgical lubricant (approximately the size of a dime) on top.
   NOTE: Two different needles are used for deposition of the catheter and for the inoculum to minimized accidental inoculation due to the mechanical manipulation necessary for catheter implantation. This also allows for the convenience of preparing fewer inoculation needles.
3. Anesthetize C57BL/6 mice by putting them in a 32 ounce glass jar containing a tea-infuser ball with cotton balls soaked in 3 ml of isoflurane or vaporizer chamber (following manufactures protocol) until unconscious but still breathing normally (1 breath/sec).
4. Then put the mouse on its back on a paper towel and spread the legs. Cover the nose of the mouse with a nose cone or 50 ml conical tube with cotton balls containing a small amount (approximately 1-2 ml) of isoflurane.
   CAUTION: Isoflurane is an inhalation anesthetic. Use in a well-ventilated area and minimize inhalation by researcher.
5. Gently palpitate the bladder to induce urination and ensure a voided bladder. Wipe periurethral area with a 100% ethanol wipe.
6. Disinfect the periurethral area with 10% povidone-iodine solution using a cotton-tip applicator. Dab the inoculation needle/syringe, point first, into the surgical lubricant.
   NOTE: Povidine-iodine is used for catheter implantation, which is a stronger aseptic solution than alcohol. Catheter implantation requires more mechanical manipulation to insert the catheter and thus a greater potential for contamination.
7. Insert the catheter into the urethral opening. Once in, use tweezers to push the (7 mm PE10) long piece toward the bladder, consequently pushing the (5 mm silicone) small catheter and depositing it into the bladder. Remove the needle, with the 7 mm tubing still attached, immediately.

11. Bacterial Inoculation

1. Follow the bacterial inoculation protocol in section 3, using the prepared inoculum from section 9. Remove the mouse from the nose cone and return it to its cage

12. At Each Time Point of Sacrifice

1. Prepare 5 ml tubes for bladder and kidney (For organs follow step 4.1) and 1.5 ml Eppendorf tube for catheters. Add 1 ml of 1x PBS into 1.5 ml Eppendorf tube for catheter samples

2. Follow steps 4.3 to 4.5. Dissect the mouse, harvesting the bladder, kidneys and catheter, placing the tissues independently into 5 ml tubes containing 1x PBS and the catheter into the 1.5 ml Eppendorf (see step 12.1).
   NOTE: Use different scissors for the external dissection and for organ harvesting and catheter retrieval to minimize contamination
3. Then follow step 4.6.

13. Bacterial Recovery

1. For organs, follow steps 5.1 and 5.2. For bacterial recovery from catheters, vortex at maximum speed for 30 sec, sonicate the catheter samples for 5 min using a bath sonicator, and then vortex at maximum speed for an additional 30 sec.
2. Make serial 1:10 dilutions out to 10^{-8} and plate each dilution for CFU enumeration on BHI plates. Incubate the plates at 37 ºC overnight and count colonies the next day.

Representative Results

The intravesical models of uncomplicated and catheter associated UTI provide flexible platforms for elucidating the molecular mechanisms of bacterial pathogenesis, the impact of these diseases on host tissue, and the development and testing of novel approaches to manage these common and costly infections. Depending on the mouse strain and pathogen, intravesical inoculation can be used to study host-pathogen interactions to elucidate factors necessary for initiating or modulating acute (**Figure 1** and **3**), chronic or recurrent (**Figure 2**) cystitis. The data shown in **Figure 1** are representative of acute stage (24 hpi) urinary tract colonization by the prototypical UPEC cystitis isolate UTI89 [44]. Following inoculation, the infections are allowed to develop for 24 hr at which point the mice are sacrificed and the bladder and kidney tissue homogenized. The tissue homogenates are serially diluted and plated for the enumeration of colonizing bacteria. Chronic UPEC colonization and bladder inflammation can be established and maintained in some mouse lines (notably C3H/HeN) in an infectious dose and uropathogen dependent manner. Chronic cystitis is defined as persistent high titer bacteriuria (>10^4 CFU/ml), bladder inflammation and high titer bacterial bladder burdens (>10^4 CFU/bladder) at sacrifice [33] . The data shown in **Figure 2** are representative CFUs occurring 4 weeks after inoculation of C3H/HeN mice with 2 x 10^7 CFUs of UTI89. Under these experimental conditions, 20-50% of infected mice develop chronic cystitis while the remaining 50-80% of mice resolve the infection. This duality of outcome is dependent on a host-pathogen checkpoint that is decided within the first 24 hpi. Activation (or not) of the checkpoint results in the observed bimodal distribution of bacterial titers, which begins to manifest in the urine at 3 dpi (days post infection) and in the bladder at sacrifice 28 dpi (**Figure 2**) [33]. Mice with a history of chronic cystitis are significantly predisposed to recurrent chronic cystitis upon challenge after the initial infection is cleared with antibiotic therapy [33]. Similarly, a history of UTI is a known risk factor for recurrent UTI in humans. Thus, this is the only known model used to study the pathogenesis of recurrent UTI. When experimentally modeling UTI in alternate mouse backgrounds, it is important to determine if the mouse strain is susceptible to UTI, recurrent UTI or CAUTI, given that some strains are more or less resistant to developing these syndromes [33].

An additional measurement of acute cystitis is IBC enumeration. IBC formation is restricted to the terminally differentiated superficial umbrella cells. IBC quantification is an informative measure of bacterial burden during acute cystitis and high levels of IBCs correlate with the risk of developing chronic cystitis [21]. IBC formation only occurs in the acute stages of disease. Following the exfoliation of the superficial umbrella cells, IBCs are no longer observed. In the C3H/HeN UTI model, IBCs can be measured 6 hours after inoculating ~1 x 10^7 CFU of UTI89 into the bladder transurethrally. Bladders are splayed with metal pins on silicone plates and stained for the expression of LacZ. LacZ, β-galactosidase, is a commonly expressed bacterial enzyme that cleaves the β linkage between galactose and other sugars. This enzyme can be used to detect bacteria by supplying X-gal, a β-galactoside analogue, which produces a blue color upon cleavage. Following staining, the bladders are imaged under a dissecting microscope with the IBCs appearing as punctae blue/purple dots on the urothelium and IBCs can be enumerated by counting the dots (**Figure 3A**). While the number of IBCs formed per animal varies, in the C3H/HeN background an average of ~50 IBCs are seen per bladder (**Figure 3B**). IBCs increase in a dose dependent manner. The number of IBCs that form varies between UPEC and mouse background strains. For example, in C57Bl/6, an inoculum of ~1 x 10^7 UTI89 results in the formation of several hundred IBCs per bladder.

Beyond the uncomplicated community acquired UTI modeled above, heath-care associated UTIs represent a significant burden to the health-care system. UTIs represent approximately 40% of all health-care associated infections and up to 95% of all health-care associated UTIs are catheter associated [45]. Here we demonstrate a bladder implant mouse model of CAUTI that represents a powerful system for the experimental analysis of this difficult clinical problem. The data presented in **Figure 4** are representative of 24 hr infections with the model *E. faecalis* strain OG1RF. When 1 x 10^7 CFU of OG1RF are intravesically inoculated into an unimplanted bladder, the infection begins to clear by 24 hpi (**Figure 4A**) and by 3 dpi the infection has resolved [41]. Conversely, inoculation of OG1RF into an implanted bladder results in colonization of both the catheter and the bladder, with 10 fold higher bladder colonization levels at 24 hpi than those observed in the unimplanted bladders (**Figure 4A** and **4B**). Additionally, this infection is maintained at >10^6 CFU in the bladder until catheter loss approximately 7 dpi [41]. All of the described experimental models are amenable to a high degree of experimental flexibility, including but not exclusive to various approaches to the macroscopic and microscopic imaging of the infected tissues and surfaces, altered inoculum sizes and disease times, competitive infections, and host immunological analyses.

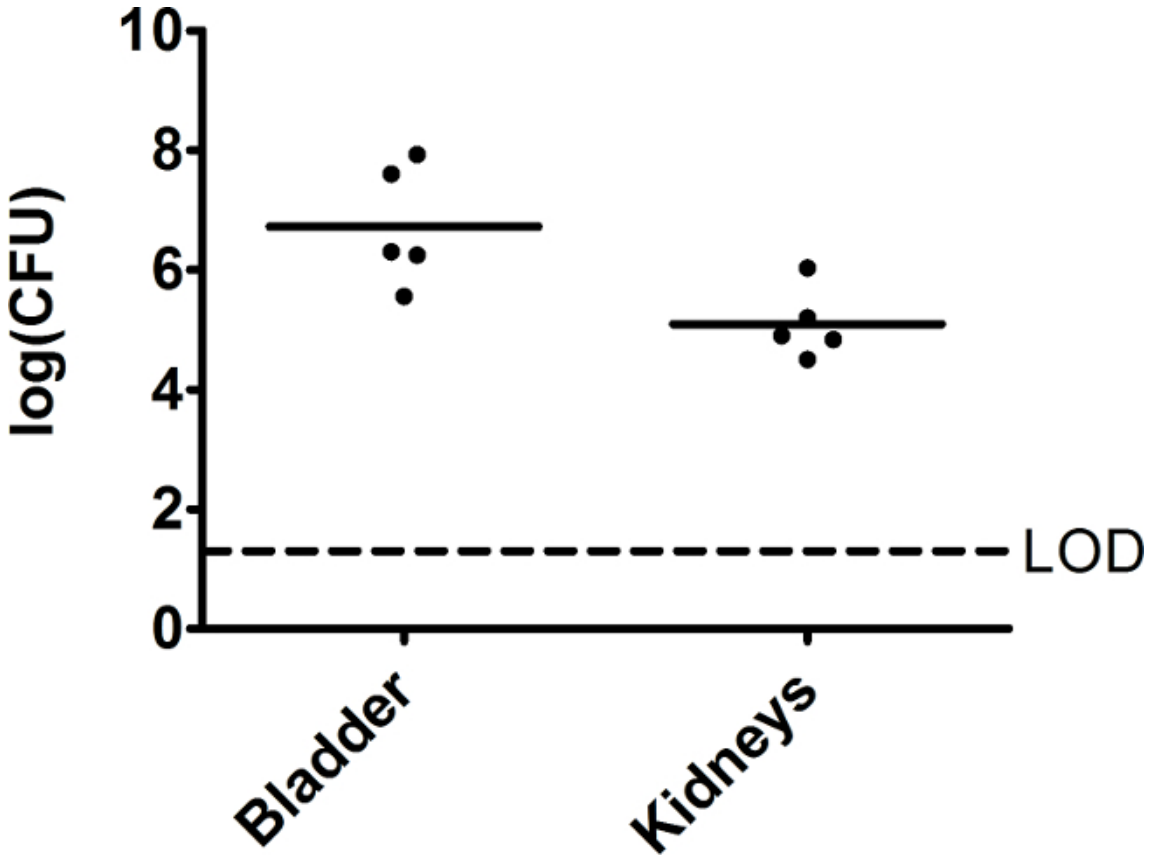

Figure 1: 24 hr urinary tract colonization. Female, 7-8 week old C3H/HeN mice were transurethrally inoculated with ~2 x 10^7 CFU of the prototypical UPEC cystitis isolate UTI89 for 24 hr. Representative CFUs recovered from the harvested bladder and kidney tissues are shown, n = 5. Dashed line denotes the lower limit of detection (20 CFU).

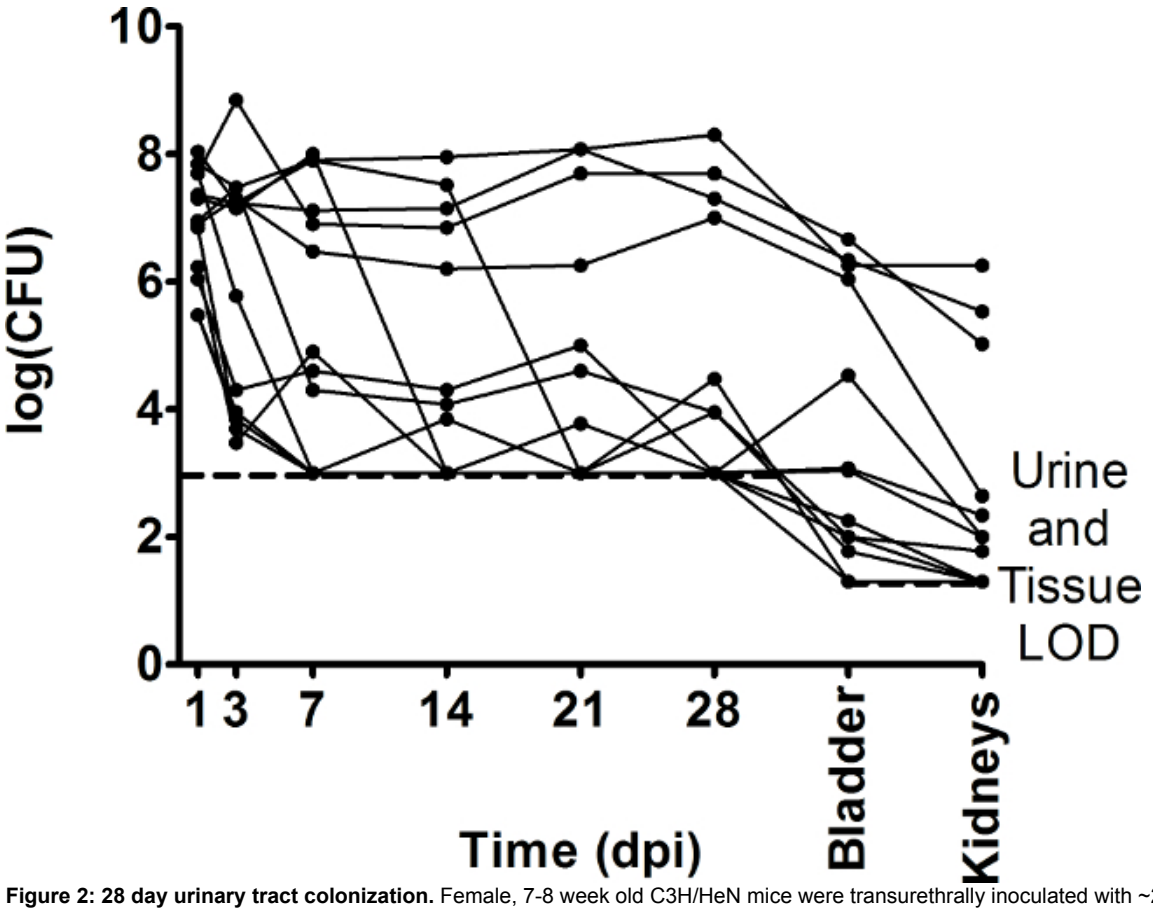

Figure 2: 28 day urinary tract colonization. Female, 7-8 week old C3H/HeN mice were transurethrally inoculated with ~2 x 10^7 CFU UTI89. Urine was collect at indicated days post infection (dpi) and plated for CFU enumeration. Representative 28 day bladder and kidney titers are also displayed, n = 8. Urine CFU lower limit of detection (1,000 CFU/ml) is denoted by the dashed line. Dotted line represents the tissue lower limit of detection, 20 CFU/tissue.

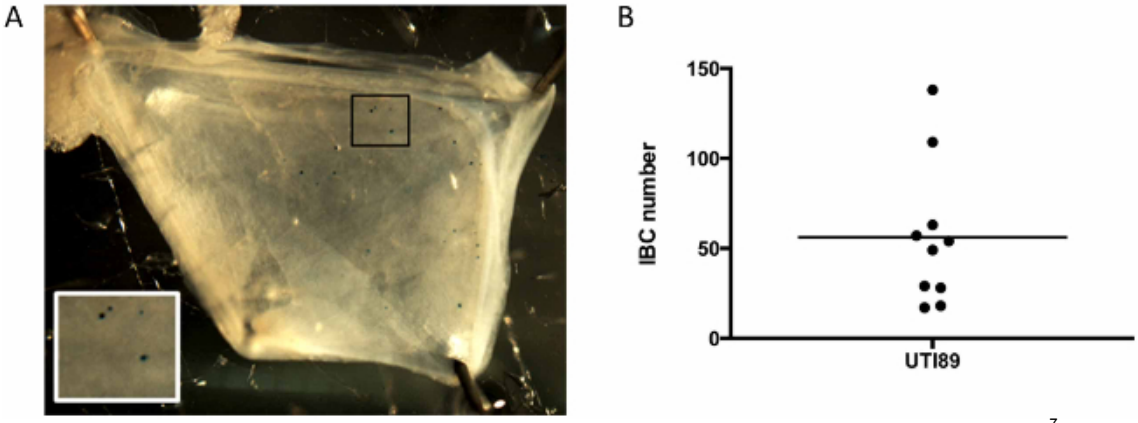

Figure 3: IBC enumeration. Female, 7-8 week old C3H/HeN mice were transurethrally inoculated with ~1 x 10^7 CFU UTI89. Bladders were harvested 6 hpi and splayed for IBC staining. (**A**) Image of splayed bladder after LacZ staining. IBCs are represented as blue dots. The inset image is a digital magnification of the section of the image in the black box. (**B**) Representative numbers of IBCs formed in C3H/HeN mice 6 hpi, n = 10.

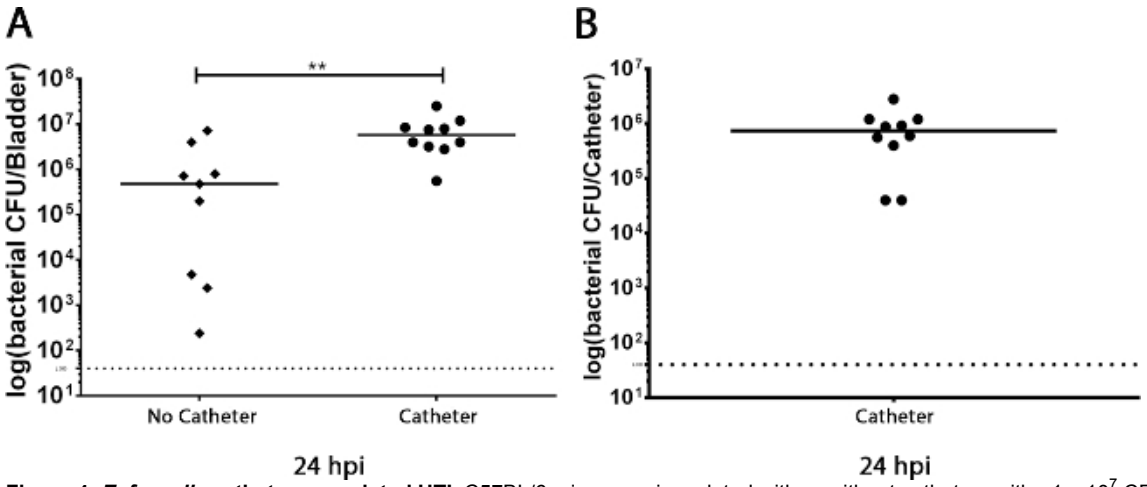

Figure 4: *E. faecalis* catheter-associated UTI. C57BL/6 mice were inoculated with or without catheters with ~1 x 10^7 CFU of the *E. faecalis* strain OG1RF for 24 hr. (**A**) *E. faecalis* bladder colonization in the presence or absence of catheter. (**B**) *E. faecalis* catheter colonization. Mann-Whitney *U* test was used for mouse experiments, p <0.05 was considered statistically significant. **, p <0.005.

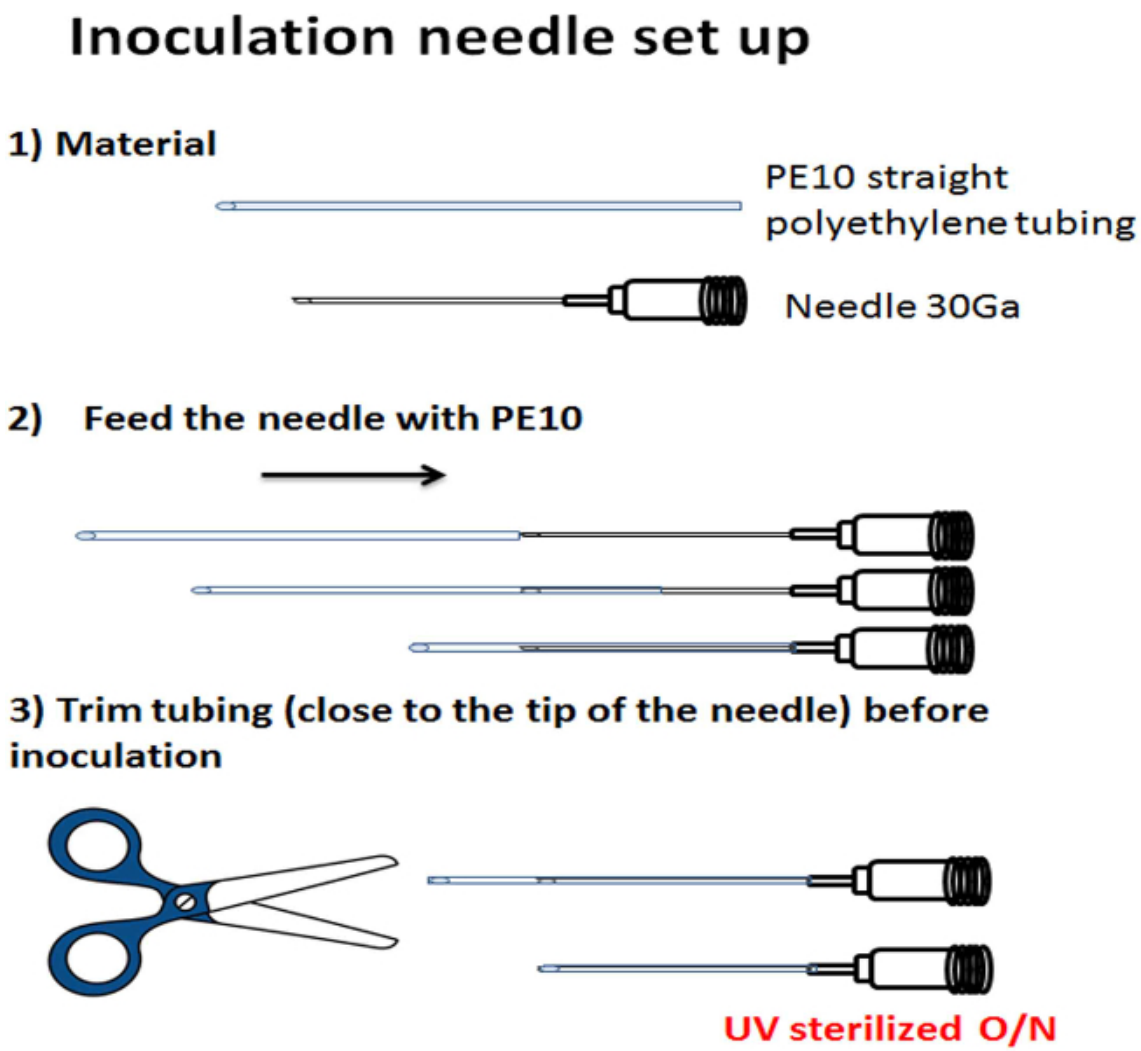

Figure S1: UTI inoculation needle preparation. Diagram displays inoculation needle preparation steps including materials needed (1), threading of the catheter on to the needle (2) and trimming the tubing before inoculation (3).

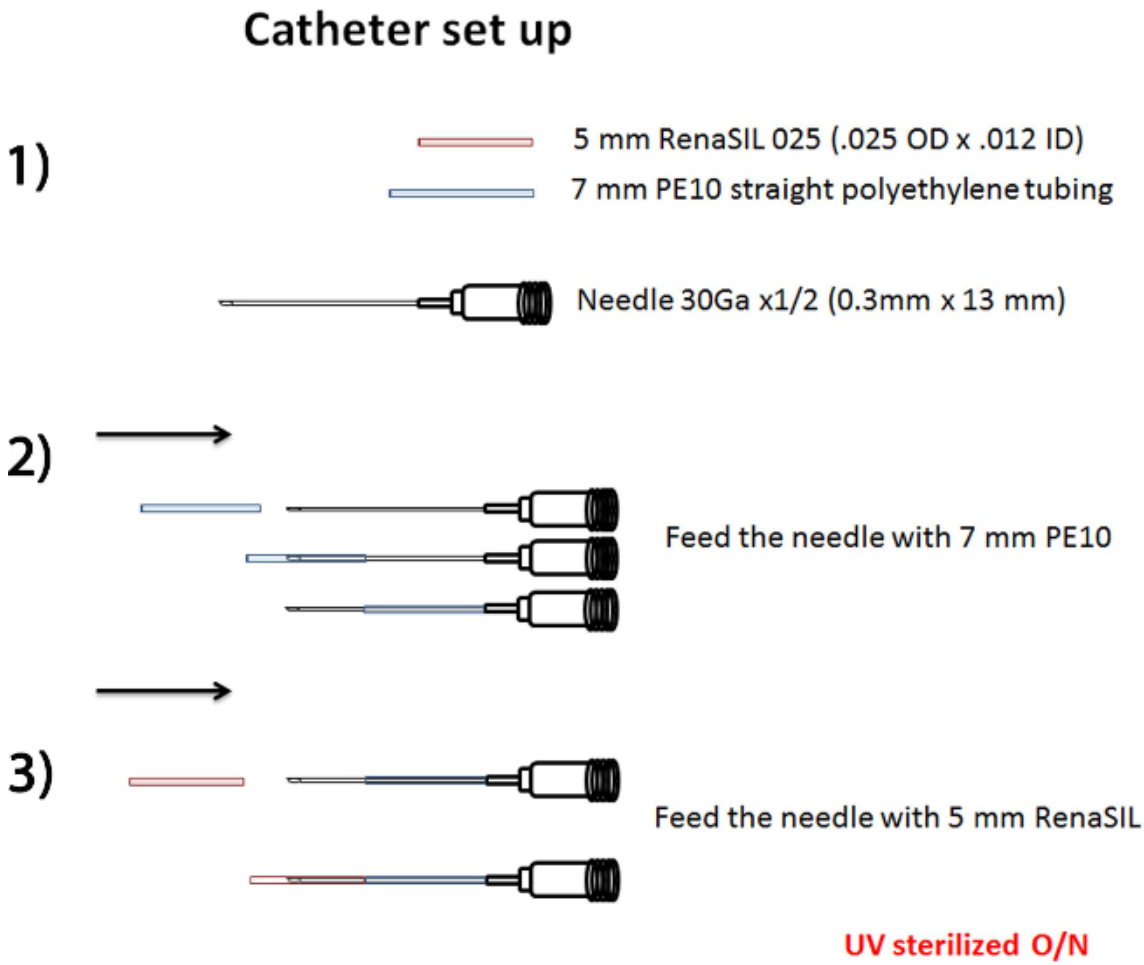

Figure S2: CAUTI inoculation needle preparation. Diagram displays inoculation needle preparation materials needed (1), threading of the inoculation catheter (2) and implantation catheter onto the needle (3).

Discussion

Uncomplicated community acquired UTI is a common and costly infection accounting for several million primary care visits every year [46]. In addition, CAUTIs are a common healthcare acquired infection that has become extremely costly to healthcare providers as the Centers of Medicare and Medicaid Services no longer reimburses providers for the added cost of treatment resulting from hospital acquired CAUTI [45]. The mouse models of UTI, both uncomplicated cystitis and CAUTI, described in these protocols provide invaluable tools for understanding the molecular basis of the host and pathogen interactions necessary for initiating, maintaining, and modulating the outcome of several forms of UTI. These models allow for the application of powerful bacterial and mouse genetics, immunological, microscopic, biochemical and chemical genetic tools to the analysis of UTI [23,47-49]. Additionally, the protocols outlined here have provided a platform for the development and testing of novel therapeutic and preventative strategies including small molecule inhibitors of bacterial virulence and novel vaccination targets and strategies [50-55].

There are several critical conditions that must be maintained for the successful application of the uncomplicated cystitis model. As with all mouse anesthetization procedures, care must be taken to prevent mouse mortality due to over-anesthetization. When preparing the inoculation catheter, the silicone tubing must be sufficiently long to cover the tip of the needle but not so long as to damage the wall of the bladder on catheterization. Additionally, due to the proximity and size of the vaginal opening, care must be taken to insert the inoculating catheter into the urethra and not the vagina. During bladder splaying for IBC enumeration, it is essential that the bladders be splayed as quickly as possible following dissection and the tissue be fixed for approximately 1 hr, but not overnight. For IBC enumeration by LacZ staining, it is also necessary that the bacterium in question produce LacZ during urothelial cell colonization. When using strains where the LacZ enzyme is not produced, it is still possible to enumerate IBCs using immunofluorescence microscopy with anti-*E. coli* antibodies or GFP expressing bacteria. One particular feature of this model is the differing patterns of disease progression and severity presented by different inbred mouse lines and the varying pathogenic potential of the prototypical UPEC strains [33,56]. These differences are both a strength and limitation of this technique as they recapitulate the natural variation of host susceptibility and pathogen diversity that has been observed in clinical and phenotypic studies while complicating experimental design and interpretation [57,58]. These variations provide sources of comparison allowing for the elucidation of host and bacterial mechanisms modulating the pathogenesis of uncomplicated UTI [33,56]. However, they also mean that, due to the varying degree of mouse line UTI susceptibility, utmost care must be taken in choosing the correct mouse line and UPEC strain for addressing the particular question an investigator is asking. For example, C57BL/6 mice are an excellent model of acute infection, presenting with high bacterial titers and large numbers of IBCs at early time-points. This strain then rapidly resolves the infection between 3 and 7 dpi with the only persistent colonization

resulting from severe kidney infections and quiescent intracellular reservoirs [33,34]. The C57BL/6 model may accurately represent the most common outcome for many UTI patients in which symptoms present and then resolve. Conversely, the C3H/HeN mouse background exhibits a high degree of susceptibility to chronic cystitis making them an ideal strain for studying recurrent UTIs . Other UTI models have also been established in CBA, DBA and C3H/HeJ mice background proving useful for studying particular aspects of UTI [33,59]. The primary experimental drawback exhibited by the uncomplicated cystitis model is the existence of multiple infection bottlenecks limiting the application of large scale genetic screening methodologies for the discovery of novel bacterial factors necessary for acute, chronic and recurrent cystitis [60].

The critical considerations for the uncomplicated cystitis model all apply to the implant based CAUTI model with two additional concerns. First, care must be taken to ensure that the insertion of the implant takes place in a sterile fashion, due to the increased susceptibility to bladder infection conferred by the implant. Second, it is essential that the catheter be held in place during removal of the implanting needle. The major limitations of the CAUTI model are that it has a decreased range of available host genetic backgrounds, the propensity for catheter loss over time and the inability to collect urine due to bladder dysfunction resulting from implantation. To compensate for implant loss and the inability to monitor the infection via longitudinal urinalysis, this model generally requires higher numbers of catheterized mice for later time points and groups of mice must be sacrificed at each desired time point. The limited host genetic backgrounds results from the prohibitive frequency at which some mouse strains, such as C3H/HeN, lose the implant (P. Guiton, Personal Communication 2010). As a result, the CAUTI protocols described above have solely been optimized in the C57BL/6 mouse strain. However, while the limitation of available mouse strains for CAUTI is unfortunate, many genomic variations have been established in the C57BL/6 background and the CAUTI model expands the bacteriological diversity available for study during UTI by altering the bladder environment to facilitate colonization by organisms that are otherwise non-pathogenic in the murine model of UTI. An example of this, is the recent discovery of the role of Fg released during catheter induced inflammation of the bladder in *E. faecalis* growth, biofilm formation, and persistence during CAUTI [42]. This example illustrates the power of the implant model of CAUTI in elucidating not only bacterial molecular mechanisms of pathogenesis but also the role of host factors during the infection. All of these features contribute to understanding this relevant clinical infection, which has been previously understudied.

In addition to the experimental approaches described above, the mouse models of uncomplicated cystitis and CAUTI are amenable to a large number of methodological modifications. In the case of the uncomplicated cystitis model, these modifications can include reducing the inoculation volume and the force applied during the instillation of the inoculum to reduce the number of bacteria introduced into the kidneys [61], varying the duration of the infection, sacrificing at time points as early as 1 hpi and using gentamicin protection assays to assess the degree to which the uropathogen has invaded the bladder epithelial cells [62]. The splayed bladders from mice sacrificed between 6 and 24 hpi can also be sectioned and stained with antibodies to various host and/or bacterial epitopes and observed under fluorescent or confocal microscopy to observe IBC structure, bacterial fluxing and filamentation and host tissue condition [25,33,63,64]. The effect of uropathogen infection and/or implantation on the local and systemic immune responses of the host can be assessed using the Bioplex cytometric bead system or ELISA based assays to determine cytokine levels in the urine or serum respectively [33,65]. Finally, the condition of the bladder tissue, location and morphology of colonizing bacteria and the structure of the biofilm deposited on the catheter can be assessed by various imaging techniques including electron microscopy [25,41].

These basic models of infection can also be adapted to mimic other clinically relevant phenomena including recurrent UTI and the development of an adaptive immune response via multiple infections. In this context, repeated infection of C57BL/6 mice results in increased resistance to acute cystitis while in the C3H/HeN model, the prior development of chronic cystitis followed by clearance of the infection via the administration of antimicrobials sensitizes these mice to develop chronic cystitis on subsequent inoculations [66]. Additionally the molecular mechanisms of risk factors for the development of UTI can be examined. Diabetes and obesity have both been shown to increase the frequency and severity of UTIs. Type 2 diabetes can be genetically modeled in mice in the FVB/NJ background and a chemically induced model of type 1 diabetes has already been shown to increase the severity of UTIs caused by both UPEC and *K. pneumophila* [67,68].

Finally, these model systems represent an ideal proving ground for the development and optimization of therapeutic modalities aimed at treating or preventing UTIs and CAUTIs. The molecular understanding of host pathogen interactions provided by these models has already facilitated the development of effective vaccination strategies aimed at targeting important virulence factors [51-55]. Additionally, small molecule inhibitors of UPEC adhesion have been shown to be effective in the treatment of uncomplicated acute and chronic UTIs as well as CAUTIs caused by UPEC [12,69,70]. Both the uncomplicated UTI and CAUTI models described in these protocols provide the experimental tools to dissect the molecular details of the host-pathogen interactions in the urinary tract. These tools can be leveraged in many ways to expand our understanding of this complex set of conditions and facilitate the development of more effective therapeutic interventions.

Disclosures

The authors declare that they have no competing financial interests.

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