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Infection and Immunity

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# Characterization of a Novel Murine Model of *Staphylococcus saprophyticus* Urinary Tract Infection Reveals Roles for Ssp and SdrI in Virulence▽

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***Staphylococcus saprophyticus*, an obligate human pathogen, is the most common Gram-positive causative agent of urinary tract infection (UTI) in young, healthy women. Despite the clinical importance of *S. saprophyticus*, little is known about how it causes disease in the urinary tract or how the host responds to the infection. Here we established an *in vivo* model to study both host and bacterial factors contributing to *S. saprophyticus* UTI. Using this model, we show that *S. saprophyticus* preferentially infects C3H/HeN murine kidneys instead of the bladder, a trait observed for multiple clinical isolates. Bacterial persistence in the kidneys was observed in C3H/HeN mice but not in C57BL/6 mice, indicating that host factors strongly contribute to the ability of *S. saprophyticus* to cause UTI. Using C3H/HeN mice as a model, histologic and immunofluorescence analyses of infected tissues revealed that *S. saprophyticus* induced epithelial cell shedding in the bladder and an inflammatory response characterized by macrophage and neutrophil infiltration in the bladder and kidneys. The inflammatory response correlated with increased production of proinflammatory cytokines and chemokines in both the bladder and the kidneys. Finally, we observed that the putative *S. saprophyticus* virulence factors Ssp and SdrI were important for persistence, but not for initial colonization, in the murine urinary tract. Thus, we characterized both host and bacterial factors involved in progression of *S. saprophyticus* UTI, and we describe a useful model system for studying factors involved in the pathogenesis of this Gram-positive uropathogen.**

Urinary tract infections (UTI) affect over 11 million women annually in the United States (11). The primary cause of UTI is the Gram-negative bacterium *Escherichia coli*. However, the Gram-positive bacterium *Staphylococcus saprophyticus* can cause up to 10 to 15% of uncomplicated UTI (33). Thus, it is estimated that *S. saprophyticus* causes up to 1 million UTI each year and is the second most common cause of UTI in sexually active women (41). Moreover, Gram-positive bacteria, such as *S. saprophyticus*, often coexist with dominant uropathogens in the urine of infected patients, although the concentrations are lower, and therefore tend to be overlooked by routine laboratory diagnostics (35). Thus, the reported estimates of the incidence of this organism may be artificially low. Interestingly, there is a seasonal pattern for *S. saprophyticus* UTI; such infections peak during late summer and fall, a pattern similar to that observed for sexually transmitted diseases (23). Other Gram-positive bacteria that cause UTI include *Enterococcus faecalis* and *Enterococcus faecium* (3). In contrast to *S. saprophyticus*, *Enterococcus* spp. cause UTI in healthy young women infrequently, but they contribute to ~19% of complicated UTI and are often nosocomially acquired (31). Despite the fact that *S. saprophyticus* is the predominant cause of Gram-positive UTI, relatively little is known about how this organism causes disease in the urinary tract.

Only two *S. saprophyticus* gene products have been shown to be virulence factors *in vivo*. Aas, a hemagglutinin-autolysin-adhesin (15) that binds to fibronectin and human ureters *in vitro* (8, 28), has been implicated in colonization of rat kidneys (7). A second protein, urease, is important for efficient colonization of the bladder and kidneys, for inflammation in the bladder, and for dissemination to the spleen in a rat model of UTI (5). Several other putative virulence factors have been characterized *in vitro*, including extracellular slime; lipoteichoic acids, which are implicated in adherence to urothelial cells (4); a cell wall-anchored protein (UafA) that may act as an adhesin for bladder cells (4, 22); a surface-associated lipase (Ssp) that forms fimbria-like surface appendages (6, 37); and a surface-associated collagen-binding protein (SdrI) that shares sequence and structural homology with the adhesive Sdr proteins of *Staphylococcus aureus* and *Staphylococcus epidermidis* (36, 45).

A major limitation in assessing the contributions of *S. saprophyticus* virulence factors to pathogenesis is the lack of a well-characterized *in vivo* model. However, an enterococcal UTI mouse model has been described in which *E. faecalis* displays a tropism for the kidney, where it can persist for at least 2 weeks (21, 42). Upon establishment of infection, a Toll-like receptor 2-independent inflammatory infiltrate composed primarily of monocytes, as determined by histological

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TABLE 1. *S. saprophyticus* strains used in this study

| Strain | Resistance | Isolation source | Reference |
|---|---|---|---|
| 7108 | None | Urine of patient with UTI | 7 |
| 7108 Δ*ssp* | Erm$^r$ | | 37 |
| 7108 Δ*sdrI* | Erm$^r$ | | 36 |
| Top 58 | None | Urine of patient with cystitis | This study |
| Top 6 | None | Urine of patient with cystitis | This study |
| Top 76 | None | Urine of patient with cystitis | This study |

staining, is recruited to areas of *E. faecalis* colonization in the kidney (21). Modest induction of the proinflammatory marker macrophage inflammatory protein 2 (MIP-2) (a mouse orthologue of human interleukin-8 [IL-8]) and suppressor of cytokine signaling 3 (SOCS-3), a modulator of cytokine induction, was observed in the kidney at 6 and 24 h postinfection (p.i.) (21). In contrast, no studies examining the host response to *S. saprophyticus* UTI have been reported.

Therefore, in this study, we sought to characterize the infection dynamics of and host response to *S. saprophyticus* in mice. Our goal was to establish a robust model to study both bacterial and host factors involved in *S. saprophyticus* pathogenesis. When developing this model, we found that *S. saprophyticus* has a preference for the kidneys over the bladder in the C3H/HeN mouse strain and that the virulence factors Ssp and SdrI are important for persistence during infection.

## MATERIALS AND METHODS

**Strains and growth conditions.** All strains used in this study are shown in Table 1. For infection, *S. saprophyticus* strains were grown statically overnight (14 to 18 h) at 37°C in brain heart infusion (BHI) medium (Difco) with antibiotics when appropriate. Clinical strains Top58 (= SJH-732), Top6 (= SJH-726), and Top76 (= SJH-735) were isolated from women with cystitis and were provided by Thomas Hooton (University of Miami) and Walter Stamm (University of Washington).

**Mouse infection and CFU enumeration.** Bacterial cultures, grown as described above, were collected by centrifugation at 6,000 × *g* for 10 min and resuspended in phosphate-buffered saline (PBS) to a density of approximately $2 \times 10^8$ CFU/ml; the final inoculum used was $1 \times 10^7$ CFU in 50 μl/mouse. Female wild-type mice that were 7 to 10 weeks old were obtained from Harlan (C3H/HeN mice) or the National Cancer Institute (C3H/HeN MTV$^-$ and C57BL/6 mice). Mice were anesthetized by inhalation of 4% isoflurane and inoculated transurethrally as described elsewhere (16, 29). At the time points indicated below, mice were euthanized, and bladders and kidneys were aseptically removed. The numbers of bacteria present in tissues were determined by homogenizing bladders or kidney pairs in PBS and plating serial dilutions on BHI agar (Bacto agar; BD) supplemented with antibiotics when appropriate. Statistical analyses were performed using the Mann-Whitney U test with GraphPad Prism software (version 4.00 for Windows; GraphPad Software). The titers below the limit of detection of the assay were defined as 1 for statistical analyses. All animals were housed with a cycle consisting of 12 h of light and 12 h of darkness and had access to standard food and water *ad libitum*. All animal studies were performed in accordance with the guidelines of the Committee for Animal Studies at Washington University School of Medicine or the Ethical Committee for Animal Experiments in Stockholm.

**Histology and immunofluorescence microscopy.** For histological analysis, bladders and kidneys were fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 5-μm-thick sections, and stained with hematoxylin and eosin (H&E). For immunofluorescence staining, organs were immediately embedded in Tissue Tek OCT compound (Sakura Finetek) and frozen for sectioning. Ten-micrometer sections were fixed in ice-cold acetone, blocked in 3% bovine serum albumin-0.3% Triton X-100 in PBS, and stained to determine the presence of bacteria using antibodies raised against *S. saprophyticus* Aas (9). Hematopoietic cells were detected with anti-CD45 antibody (BD Pharmingen). Secondary antibodies were labeled with Alexa Fluor (Molecular Probes). Tissue was counterstained with bis-benzimide (Sigma) to determine nuclear morphology. All imaging was performed at the Washington University Molecular Microbiology Imaging Facility with a Zeiss Axioskop 2 MOT Plus fluorescence microscope operated by Axiovision software. All images were obtained using matched acquisition settings and were prepared using Adobe Photoshop.

**Cytokine measurement.** *S. saprophyticus* or PBS (mock infection) was inoculated into mouse bladders, and organs were homogenized in 1 ml PBS at the times p.i. indicated below. Homogenates were spun at 14,000 rpm for 5 min, and the supernatants were frozen at −20°C until the assay was performed. Cytokine expression in organ supernatants was measured using a Bio-Plex multiplex cytokine bead kit (Bio-Rad). In preliminary studies, 23 proinflammatory cytokines were assessed by examining the bladders and kidneys of mice infected with either Gram-negative uropathogenic *E. coli* (UPEC) strain UTI89 (30), Gram-positive *E. faecalis* strain 0852 (21), or *S. saprophyticus* UTI strain 7108. IL-2, IL-3, IL-4, IL-6, IL-9, IL-10, IL-13, granulocyte-macrophage colony-stimulating factor (GM-CSF), and gamma interferon (IFN-γ) were not induced significantly in infected mice compared to mock-infected animals (data not shown). Thus, a custom 12-plex kit was developed; this kit contained antibodies to mouse IL-6, KC, IL-12p40, IL-12p70, tumor necrosis factor alpha (TNF-α), IL-1β, granulocyte colony-stimulating factor (G-CSF), IL-17, eotaxin, monocyte chemoattractant protein 1 (MCP-1), MIP-1β, and RANTES, which were induced during murine UTI (17). Kidney supernatants were diluted 10-fold to obtain the protein concentration recommended by the manufacturer. Two or three organs were pooled for each measurement, as indicated below. Statistical analyses were performed using the one-sample Student *t* test (GraphPad Prism).

**Flow cytometry.** For flow cytometric analysis, bisected bladders or quartered kidneys were incubated for 1 h at 37°C with 1 mg/ml collagenase D (Sigma) and 100 μg/ml DNase (Sigma) and then manually passed through a 100-μm cell strainer to obtain a single-cell suspension. Cell suspensions were treated with red blood cell lysing buffer (Sigma) to lyse red blood cells, washed in PBS, and fixed in 1% paraformaldehyde. Samples were stained with anti-F4/80-fluorescein isothiocyanate (FITC) (eBiosciences, San Diego, CA) and anti-Gr1-allophycocyanin (APC) or anti-CD45-phycoerythrin (PE) antibodies (BD Pharmingen, San Jose, CA). A BD FACScan cytometer (BD Biosciences) was used to acquire flow cytometric data, and data were analyzed using FlowJo software (Treestar). Statistical analyses were performed using the Student *t* test (GraphPad Prism).

## RESULTS

***S. saprophyticus* is rapidly cleared from the bladder but persists in the kidneys of C3H/HeN mice.** To establish a comprehensive *in vivo* model of *S. saprophyticus* pathogenesis, we first characterized the infection kinetics of strain 7108, which was isolated from a patient with an acute UTI (7) (Table 1). The murine model for uropathogenic *E. coli* (UPEC) UTI utilizes a 50-μl inoculum (16), whereas the model for *E. faecalis* UTI required a 200-μl inoculum to obtain reproducible infection (21). We empirically determined that $10^7$ CFU of *S. saprophyticus* in a 50-μl inoculum was the lowest dose that established consistent infection in 100% of the animals when it was transurethrally instilled into the bladders of C3H/HeN female mice (data not shown). Therefore, C3H/HeN mice were infected with $10^7$ CFU in 50 μl, and the numbers of bacterial CFU in the urinary tract were assessed over a 2-week time course.

In the bladder (Fig. 1A), the majority of the inoculum was cleared by 6 h postinfection (p.i.), at which time the geometric mean level of recovered bacteria was $3.8 \times 10^2$ CFU/bladder. The bacterial titers in the bladder at 1 h and 3 h p.i. were $3.6 \times 10^2$ and $5.1 \times 10^2$ CFU/bladder, respectively, for C3H/HeN mice (see Fig. 3A), indicating that *S. saprophyticus* clearance is very rapid. The bacterial titers in the bladder decreased over time and were below the level of detection by 14 days p.i. In contrast, the level of *S. saprophyticus* in the kidneys was $4.3 \times 10^4$ CFU/mouse at 6 h p.i. (Fig. 1B). The bacterial titers in the kidneys decreased gradually but persisted at $\sim 10^3$ CFU/mouse at 14 days p.i. At every time point examined, the number of *S.*

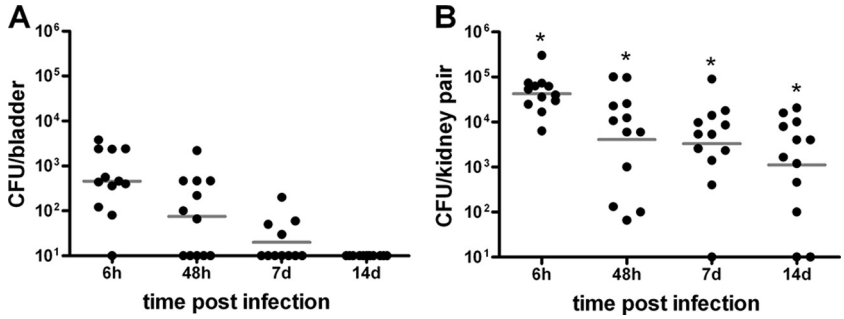


FIG. 1. *S. saprophyticus* strain 7108 is rapidly cleared from the bladder but persists in the kidneys of C3H/HeN mice. *S. saprophyticus* was instilled into the bladders of C3H/HeN mice (12 mice/time point). Bladders (A) and kidneys (B) were removed and homogenized, and 10-fold serial dilutions were plated to determine the number of CFU/organ. Each circle indicates the data for one mouse, and the horizontal lines indicate the geometric means. The data are composite data for two experiments. An asterisk indicates that the kidney titer was significantly higher than the bladder titer at a time point ($P < 0.005$, Mann-Whitney U test).

*saprophyticus* bacteria recovered from the kidneys was significantly higher than the number recovered from the bladder ($P < 0.005$), indicating that there was preferential colonization of and/or persistence in the kidneys compared to the bladder. Preferential colonization of the kidneys likely did not result from a retrograde reflux induced by the 50-μl inoculum, since inoculation with a total of $10^7$ CFU in five separate 10-μl instillments over the course of 5 h resulted in equivalent bladder and kidney titers at 48 h p.i. (data not shown).

**Multiple *S. saprophyticus* clinical isolates preferentially colonize the kidneys of C3H/HeN mice.** To ascertain whether the kidney tropism observed during *S. saprophyticus* strain 7108 infection was specific for this strain or a trait of all *S. saprophyticus* strains, we assessed three additional low-passage *S. saprophyticus* clinical isolates (Table 1) in this newly established mouse model. In each infection, the bacterial titers in the bladder were $\sim 10^1$ to $10^2$ CFU/mouse and the bacterial titers in the kidneys were $\sim 10^4$ CFU/mouse (Fig. 2) at 48 h p.i. Thus, all of the *S. saprophyticus* clinical strains tested had a statistically significant ($P < 0.005$) preference for infection of the murine kidneys.

**Host factors determine the efficiency of *S. saprophyticus* clearance from the kidneys.** To test whether kidney tropism is host or bacterium driven, we examined *S. saprophyticus* infection in a second mouse strain, C57BL/6, which responds to UPEC infection with kinetics different than those of C3H/HeN mice (16, 34, 38). C3H/HeN or C57BL/6 mice were infected with $10^7$ CFU of *S. saprophyticus*, and the bacterial titers in the bladder and kidneys were measured over time. The initial low levels of colonization of the bladder at 1 h p.i. were similar for the two strains (Fig. 3A). In contrast to the results for *S. saprophyticus* infection of C3H/HeN mice, little or no bacterial colonization of C57BL/6 kidneys was observed by 3 h p.i. (Fig. 3B). Furthermore, by 48 h p.i., the bladders and kidneys of all C57BL/6 mice were sterile, indicating that *S. saprophyticus* was not able to colonize the kidneys of C57BL/6 mice. No increases in cytokine production were observed in the kidneys of *S. saprophyticus*-infected C57BL/6 mice compared to the cytokine production in the kidneys of C3H/HeN mice (data not shown), suggesting that the lack of recoverable bacteria in C57BL/6 mice was not the result of a more robust host inflammatory response in this strain. In the remainder of this study we used the C3H/HeN mouse model to assess the host response to infection; however, these results suggest that host factors may play a large role in governing bacterial colonization, persistence, and/or clearance during *S. saprophyticus* UTI.

***S. saprophyticus* induces more inflammation in the kidneys than in the bladders of C3H/HeN mice.** Both UPEC infection and *E. faecalis* infection induce inflammation in the bladders and kidneys of C3H/HeN mice, as assessed by H&E staining,

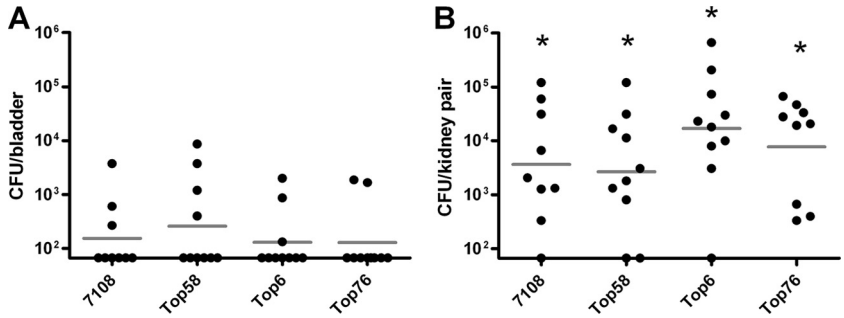


FIG. 2. *S. saprophyticus* strains preferentially colonize the kidney. Clinical isolates (indicated on the *x* axis) (Table 1) were inoculated into the bladders of C3H/HeN mice (10 mice/group) using 50 μl containing $10^7$ CFU. Bladders and kidneys were removed and homogenized, and 10-fold dilutions were plated to determine the number of CFU/organ at 48 h postinfection. Each circle indicates the data for one mouse, and the horizontal lines indicate the geometric means. The data are composite data for two experiments. An asterisk indicates that the kidney titer was significantly higher than the bladder titer at a time point ($P < 0.005$, Mann-Whitney U test).

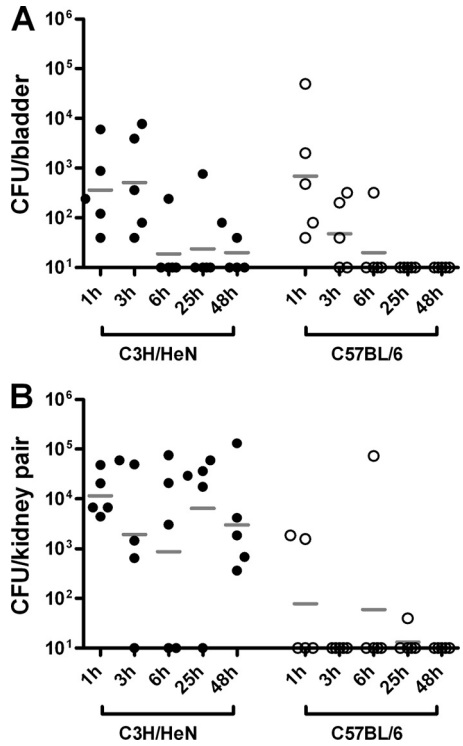


FIG. 3. *S. saprophyticus* is rapidly cleared from the C57BL/6 murine urinary tract. Female C3H/HeN or C57BL/6 mice (5 mice/time point) were infected with $10^7$ CFU of *S. saprophyticus* strain 7108. Bladders and kidneys were homogenized at 1, 3, 6, 25, and 48 h postinfection, and serial dilutions of organ homogenates were plated to determine the number of CFU/organ. Each circle indicates the data for one mouse; the filled circles indicate the data for C3H/HeN mice, and the open circles indicate the data for C57BL/6 mice. The horizontal lines indicate the geometric means. The data are representative of the data obtained in two separate experiments performed with 5 mice/experiment/mouse strain/time point.

immunofluorescence staining for neutrophils or macrophages in infected tissues, and induction of proinflammatory cytokines (21, 39). Therefore, we expected that *S. saprophyticus* would also induce inflammation in the C3H/HeN strain. Light microscopic assessment of H&E-stained tissue sections, obtained 48 h after infection with *S. saprophyticus* strain 7108 or Top58, showed that there was focal epithelial exfoliation in the bladder, characterized by the absence of large binucleate superficial umbrella cells in the bladder lumen; little or no inflammatory infiltrate was observed (Fig. 4B and C). In the kidney, a focal inflammatory cellular infiltrate adjacent to the renal pelvis was observed (Fig. 4E and F). No epithelial exfoliation or inflammation was observed in the bladders or kidneys of PBS mock-infected mice (Fig. 4A and D). Consistent with these histological findings, immunofluorescence microscopy showed that *S. saprophyticus* was closely associated with superficial umbrella cells in the bladder lumen and predominantly localized in the renal pelvis (see Fig. 6I to L; data not shown) in the kidneys. Colonization was observed less frequently in the renal pyramid and renal cortex in the kidney (data not shown). Thus, the increase in the immune cell infiltrate in the kidneys is consistent with the preferential colonization of the kidneys by *S. saprophyticus*.

***S. saprophyticus* induces inflammatory cytokine secretion more strongly in the kidneys.** Our current understanding of the host response to Gram-positive UTI is limited to the presence of a primarily monocytic infiltrate observed in *E. faecalis*-infected murine kidneys and modest concomitant induction of MIP-2 and SOCS-3 in kidney tissues (21). IL-8 can be detected in the urine of patients infected with many uropathogens, including *E. faecalis* and other Gram-positive cocci (32). *In vitro*, human mononuclear cells secrete IL-1β and IL-6 in response to *S. saprophyticus* (27). To gain greater insight into the host response to *S. saprophyticus* UTI, we examined soluble factors induced *in vivo* during infection.

We simultaneously examined the levels of 12 cytokines and chemokines (TNF-α, MCP-1, MIP-1β, IL-1β, IL-6, IL-12p70, IL-12p40, IL-17, G-CSF, KC, RANTES, and eotaxin [see Materials and Methods]) in organ homogenate supernatants from *S. saprophyticus*-infected and PBS mock-infected mice over time. The levels of TNF-α, MIP-1β, IL-1β, IL-6, IL-12p70, IL-17, and G-CSF in *S. saprophyticus*-infected C3H/HeN bladder homogenate supernatants increased in a time-dependent manner, peaking between 48 h and 7 days p.i.; the levels of MCP-1 and KC peaked at 6 h p.i. (Fig. 5A). In the kidneys, higher levels of all of the cytokines examined except eotaxin were produced during the course of infection in *S. saprophyticus*-infected mice than in mock-infected mice, and the levels peaked at 48 h p.i (Fig. 5B). In *S. saprophyticus*-infected kidneys, TNF-α, IL-1β, IL-6, IL-17, G-CSF, and RANTES were the most highly induced molecules; the levels were 100-fold higher than the levels in mock-infected animals and generally were more than 10-fold higher than the levels in the bladder. The more robust immune response in the kidneys may partially reflect the higher bacterial titers recovered from for these organs.

**Neutrophils infiltrate into the bladder and kidneys during *S. saprophyticus* infection.** Bladder and kidney epithelial cells readily produce IL-6 and IL-8 in response to *S. saprophyticus* infection (13). Macrophages secrete the proinflammatory cytokines TNF-α, IL-12, and IL-6 in addition to the chemokine MIP-1β (24, 25). MIP-1β is chemotactic for monocytes/macrophages and other proinflammatory cells, such as neutrophils (25). In addition, MIP-1β, KC, and G-CSF are important for neutrophil recruitment and activation. The increased levels of macrophage-secreted and -recruiting cytokines, as well as neutrophil-associated cytokines, suggested that these cells may be recruited to the sites of infection during *S. saprophyticus* UTI.

To assess whether monocytes/macrophages or neutrophils are recruited to the urinary tract upon *S. saprophyticus* infection, we dissociated whole bladders and kidneys and stained them with anti-CD45 antibody to identify immune cell populations; with anti-Gr1 antibody, which recognizes an epitope in the surface proteins Ly6G and Ly6C present on polymorphonuclear cells (PMN) and monocytes, respectively (2); and with anti-F4/80 antibody, which recognizes a monocyte- and macrophage-specific marker (1, 10). Using flow cytometry, we counted $F4/80^+$ cells (monocytes/macrophages) and $Gr1^+$ $F4/80^-$ cells (PMN) and expressed the results as percentages of the total $CD45^+$ cell populations in the bladder and the kidneys (Fig. 6A to D). We found that the percentage of monocytes/macrophages in the *S. saprophyticus*-infected bladders was modestly higher at 6 h p.i. and then decreased over time

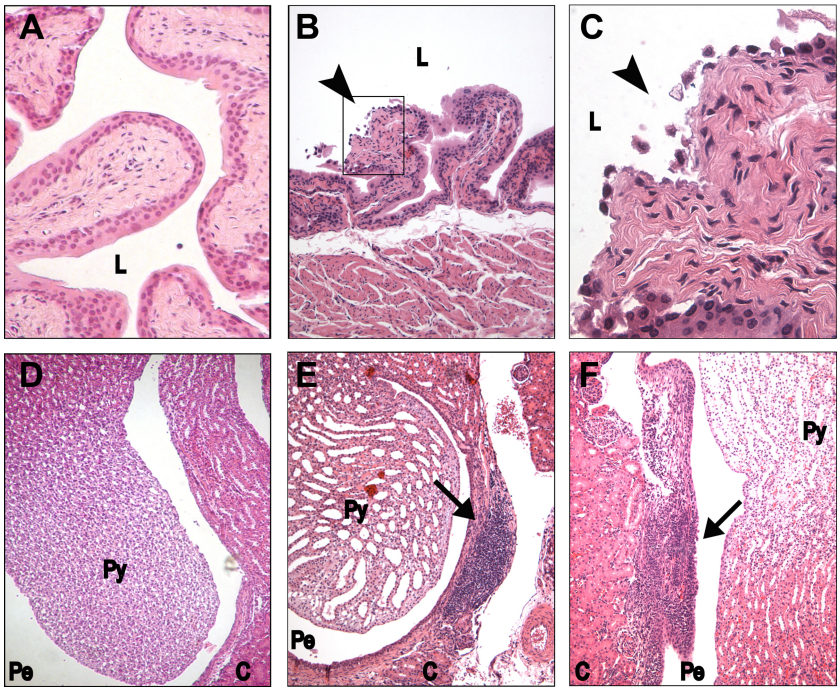


FIG. 4. *S. saprophyticus* strain 7108 induces inflammatory infiltration in the kidney. Mice were inoculated with either PBS (A and D), *S. saprophyticus* 7108 (B, C, and E), or *S. saprophyticus* Top58 (F). Bladders (A to C) and kidneys (D to F) were harvested and prepared for hematoxylin and eosin staining as described in the text. An inflammatory infiltrate was observed in *S. saprophyticus*-infected kidneys at 48 h p.i. (panels E and F, arrows). Conversely, no infiltrate was observed in the bladder at the same time point (B and C), but areas of epithelial exfoliation were observed (arrowheads). (A) The bladder epithelium from PBS mock-infected mice was intact, and no inflammatory infiltrate was observed in the bladders or kidneys of mock-infected animals (A and D). The area showing epithelial exfoliation in a box in panel B (magnification, ×10) is shown at a higher magnification (×40) in panel C. The magnification of all other images is ×10. L, bladder lumen; Pe, renal pelvis; Py, pyramid; C, cortex.

compared to the findings for PBS mock-infected animals (Fig. 6G). There was no difference between the percentages of kidney-infiltrating F4/80$^+$ cells in *S. saprophyticus*-infected and PBS mock-infected mice (Fig. 6H). In contrast, a small increase in the percentage of bladder-associated neutrophils (Fig. 6E) and a significant neutrophil infiltrate in the kidneys (Fig. 6F) were observed by 48 h p.i., when cytokine induction was also maximal (Fig. 5). After peaking at 48 h p.i., the number of neutrophils gradually decreased over time. The majority of the cellular immune infiltrate observed in the bladders and kidneys occurred focally in close proximity to *S. saprophyticus* bacterial cells (Fig. 6I to L and data not shown). Together, these data indicate that, like the response in UPEC UTI, PMN also predominate in the response to *S. saprophyticus* infection (40).

***S. saprophyticus* mutants are defective in colonization *in vivo*.** To examine the versatility of the *S. saprophyticus* murine model characterized here, we tested putative virulence factors that were previously characterized *in vitro*. Mice were infected with equal numbers of wild-type bacteria or strains with mutations in the *ssp* (Ssp$^-$) or *sdrI* (SdrI$^-$) gene. Both mutants colonized the mouse urinary tract as well as the wild type at 6 h p.i. (Fig. 7A), whereas by 48 h p.i. significantly fewer bacteria were recovered from the bladders and kidneys of mice infected with the Ssp$^-$ and SdrI$^-$ strains than from the bladders and kidneys of mice infected with wild-type bacteria (Fig. 7B). The virulence defect of both the Ssp$^-$ and SdrI$^-$ mutants in the kidneys was still observed at day 7, when significantly fewer mutant bacteria than wild-type bacteria were recovered. The *in vitro* phenotypes of Δ*ssp* and Δ*sdrI* mutants, including their lipase activity and collagen binding, respectively, are complemented when a copy of the wild-type gene is supplied on a plasmid (36, 37). Infection of mice with the Δ*ssp* or Δ*sdrI* mutant strain harboring the complementing plasmid resulted in loss of the plasmid by 48 h p.i. (data not shown); thus, complementation *in vivo* could not be assessed. Together, these data indicate that Ssp and SdrI are not required for initial *S. saprophyticus* colonization of the urinary tract but are necessary for persistence in both the bladder and kidneys.

## DISCUSSION

Urinary tract infection is one of the most common infectious diseases afflicting women in the developed world (46). It encompasses a number of disease states ranging from acute to recurrent infection, manifests in the bladder and/or kidneys, and includes symptoms that range from mild to painful and debilitating. Importantly, all of these disease states can be caused by a number of uropathogens, including both Gram-negative and Gram-positive bacteria. Most studies examining the pathogenic mechanisms that contribute to UTI have been performed in the context of Gram-negative uropathogenic *E. coli* (UPEC) infections. In light of recent studies that revealed significant differences in pathogenic mechanisms between

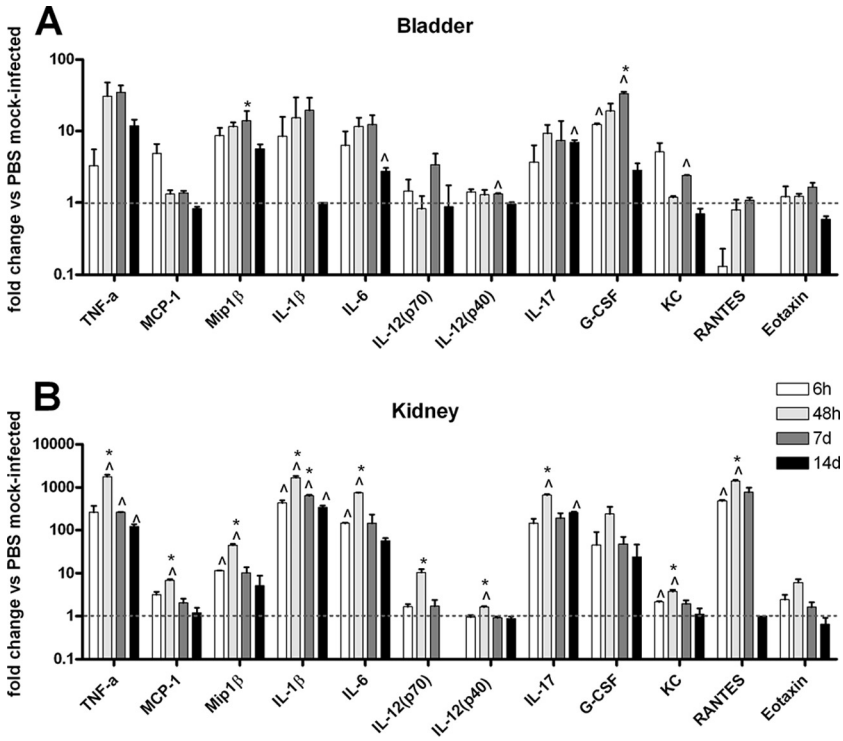


FIG. 5. *S. saprophyticus* infection induces a soluble proinflammatory innate immune response in the urinary tract of C3H/HeN mice. *S. saprophyticus* strain 7108 ($10^7$ CFU) was instilled into the bladders of C3H/HeN mice (5 mice/time point). Bladders (A) and kidneys (B) were removed at 6 h, 48 h, 7 days, and 14 days postinfection and homogenized, and supernatants were collected. The cytokine levels in organ homogenate supernatants are expressed as fold changes compared to the levels in PBS mock-infected mice (the fold change for mock-infected mice was defined as 1 and is indicated by a dashed line). The error bars indicate the standard deviations. Cytokine levels were measured in three separate experiments (48 h), two separate experiments (7 days), or one experiment (6 h and 14 days). The results of one representative experiment are shown. Cytokines significantly induced by *S. saprophyticus* in this experiment are indicated by carets, and cytokines significantly induced in multiple experiments are indicated by asterisks. Statistical significance was determined using a one-sample *t* test ($P < 0.1$). The lack of significance when induction appears to be high is a result of a small sample size.

UPEC and *Enterococcus* in the urinary tract (20, 21), we sought to establish a model with which to study the most common Gram-positive uropathogen, *S. saprophyticus.*

Here, we show that *S. saprophyticus* colonizes the C3H/HeN murine kidneys nearly 100-fold more efficiently than it colonizes the bladder at all time points examined. This finding is similar to the results obtained for the other major Gram-positive uropathogen, *E. faecalis* (21, 42). This observation is also consistent with an early report of murine *S. saprophyticus* UTI (12), but it contrasts strikingly with the bladder tropism displayed by some UPEC strains (19, 29). Although UTI symptoms consistent with cystitis reportedly are common during *S. saprophyticus* infection (14), it is possible that these symptoms actually result from infection of the kidneys. Indeed, *S. saprophyticus* causes 13% of upper UTI (14). Another study demonstrated that significantly more patients infected with *S. saprophyticus* than patients infected with UPEC reported back pain symptoms, a hallmark of kidney infection (18). Further, although upper UTI symptoms are traditionally diagnostic criteria for pyelonephritis, a lack of these symptoms has not been shown to preclude the possibility of a bacterial burden in the kidneys. Together, these data suggest that our findings for this murine infection may accurately model Gram-positive infection tropisms in human Gram-positive UTI.

Given the preference of *S. saprophyticus* for the kidneys, we performed the first comprehensive assessment of soluble cytokine mediators responding specifically to *S. saprophyticus* during UTI. In addition to the previously reported high-level induction of IL-1β and IL-6 (27), this study revealed that the levels TNF-α, IL-17, G-CSF, and RANTES secreted in the kidneys were ~100-fold greater than the levels secreted in the bladder at the height of induction. Low-level induction of MCP-1, MIP-1β, IL-12, and KC was also observed. These results indicate that the host response to *S. saprophyticus* is commensurate with the bacterial titer in the organ investigated. Further, there is no evidence that there is an early and robust immune response in the bladder, like that seen in Gram-negative UPEC infection (17), which is consistent with the low bacterial titers observed during murine *S. saprophyticus* UTI. However, modest neutrophil influx and bladder epithelial exfoliation in response to *S. saprophyticus* infection were observed, possibly resulting from an innate defense to shed infected cells during micturation (26). Alternatively, inflammation and/or epithelial shedding may be important for revealing bacterial receptors that are necessary for colonization. Interestingly, *S. saprophyticus* does not efficiently infect C57BL/6 mice, in contrast to C3H/HeN mice. The defect in colonization is not likely a result of an enhanced inflammatory response or faster clearance in C57BL/6 mice, since no increases in the levels of soluble factors were observed in this host background

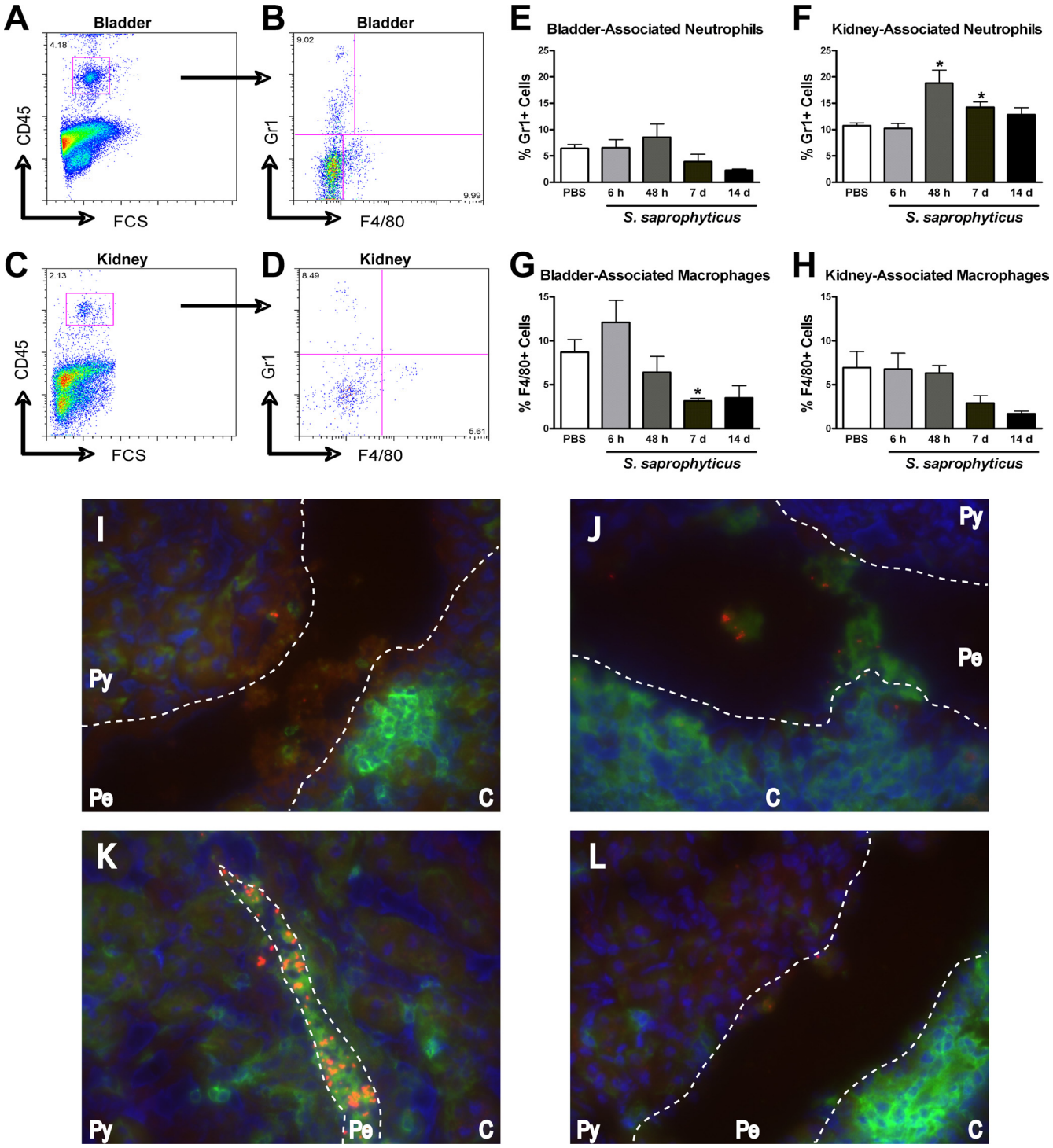


FIG. 6. Immune infiltrates in the murine urinary tract in response to *S. saprophyticus* strain 7108. C3H/HeN mice were infected with *S. saprophyticus*, and organs were removed at the time points indicated, fixed, and either dissociated for flow cytometry or sectioned for immunofluorescence microscopy. (A to D) Representative dot plots resulting from cellular infiltration analysis. $CD45^+$ cells, which were ~2 to 4% of the total bladder cells (A and B) or kidney cells (C and D), were gated (A and C), and the percentages of $Gr1^+$ neutrophils and $F4/80^+$ macrophages were determined (B and D). (E to H) Immune infiltrate as assessed by determining the percentage of $CD45^+$ immune cells in each organ which costained with the neutrophil/monocyte marker Gr1 or the monocyte/macrophage marker F4/80. The data are composite data for two separate experiments (2 or 3 mice/time point/experiment). The samples used for PBS mock-infected organs were pooled samples from 6 h, 48 h, and 7 days p.i. The error bars indicate the standard deviations. Statistical significance (indicated by an asterisk) was determined by a paired *t* test ($P < 0.05$). (I to L) Kidney sections from mice inoculated with *S. saprophyticus* for 6 h (I and J) or 48 h (K and L) stained with anti-Aas (red) and anti-CD-45 antibodies (green). Tissue sections were counterstained with Hoescht dye for nuclear labeling (blue). Magnification, ×40. The renal pelvis (Pe), pyramid (Py), and cortex (C) areas are outlined with dashed lines. In negative controls for each preparation, in which the primary anti-Aas and anti-CD-45 antibodies were not included, there was no cellular or bacterial staining (data not shown).

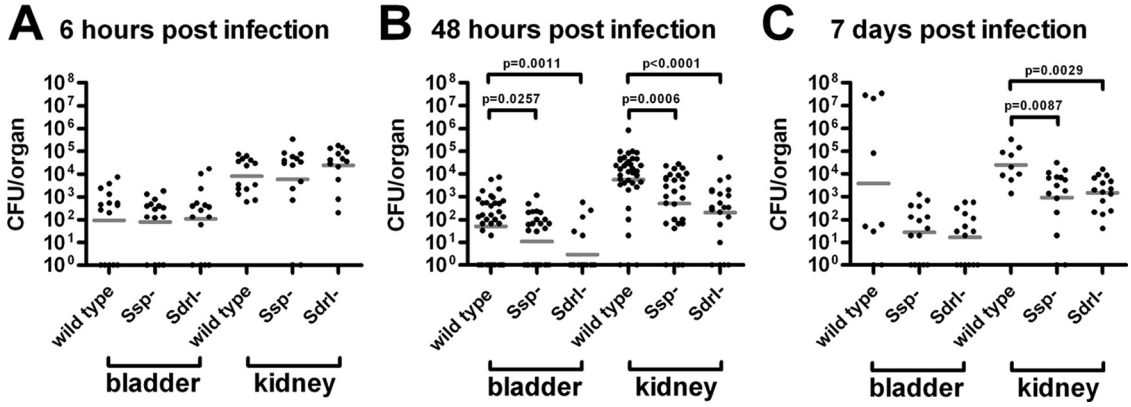


FIG. 7. Putative *S. saprophyticus* virulence factors are necessary for infection of C3H/HeN mice. Female C3H/HeN mice were infected with $10^7$ CFU of *S. saprophyticus* wild-type strain 7108 or isogenic strains with a mutation in *ssp* ($Ssp^-$) or *sdrI* ($SdrI^-$). Bladders and kidneys were homogenized at 6 h, 48 h, and 7 days postinfection, and serial dilutions of organ homogenates were plated for determination of the number of CFU. The data are composite data for two (6 h and 7 days) and four (48 h) experiments (5 to 10 mice/time point/experiment). The horizontal lines indicate the geometric means. Statistical significance was determined by the Mann-Whitney U test.

(data not shown). Thus, it is possible that C57BL/6 mice lack a receptor required for *S. saprophyticus* colonization.

The soluble response to *S. saprophyticus* infection included production of macrophage-secreted proinflammatory cytokines IL-1β, IL-6, TNF-α, G-CSF, and IL-12, as well as the monocyte/macrophage chemokines MIP-1β and MCP-1. The preponderance of macrophage-associated cytokines and chemokines produced in response to *S. saprophyticus* infection in the absence of a significant macrophage infiltrate in the infected organs suggests that resident macrophages may be activated during infection and play critical roles in the host response to Gram-positive pathogens. Indeed, the kidney was recently described to have a network of resident immune cells that could respond rapidly to infection (44). The presence of PMN migration and maturation signals, such as KC and G-CSF, along with MIP-1β, IL-6, and TNF-α, which can also be secreted by neutrophils, is consistent with our observation that PMN are recruited to *S. saprophyticus*-infected bladders and kidneys. Further, higher numbers of PMN were observed in the urine of *S. saprophyticus*-infected mice than in the urine of mock-infected animals (data now shown). However, the number of PMN was less than the number in the urine of UPEC-infected mice, which display robust neutrophil recruitment early in infection (17, 40). These data suggest that although the primary innate immune cell responses in UPEC and Gram-positive UTI may not differ in cell type, they may differ dramatically in magnitude. One explanation for this difference may be a reduced invasive capacity of *S. saprophyticus* compared to the invasive capacity of UPEC, for which urothelial cell invasion profoundly enhances the inflammatory response in the urinary tract (40). Furthermore, the proinflammatory response leading to PMN infiltration during UPEC infection is mediated in part via Toll-like receptor 4 (TLR4) signaling in response to lipopolysaccharide (LPS) (39, 43). Gram-positive uropathogens lack LPS, and *E. faecalis* pathogenesis in the urinary tract is not altered by the absence of TLR4 or TLR2 (which recognizes Gram-positive lipoteichoic acid) (21). Thus, examination of the *in vitro* interactions between *S. saprophyticus* and macrophages or neutrophils and the signaling pathways involved, along with cellular depletion studies *in vivo*, together should shed light on the contributions of each type of cell and the overall innate immune response to *S. saprophyticus* UTI.

This report describes a new murine model for the study of bacterial and host factors that contribute to *S. saprophyticus* pathogenesis in the urinary tract. Despite the clinical importance of this Gram-positive uropathogen, very little is known about how it so specifically and productively infects the urinary tract. To test the efficacy of our model, we examined the contributions of the putative virulence factors SdrI and Ssp to murine UTI. We found that while neither Ssp nor SdrI is required for the initial colonization of the urinary tract, both proteins are necessary for persistence in the bladder and kidney. Similar contributions to virulence have been reported for urease; urease mutants were associated with a ~10-fold reduction in rat bladder colonization (5). Additional studies are required to elucidate the true roles of *S. saprophyticus* Ssp and SdrI in UTI pathogenesis.

Taken together, our data support the conclusion that this new model of Gram-positive UTI is a relevant approximation of human Gram-positive UTI. We show that *S. saprophyticus*, like the other major Gram-positive uropathogen *E. faecalis*, displays kidney tropism during UTI. However, the host responses to *S. saprophyticus* and *E. faecalis* are different, which may in part explain why these uropathogens target different host populations. Further, our studies using bacterial mutants underscore the utility of this murine model for revealing important contributions of the bacterial factors necessary for *S. saprophyticus* to cause a productive urinary tract infection. This model should, therefore, be useful for further study of the contributions of both *S. saprophyticus* virulence factors and the virulence factors of other prevalent Gram-positive urinary tract pathogens.

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