Longitudinal multi-omics analyses link gut microbiome dysbiosis with recurrent urinary tract infections in women

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Recurrent urinary tract infections (rUTIs) are a major health burden worldwide, with history of infection being a significant risk factor. While the gut is a known reservoir for uropathogenic bacteria, the role of the microbiota in rUTI remains unclear. We conducted a year-long study of women with (n = 15) and without (n = 16) history of rUTI, from whom we collected urine, blood and monthly faecal samples for metagenomic and transcriptomic interrogation. During the study 24 UTIs were reported, with additional samples collected during and after infection. The gut microbiome of individuals with a history of rUTI was significantly depleted in microbial richness and butyrate-producing bacteria compared with controls, reminiscent of other inflammatory conditions. However, Escherichia coli gut and bladder populations were comparable between cohorts in both relative abundance and phylogroup. Transcriptional analysis of peripheral blood mononuclear cells revealed expression profiles indicative of differential systemic immunity between cohorts. Altogether, these results suggest that rUTI susceptibility is in part mediated through the gut-bladder axis, comprising gut dysbiosis and differential immune response to bacterial bladder colonization, manifesting in symptoms.
of the gut microbiota and \textit{E. coli} strain dynamics in rUTI, susceptibility to infection and host immune responses that may impact these dynamics. Using multi-omic techniques, we determined that (1) compared with healthy controls, women with a history of rUTI had a distinct, less diverse gut microbiota, depleted in butyrate producers and exhibiting characteristics of low-level inflammation; (2) differential immunological biomarkers suggest that rUTI women may have a distinct immune state; (3) \textit{E. coli} strains were transmitted from the gut to the bladder in both cohorts, although no UTI symptoms occurred in healthy controls; and (4) UTI-causing \textit{E. coli} strains often persistently colonized the gut and were not permanently cleared by repeated antibiotic exposure. Thus, susceptibility to rUTI is in part mediated through a syndrome involving the gut–bladder axis, comprising a dysbiotic gut microbiome with reduced butyrate production and apparent alterations of systemic immunity. Our work shows that UPEC strains persist in the gut despite antibiotic treatment, which itself may exacerbate gut dysbiosis.

**Results**

**Frequent antibiotic use and \textit{E. coli} infections in rUTI cohort.** Women with a history of rUTI were recruited to the UMB study, along with an age- and community-matched control cohort comprising healthy women (Methods). A total of 16 controls and 15 women with rUTI participated in the year-long study, providing both monthly home-collected stool samples and blood, urine and rectal swabs at enrolment and subsequent clinic visits for UTI treatment (Fig. 1a). Participants completed monthly questionnaires on diet, symptoms and behaviour (Supplementary Data). There was a greater proportion of white women in the rUTI cohort, and self-reported antibiotic use was higher in this group in line with UTI treatment; otherwise, few dietary or behavioural differences were apparent (Extended Data Table 1).

A total of 24 UTIs occurred during the study, all in women with rUTI who each had experienced between no and four UTIs (Fig. 1b). Nineteen were diagnosed by clinicians while five were inferred through self-reported symptoms and antibiotic use in the questionnaire during monthly sample collection. UTIs were typically treated with ciprofloxacin or nitrofurantoin. No significant temporal risk factors for UTI were identified amongst dietary or behavioural variables. Sexual intercourse is a well-known risk factor for UTI\textsuperscript{27,28}, and all 19 clinically diagnosed UTIs occurred following at least one reported sexual encounter in the previous 2 weeks (Extended Data Fig. 1).

Urine samples collected at the time of clinical UTI diagnoses were plated on MacConkey agar, with bacterial growth detected (>0 colony-forming units (CFU) ml\textsuperscript{-1}) in the majority (15/19, 79%; Supplementary Table 1). To determine the cause of infection we sequenced 13 urine cultures, as well as uncultured urine, from all UTI diagnoses, defaulting to results from cultures when available. \textit{E. coli} dominated 12/13 (92%) sequenced outgrowths, the remaining sample being dominated by \textit{Klebsiella pneumoniae}. Sequencing of uncultured urine from the remaining UTI samples identified uropathogens in a further four samples, including \textit{E. coli} (two), \textit{Enterococcus faecalis} and \textit{Staphylococcus saprophyticus}, while two yielded no bacterial sequence (Supplementary Table 1). Based on sequencing we defined 14 \textit{E. coli} UTIs, comprising 82% of infections for which a bacterial cause could be inferred, broadly reflecting previous estimates of the proportion of all UTIs caused by \textit{E. coli}\textsuperscript{2}.

rUTI gut is depleted in microbial richness and butyrate producers. It is increasingly recognized that the gut microbiota plays a role in a range of autoimmune and inflammatory diseases\textsuperscript{3,4}, as well as susceptibility to infection\textsuperscript{5}, and can alter inflammation in distal organs\textsuperscript{6}. While previous studies have highlighted differential abundances of non-uropathogenic gut taxa as risk factors for bacteriuria in patients having undergone kidney transplant (reduced \textit{Faecalibacterium} and \textit{Romboutsia})\textsuperscript{7} and UTIs in children (reduced \textit{Peptostreptococcaceae})\textsuperscript{8}, it is unclear whether these are risk factors for recurrence in otherwise healthy adult women. To explore this, we sequenced and analysed the metagenomes of 367 longitudinal stool samples from both women with rUTI (\(n=197\)) and controls (\(n=170\)) (Fig. 1b and Methods). Rectal swabs, collected during clinic visits, were not used to determine microbiome profiles.

There were broad differences in gut microbiota composition between cohorts (Fig. 2a–c). We fit linear mixed models with individual-level random effects to determine differences in diversity and composition between cohorts, adjusting for recent antibiotic use (Methods). Gut microbial richness was significantly lower, on average, in women with rUTI (\(P=0.05\); Fig. 2c). At the phylum level, we saw elevated levels of \textit{Bacteroidetes} (false discovery rate (FDR) = 0.003) and a lower relative abundance of \textit{Firmicutes} (FDR = 0.02) in women with rUTI. We identified 22 differentially abundant taxa (FDR < 0.25) at lower taxonomic levels, 16 of which were depleted in women with rUTI (Supplementary Table 2 and Fig. 2b), including \textit{Faecalibacterium} as previously reported\textsuperscript{1}.

Several of the taxa reduced in the rUTI gut, including \textit{Faecalibacterium}, \textit{Akermansia}, \textit{Blautia} and \textit{Eubacterium hallii}, are associated with short-chain fatty acid (SCFA) production, including propionate and butyrate, which exert an anti-inflammatory effect in the gut through the promotion of intestinal barrier function and immunomodulation\textsuperscript{3,21}. \textit{Blautia} was additionally identified as the only taxon significantly depleted at the time of UTI relative to non-UTI samples (FDR = 0.01). Cumulatively, SCFA producers,
particularly butyrate producers, were significantly less abundant in women with rUTI \( (P = 0.001); \text{ Fig. 2d and Extended Data Fig. 2}. \) Four Kyoto Encyclopedia of Genes and Genomes orthogroups \(^{22}\) representing components of butyrate production pathways were significantly reduced across the rUTI cohort (Supplementary Table 3). Functional analysis with HUMAnN2 \(^{23}\) additionally revealed pathways depleted in the rUTI cohort, including those associated with sugar degradation and biosynthesis of metabolite intermediates and amino acids (Supplementary Table 4), many of which were also found to be differentially abundant in a study of patients with irritable bowel disease.\(^{24}\) Within the rUTI group, the frequency of infections was associated with microbial richness or the relative abundance of butyrate producers, but not with neither microbial richness nor the relative abundance of butyrate producers between women with UTIs under different antibiotic exposures (Extended Data Fig. 4a,b). We observed no differences in either richness or the abundance of butyrate producers between women with rUTI under different antibiotic exposures (Extended Data Fig. 4a,b). Within the rUTI group, the frequency of infections was associated with neither microbial richness nor the relative abundance of butyrate producers. The microbial richness of women suffering UTIs during the study did not differ significantly from that of women with rUTI not reporting infections \( (P = 0.4; \text{ Fig. 2b–d}). \) While we did not detect a lasting impact from individual antibiotic courses—there were few long-term trends among women with rUTI over the study (Extended Data Fig. 4c)—it is still possible that repeated antibiotic use over years may have contributed to the observed rUTI dysbiosis.

rUTI gut dysbiosis shares broad similarities with inflammatory bowel disease. The depletion of butyrate-producing taxa and exposure were not significantly different from baseline levels \( (P = 0.2). \) Furthermore, we saw no association between the reported number of antibiotic courses and average richness (Fig. 2c), and no differences in overall gut microbiome stability between cohorts, despite more frequent antibiotic treatment among women with UTI (Extended Data Fig. 3). We observed no differences in either richness or the abundance of butyrate producers between women with rUTI under different antibiotic exposures (Extended Data Fig. 4a,b). Within the rUTI group, the frequency of infections was associated with neither microbial richness nor the relative abundance of butyrate producers. The microbial richness of women suffering UTIs during the study did not differ significantly from that of women with rUTI not reporting infections \( (P = 0.4; \text{ Fig. 2b–d}). \) While we did not detect a lasting impact from individual antibiotic courses—there were few long-term trends among women with rUTI over the study (Extended Data Fig. 4c)—it is still possible that repeated antibiotic use over years may have contributed to the observed rUTI dysbiosis.

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microbial richness, key characteristics of rUTI dysbiosis, is also observed in other gut inflammatory conditions, including nosocomial diarrhoea, IBS and inflammatory bowel disease (IBD), particularly Crohn’s disease, and thus may be indicative of gut inflammation in women with rUTI. While IBD is a multifactorial disorder for which the causative role of gut microbes is incompletely understood, mouse models have helped demonstrate a causal relationship between gut dysbiosis and inflammation. We compared our data to longitudinal gut microbiome data from adults with and without IBD in the Human Microbiome Project 2 (HMP2) study, which shared the same extraction and sequencing protocols (Methods). Relative to each study’s control group, we found that the ten most significantly depleted species in the rUTI gut, including butyrate producers Faecalibacterium prausnitzii and E. hallii, were also depleted in the IBD gut. We further observed a significant overall correlation in the estimated change of species-level abundances associated with rUTI and IBD (Extended Data Fig. 5), suggesting more general similarities.

There were also some notable differences. Bacteroides, significantly elevated in the rUTI group, did not differ between cohorts in the HMP2 study (Extended Data Fig. 5), and was also decreased among patients with IBD in other studies. E. coli was significantly elevated in patients with IBD in the HMP2 study, but showed no difference in average relative abundance between our cohorts (Fig. 2e). Diminished Bacteroides alongside elevated Enterobacteriaceae was also observed in patients with nosocomial diarrhoea. Diarrhoea, also a symptom of IBD, is associated with reduced gut transit time and is known to enrich for organisms common in the upper gastrointestinal tract, including Enterobacteriaceae, at the expense of anaerobic organisms such as Bacteroides. As such, women with rUTI and low-level inflammation and no diarrhoea may lack the depletion of Bacteroides and elevation of Enterobacteriaceae observed in diarrhoea-associated conditions. It is also possible that the considerable differences in treatment regimens—that is, antibiotics versus anti-inflammatory agents—contribute to divergences of a common underlying inflammatory signal.

Differential host immune response potentially linked to rUTI. rUTI dysbiosis also shares similarities with immunological syndromes affecting distal sites. For example, depletion of butyrate producers has been associated with rheumatoid arthritis, a systemic autoimmune disease that can be partially ameliorated in animal models with oral butyrate supplementation. Patients with chronic kidney disease also exhibit similar dysbiosis, including reduced Parasutterella and Akkermansia, the latter of which is inversely correlated with levels of interleukin-10, an anti-inflammatory cytokine. We hypothesized that rUTI dysbiosis may also have an immunomodulatory role, potentially eliciting a differential immune response to bacterial invasion of the bladder. Thus, we explored immunological biomarkers from blood samples collected at enrolment and UTI, quantifying (1) a Lumines panel of human cytokines, chemokines and growth factors involved in inflammation and T cell activation, and (2) cell types and the transcriptional activity of peripheral blood mononuclear cells (PBMCs) (Methods).

Of the 39 Lumines analytes, one chemokine, plasma eotaxin-1, was higher in women with rUTI versus controls at enrolment, and is associated with intestinal inflammation. Levels of eotaxin-1 are increased in colonic tissue of patients with active IBD. Subsequent human eotaxin-1 ELISAs validated these results, highlighting an additional link to dysbiosis-driven perturbation of the immune state; however, since this result did not hold after adjusting for race, we could not rule out potential demographic confounders. Eotaxin-1 was also higher in the blood plasma of women with rUTI at the time of UTI versus enrolment (P = 0.04; Extended Data Fig. 6b).

Our small cohort size provided limited statistical power to identify differential expression between cohorts based on PBMC RNA sequencing (RNA-seq) data, and no large-scale differences were observed (Extended Data Fig. 6a). However, we found two genes that were upregulated in the PBMCs of the rUTI cohort (FDR < 0.1): ZNF266 and the long non-coding RNA LINCO00944 (Supplementary Table 5). ZNF266 has previously been linked to urological health, as a potential PBMC biomarker for overactive bladder and incontinence in women. LINCO00944 has been associated with inflammatory and immune-related signaling pathways, as well as tumour-invading T lymphocytes in breast cancer and markers for programmed cell death. Resting natural killer (NK) cells were significantly reduced at the time of UTI relative to baseline levels (P = 0.02; Extended Data Fig. 6c). NK cells help suppress bladder infection by UPEC in mice, so the loss of these cells in the periphery may suggest migration to the bladder concurrent with rUTI.

**Gut and bladder E. coli dynamics are similar between cohorts.** Previous work has implicated gut dysbiosis and depletion of butyrate-producing bacteria in enhanced susceptibility to gut colonization by pathogens, including Salmonella and C. difficile. While we could not quantify absolute species abundances, we observed no significant difference in the average relative abundance of E. coli between cohorts (Fig. 2e), suggesting that the rUTI dysbiotic gut is no more hospitable to E. coli colonization than that of controls. Further, we found no relationship between the relative abundances of Escherichia and butyrate producers in either cohort, suggesting that depletion of butyrate-producing bacteria does not enhance gut colonization by Escherichia (Extended Data Fig. 7). We considered the possibility that a temporal increase, or bloom, in E. coli relative abundance is a rUTI risk factor. Of the samples collected in the 14 days preceding an E. coli UTI, 75% exhibited E. coli relative abundance at or above average levels in the gut (Extended Data Fig. 8a,b). However, elevated E. coli levels were not predictive of UTIs: none of the 22 E. coli blooms (defined as E. coli relative abundance tenfold higher than intrahost mean) occurred in the 2 weeks before UTIs. Thäner et al. identified intestinal blooms of uropathogens preceding some UTIs, but similarly noted that blooms often occurred in the absence of infection, leading us to conclude that elevated levels of E. coli may facilitate transfer to the bladder but rarely manifest in infection. However, without frequent urine collection, we cannot rule out asymptomatic bladder colonization.

Although we did not detect differences in E. coli species dynamics, we hypothesized that rUTI dysbiosis may manifest in a qualitatively different E. coli population in the gut, contributing to increased rUTI susceptibility. We applied StrainGE to explore E. coli strain-level diversity within stool metagenomes (Methods), and classified strains by phylogroup. Patterns of strain carriage were similar in the rUTI (Fig. 3) and control (Extended Data Fig. 9) cohorts. Both the number of strains per sample and phylogroup distribution were comparable between cohorts (Fig. 4 and Extended Data Fig. 8c,d). While most E. coli strains (62%) were observed in one sample only, 22% were ‘persistent’, observed in at least one-quarter of their carrier’s samples. Persistent strains were more likely to originate from phylogroups B2 and D (P = 0.01), regardless of cohort, and were slightly more common in controls (odds ratio (OR) = 2.1 (0.9, 5.2), P = 0.1), at odds with the hypothesis of differential colonization resistance to phylogroups associated with UPEC between cohorts.

We then applied StrainGE to all urine samples, seeking to elucidate differences in strain dynamics in the bladder. We found that 79% (11/14) of E. coli UTIs were caused by phylogroup B2 (n = 7) or D (n = 4) strains (Supplementary Table 1), approximately in line with previous studies. Of the 24 healthy enrolment urine samples yielding sufficient bacterial DNA for sequencing and profiling (Supplementary Table 6), we detected E. coli strains in 54% (13/24),...
including over half of samples (7/13) from control participants, despite the absence of symptoms. All but one of these strains also belonged to phylogroup B2 or D. Control urine samples carried E. coli strains that were phylogenetically similar to UTI-causing strains based on StrainGE predictions (Fig. 4 and Methods), despite divergent clinical outcomes.

Mapping of urine metagenome assemblies to a curated virulence factor database showed that UTI-causing strains were enriched in virulence factors (including iron uptake systems (sit, chu, iro, ybt operons), colibactin (clb) and type6 secretion systems) relative to an E. coli species-wide database, though many of these were also present in the one urine sample from a control participant for which we had sufficient coverage to assess gene content (Methods and Supplementary Table 7). This transition of a probable urovirulent strain to the bladder of healthy women without eliciting UTI symptoms is consistent with previous studies that were unable to identify genetic markers of urovirulence in mice, or to consistently discriminate between UTI and asymptomatic bacteriuria strains in women. Nevertheless, the divergence in clinical outcomes after bacterial bladder invasion may still arise due to phenotypic differences in E. coli strains reaching the bladder that are not readily apparent in genome comparisons. rUTI dysbiosis could have an impact on UPEC gene expression; it has been shown that higher SCFA levels are associated with downregulation of E. coli virulence factors, including fimbrial and flagellar genes. However, such transcriptional analyses fall outside the scope of this study.

Antibiotic treatment fails to clear UTI-causing strains from gut. While it is well known that UTIs are most commonly caused by UPEC resident in the gut, the longitudinal dynamics of these strains within the gut are less well understood despite the importance of such insights into development of rUTI prophylaxis. We applied StrainGE to all urine samples to identify UTI-causing strains and their gut dynamics, in particular at the time of UTI and after antibiotic
We anticipated that antibiotic exposure—particularly ciprofloxacin—would impact gut carriage of *E. coli* strains, and may explain the lower frequency of persistent colonizers in the rUTI group. Indeed, *E. coli* strains were detected by StrainGE significantly less frequently in stool samples from the 2 weeks following antibiotic use (OR = 0.3 (0.13, 0.68), P = 0.004). However, many strains apparently cleared by antibiotics were observed again at later time points; in fact, none of the UTI-causing strains observed in the gut was permanently cleared following antibiotic exposure. It has previously been shown that coexistence of susceptible and resistant strains of the same lineage through acquisition/loss of mobile resistance elements can allow rapid adaptation by UPEC populations to repeated antibiotic exposure and persistence in the gut\(^5\). While low-level persistence that is undetectable from sequencing data is a possibility, we plated a subset of post-treatment stool samples onto MacConkey agar to culture *E. coli*. In many cases we observed no growth, suggesting absence rather than low-level persistence (Supplementary Table 8). Furthermore, profiling of 12 UTI-causing strains isolated from proximate stool samples demonstrated that the majority were susceptible to the antibiotics to which they were exposed (Supplementary Table 9). While a single stool sample is not completely representative of the gut microbiota, this suggests that UTI-causing strains may be frequently reintroduced to the gut from alternative sources following antibiotic clearance of the bladder and gut.

**Discussion**

Our study design, data collection and culture-independent metagenomic sequencing approach allowed us to characterize dynamics of the gut–bladder axis in both healthy women and those with rUTI. We propose that rUTI susceptibility is dependent, in part, on perturbation of the gut–bladder axis, which represents a previously undescribed syndrome comprising gut dysbiosis and differential host immunology. While this study was not designed to identify causal links between gut dysbiosis, immune response and rUTI susceptibility, the proposed model is consistent with our findings and provides a benchmark to be tested in future studies. Compared to healthy controls, women suffering from rUTI exhibited gut dysbiosis characterized by depleted levels of butyrate-producing bacteria and diminished microbial richness. This dysbiosis did not appear to impact *E. coli* dynamics within the gut; relative abundances and strain types were similar between cohorts, suggesting that gut carriage of urovirulent bacteria in itself is not a risk factor for rUTI. Notably, *E. coli* was commonly identified in the urine of healthy women, including strains arising from UPEC-associated clades and harbouring similar virulence factors. Based on our observations, rUTI gut dysbiosis is consistent with low-level gut inflammation and is reminiscent of other disorders in which microbiome-mediated immunomodulation plays a role in disease severity.

Our study has a number of limitations. First, due to the limited collection of urine samples in the control group, it was not possible to robustly compare (1) the composition of the urine microbiome and (2) the frequency of (asymptomatic) strain transfer from gut to bladder between cohorts. Second, we did not assess the role of other potential reservoirs, such as the vagina, which could explain UTIs caused by strains never observed in the gut. Third, while StrainGE offers a high-resolution view of *E. coli* strain dynamics in the gut and bladder, we cannot rule out the presence of additional, low-abundance strains that could not be detected from the depth of metagenomic data generated. Finally, the small cohort size and infrequent blood sample collection provided limited power to assess differential expression in PBMCs. While we identified some indications of immunological differences between cohorts, our findings warrant further investigations to explore microbiome–host mucosal immune interactions in the context of rUTI susceptibility.

While identification of the origins of rUTI dysbiosis is outside the scope of this study, repeated antibiotic exposure is a plausible mechanism through which dysbiosis is maintained. The relatively short study period precluded us from establishing whether dysbiosis
is the direct result of long-term antibiotic perturbation. In addition to the potentially detrimental impact of antibiotic use on the gut microbiota, we found that treatment also failed to clear UTI-causing strains from the gut in the long term. rUTI treatment protocols targeting UPEC strains in the gut with minimal disruption to other gut microbiota, as small molecule therapeutics\textsuperscript{14},\textsuperscript{15}, may offer improved prospects. While more evidence is required to fully characterize the causal mechanisms between dysbiosis and infection, our work highlights the ineffectiveness and potential detrimental impact of current antibiotic therapies, as well as the potential for microbiome therapeutics (for example, faecal microbiota transplants\textsuperscript{16}) to limit infections via restoration of a healthy bacterial community in the gut.

Methods

Study design and sample collection. Enrolment. This study was conducted with the approval and under the supervision of the Institutional Review Board of Washington University School of Medicine in St. Louis, MO. Women from the St. Louis, MO area reporting three or more UTIs in the past 12 months were recruited into the rUTI study arm, while women with no history of UTI (at most one UTI ever) were recruited into the control arm via the Department of Urological Surgery at Barnes-Jewish Hospital in St. Louis, MO. We excluded women who (1) had IBD or urological developmental defects (for example, ureteral reflux, kidney agenesis and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and we excluded women who (1) had IBD or urological developmental defects (for example, ureteral reflux, kidney agenesis and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on), (2) were pregnant, (3) were taking antibiotics as prophylaxis for rUTI symptoms, (4) were smokers, (5) had IBD or other gastrointestinal conditions, (6) had a history of HIV/AIDS, (7) were taking immunosuppressant drugs, and so on). Women visiting the clinic during the study with UTI symptoms provided rectal swabs, blood, and urine samples and were requested to submit stool samples as soon as possible (within 24 h) after the clinic visit, as well as at a 2-week follow-up time point.

All participants provided monthly stool samples for 12 months. Samples were collected at home and submitted via mail following procedures developed in the HMP2 protocol\textsuperscript{33}. Briefly, participants collected a fresh faecal sample in a disposable toilet hat and then aliquoted two teaspoon-sized scoops of stool into one tube containing PBS and another containing 100% ethanol. Samples were kept overnight at 37 °C to enumerate CFU. After outgrowth, plates were incubated at 37 °C for 24 h and then transferred to LB plates, and the supernatant was then flash-frozen and stored at −80 °C until DNA extraction. Plasma eotaxin (CCL11) levels from patients with rUTI and controls were measured using the Eotaxin (CCL11) Human Simple Step ELISA kit (Abcam, ab185985), according to the manufacturer's protocol. Briefly, plasma samples were diluted in sample diluent and 50 µl of each sample and antibody
cocktail were added to 96-well plate strips. Plates were seeded and incubated with shaking for 1 h at room temperature. Wells were washed three times with 1× wash buffer and inverted to remove excess liquid; 100 µl of TMB substrate was added to each well, plates were covered to protect from light and incubated with shaking for 10 min. Stop solution (100 µl) was added to each well and plates were incubated with shaking for 1 min. Optical density (OD450) was measured and recorded to determine the concentration of Eotaxin (in pg/ml).

Sequence data analysis. Community profiling and metrics. Bacterial community composition was determined using MetaPhlAn2 (v2.7.0 with db v2.0)23 on KneadData-processed sequences. Functional profiling was performed using HUMAnN2 (v2.8.1, database downloaded October 2016)24 on KneadData-processed sequences. Diversity metrics and Bray–Curtis (BC) distances were derived from MetaPhlAn2 relative abundance output using the veganc package in R (https://cran.r-project.org/web/packages/vegan/).

PBMC RNA-seq analysis. Sequences from PBMC-extracted messenger RNA were aligned to the human reference genome (hg19, Bioproject PRJNA31257) using the STAR aligner25. Picard-Tools (https://broadinstitute.github.io/picard/) was used to track duplicate reads. Read counts per gene were generated with subread featureCounts26. Read counts were normalized to counts per million using edgeR27.

Functional analyses. To determine the significance of T6SS presence in certain phylogroups. Again, an enrichment analysis was performed using Fisher’s exact test to determine the significance of virulence factor enrichment in a certain phylogroup. T6SS identity and analytical pipeline. Other VFDB hits from blastn were filtered for 90% identity and 90% coverage. All pairs with a common callable genome ≥90% identity and ≥90% coverage were fitted using DESeq2 (ref. 9). Baseline healthy control samples were compared to baseline rUTI samples. Due to limited sample numbers and potential confounding, we included only samples collected from Caucasian women in this analysis. Results driven by single outlying data points were not considered.

E. coli strain profiling. To track E. coli strain dynamics we used Strain Genome Explorer (StrainGE), which we extensively benchmarked for use on low-abundance species in the context of typical Illumina sequencer error28. We applied the StrainGST module of StrainGE to identify representative E. coli strains in all stool, urine, and rectal swabs, using an E. coli reference database generated from RefSeq complete genome sequences detailed in van Dijk et al.29. Strains mapping to the same representative reference genome in this database typically have an average nucleotide identity (ANI) of at least 99.9%. To provide further evidence that same-strain calls from sample pairs from the same host were indeed matches, we ran the StrainGR module of StrainGE, which calculates alignment-based similarity metrics. We used benchmarking thresholds to determine single strain matches; strain pairs with a common callable genome >0.5%, Jaccard gap similarity >0.95 and average callable nucleotide identity >99.95% were deemed matches.

Determination of UTI-causing strains. Urine samples provided at the time of UTI diagnosis were plated on MacConkey agar. Sequence data were generated from DNA extracted from uncultured urine and/or outgrowth on selective media. The cause of UTI was deemed to be the most abundant uropathogen, using outgrowth data where available or uncultured urine otherwise. Species were determined to be uropathogens based on UTI prevalence studies (for example, ref. 1).

Determination of virulence factors. Urine metagenomes for which E. coli represented the dominant species were assembled using SPAdes30. To detect virulence factors in E. coli references (E. coli strain profiling) and assembled genomes from study samples, we used the Virulence Factor Database (VFDB) for E. coli and the type 6 secretion system (T6SS) database (SecReT6) in genome-wide BLAST+ searches. Although VFDB contains T6SS genes, we removed these in favour of the T6SS-specific database for a T6SS-specific analytical pipeline. Other VFDB hits from blastn were filtered for ≥90% identity and ≥90% coverage. All E. coli genomes were separated by phylogroup for enrichment analysis, where Fisher’s exact test was used to determine the significance of virulence factor enrichment in a certain phylogroup. T6SS hits were filtered for ≥90% identity and ≥90% coverage, and the system was considered present when at least 12 different adjacent gene segments were present. Again, an enrichment analysis was performed using Fisher’s exact test to determine the significance of T6SS presence in certain phylogroups.

Identification of differences at the cohort level and time of UTI. We fit mixed-effects linear regression models to compare the structure, diversity and function of the gut microbiome between cohorts, following similar approaches employed by previous studies (for example, ref. 31). For this purpose we used sequence data from all collected stool samples but did not include rectal samples. An arcsine square root transformation was applied to relative abundance values. Features (transformed relative abundances, diversity, microbial richness) were fit as a function of host (random effects term), cohort (categorical variable) and terms for antibiotic use and race (categorical variable) to adjust for potential confounding effects. To assess change in relative abundances at relevant time points, we also fit models including covariates for ‘pre-UTI’ (14 days preceding UTI diagnosis), ‘time of UTI’ (3 days either side of UTI diagnosis) or ‘post antibiotics’ (<14 days post antibiotic exposure) as binary variables. All taxa with >10% non-zero values were fitted using the lme4 function in R. Significance of associations was determined using Wald’s test, and P-values were adjusted for multiple hypothesis testing using Benjamini–Hochberg correction at each taxonomic level.

The relative abundance of SCFA producers was additionally compared between cohorts; butyrate- and propionate-producing species were determined based on functional capacity to produce butyrate and propionate32. These species’ relative abundances were then aggregated and compared as above.

We compared the stability of the microbiome between cohorts by assessing the distributions of within-host pairwise BC dissimilarities between individuals. Since women with rUTI had undergone, on average, slightly more frequent sampling than controls, due to additional follow-up samples after UTI diagnoses, this metric was reframed as follows. Sequences from earlier collection dates were removed, and we observed no significant trend between BC dissimilarity and time between samples, suggesting no detectable long-term trends. Furthermore, we detected no difference in the distribution of time-adjusted BC distances (BC divided by number of days between samples) across cohorts.

IBD comparisons. To compare rUTI dysbiosis with an IBD gut state, we downloaded MetaPhlAn2 output from the HMP2 study33 (ibmdnmdb.org). We extracted longitudinal samples from adults with IBD (diagnosis ‘UC’ or ‘CD’) and non-IBD controls (diagnosis ‘nonIBD’). We fit linear mixed-effects models with standardized relative abundances as a function of host (random effects term), race (race ‘white’, ‘black’ and ‘other’) and recent antibiotic use. Fitted coefficients for the IBD and rUTI cohorts are plotted in Extended Data Fig. 5.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Metagenomic sequence data are available from the Sequence Read Archive under Bioproject PRJNA400628. PBMC RNA-seq data are available from the database of Genotypes and Phenotypes (dbGaP) under project no. phs002728. Questionnaire data and output files from MetaPhlAn2, Humann2 and StrainGE are available from github.com/cworby/UMB-study. Source data are provided with this paper.

Code availability. Custom R scripts used to analyse outputs are available from github.com/cworby/UMB-study.

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**Author contributions**

Study design was undertaken by H.L.S., K.W.D., S.J.H. and A.M.E. Study coordination was carried out by H.L.S., K.B., S.B.C. and A.K. Experiments were performed by H.L.S., J.S.P., C.L.P.O., V.L.M. and A.E.P. Data analysis was undertaken by C.J.W., H.L.S., T.J.S., L.R.v.D., R.A.B., B.S.O., B.J.H., C.A.D. and W.-C.C. Consultation and supervision of analyses were the responsibility of B.J.W., A.L.M., T.J.H., T.M.H., A.L.K., H.H.L., K.W.D., S.J.H. and A.M.E. C.J.W., A.L.M., K.W.D., S.J.H. and A.M.E. prepared the original draft. Review and approval of the final manuscript was provided by all authors.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41564-022-01107-x.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41564-022-01107-x.

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Extended Data Fig. 1 | Sex precedes all clinical UTI events. Survey reports of intercourse frequency in the previous two weeks. Responses are partitioned by (i) control women, (ii) rUTI women at time of UTI, and (iii) rUTI women at non-UTI time points.
Extended Data Fig. 2 | SCFA producing bacteria are depleted in the rUTI gut. Cumulative relative abundances of (a) butyrate and (b) propionate producing bacterial species in rUTI and control samples. Box plots display the median (center line), 25th and 75th percentiles (box), as well as the 5th and 95th percentiles (whiskers). Within-host average relative abundances of individual species for (c) butyrate and (d) propionate producers are also shown. Horizontal lines denote the mean relative abundance in rUTI (red) and control (blue) women.
Extended Data Fig. 3 | Bray Curtis dissimilarity across stool samples. (a) For each patient, the distribution of Bray-Curtis dissimilarities between all stool samples, ordered by increasing mean patient values within each cohort. (b) Bray-Curtis distributions between samples taken at the time of UTI vs. healthy time points (red), compared to all pairwise healthy sample comparisons. Box plots show the median (center line), 25th and 75th percentiles (box), as well as the 5th and 95th percentiles (whiskers).
Extended Data Fig. 4 | rUTI dysbiosis is not driven by antibiotic use during the study. We grouped rUTI women according to their antibiotic exposures at any point during the UMB study: (i) ciprofloxacin (n = 6); (ii) non-ciprofloxacin antibiotics (n = 6); (iii) no antibiotics (n = 3); (iv) any antibiotics (n = 12). Groups were compared against each other and against the control cohort (n = 16) for (a) overall microbial richness and (b) relative abundance of butyrate producers. Crosses represent mean values for individuals, boxplots denote the IQR and 95% central quantiles for each group. Wilcoxon rank sum tests (two-sided) were applied to group pairs to derive p-values. (c) Temporal trends of microbial richness (black) and relative abundance of butyrate producers (red) in all rUTI participants using antibiotics during the study. For each individual, linear models were fit to observations (points) over time; fitted trends are shown, with coefficients & p values reported at the top of each panel. Dashed vertical lines denote antibiotic usage. Participant mean values are represented by horizontal lines.
Extended Data Fig. 5 | Most species depleted in the rUTI gut are also depleted in the IBD gut. We compared discriminatory taxa in rUTI women to those in IBD patients using data from adult participants in the HMP2 study. For each study, we fitted mixed effects models to standardized Metaphlan2 relative abundances as a function of categorical disease group (rUTI or IBD respectively, vs. each study’s control cohort), including covariates for race and antibiotic use. The disease group coefficients are plotted against each other for each species, with circle pairs representing the average relative abundance in each study. Species with uncorrected p values <0.05 in either study are labelled. Species not present in at least 10% of samples in either study are excluded. IBD comprises patients with either CD or UC.
Extended Data Fig. 6 | Immunological differences between cohorts. (a) PCA plot of gene expression across cohorts, based on PBMC RNA Seq data. Samples are partitioned into healthy controls (n = 13), rUTI patient baseline (enrollment; n = 12) and rUTI patient at time of UTI (n = 17). (b) Plasma eotaxin-1 levels in control women, and rUTI women at healthy enrollment and time of UTI. (c) Relative abundance of NK cells in control and rUTI women based on CIBERSORT output. Box plots display the median (center line), 25th and 75th percentiles (box), as well as data points within 1.5 IQR of the upper & lower quartiles (whiskers), and outliers beyond this range (dots).
Extended Data Fig. 7 | Limited relationship between non SCFA-producing taxa with butyrate producers. For all non SCFA-producing genera detected across all samples, the correlation coefficient between its relative abundance and the relative abundance of butyrate producers was calculated and plotted against its mean relative abundance across (a) control (n = 170) and (b) rUTI (n = 197) samples. Genera with an absolute correlation coefficient greater than 0.25 are labeled, along with Escherichia, represented by the red point.
Extended Data Fig. 8 | *E. coli* relative abundance around the time of UTI and phylogroup distributions. For all stool samples taken within 3 days of a UTI event, the log fold change is given relative to (a) the median *E. coli* relative abundance in the corresponding patient, excluding samples taken at the time of UTI, and (b) the relative abundance of *E. coli* in the preceding stool sample. ‘X’ denotes samples for which there was no prior sample available. (c) Number of detected *E. coli* strains by sample type. (d) Number of detected StrainGST reference strains vs. relative abundance of *E. coli*. 
Extended Data Fig. 9 | Strain dynamics in control women. Strain dynamics within all control participants; analogous to Fig. 3. (a) Phylogenetic tree comprising strains called by StrainGE across all stool and urine samples, colored by phylogroup. Bars show number of unique participants with at least one strain observation; bars are bolded if the strain was identified in at least one urine sample. Each strain identified in control women is uniquely identifiable by the phylogroup (colour) and ID (numeral) indicated right. (b) Each panel represents longitudinal strain dynamics within one patient. Numerals refer to strain identifiers in (a). All fecal strains are connected to their most recent previous observation in fecal samples. Diamonds denote clinical rectal swabs. Strains identified in urine outgrowth depicted if available; otherwise raw urine strains are shown. Fecal or urine samples with no detected *E. coli* strains represented by open grey symbols. Vertical dashed lines represent self-reported antibiotic use.
Extended Data Table 1 | Cohort Characteristics. Demographic, behavioral and dietary characteristics of the rUTI and control women who completed the year-long study. Fisher’s exact tests (two-sided) were used to compare frequencies between cohorts. * recorded recent consumption of item in at least 50% of questionnaire responses during study.

|                         | rUTI (n=14) | Control (n=14) | p      |
|-------------------------|-------------|----------------|--------|
| Age (mean, years)       | 25.6        | 29.3           | 0.77   |
| Race—white              | 12 (86%)    | 6 (46%)        | 0.046  |
| No. UTIs during study   | 1.6         | 0              |        |
| Intercourse frequency   | 2           | 1.6            | 0.22   |
|                         |             |                |        |
| Antibiotic use during   |             |                |        |
| study                   |             |                |        |
| Cumulative antibiotic   | 2.6         | 0.9            | 0.04   |
| use (doses per patient) |             |                |        |
| Total doses:            |             |                |        |
| Nitrofurant (macrobid)  | 11          | 0              |        |
| Fluoroquinolone (ciprofloxacin) | 8      | 0            |        |
| Beta-lactam (incl. amoxicillin, cephalaxin) | 6 | 3 |        |
| Macrolide (azithromycin) | 1          | 5              |        |
| Sulfonamide (bactrim, sulfamethoxazole) | 3 | 0           |        |
| Tetracycline (doxycycline) | 0          | 2              |        |
| Unspecified             | 7           | 2              |        |
|                         |             |                |        |
| Usually consumers*      |             |                |        |
| Tea or coffee (no sugar) | 11 (79%)   | 3 (21%)        | 0.01   |
| Soft drinks, tea/coffee | 10 (71%)   | 12 (86%)       | 0.65   |
| with sugar              |             |                |        |
| Diet soft drinks, tea   | 6 (43%)     | 2 (14%)        | 0.21   |
| coffee with sugar       | 6 (43%)     | 6 (43%)        | 0.45   |
| Alcohol                 | 11 (79%)    | 10 (71%)       | 1      |
| Yoghurt/tacoative       | 9 (64%)     | 7 (50%)        | 0.7    |
| bacterial culture       |             |                |        |
| Dairy                   | 14 (100%)   | 14 (100%)      | 1      |
| Probiotic (not yoghurt) | 3 (21%)     | 0 (0%)         | 0.22   |
| Fruit                   | 14 (100%)   | 14 (100%)      | 1      |
| Vegetables              | 14 (100%)   | 13 (92%)       | 1      |
| Beans (incl. tofu, soy) | 10 (71%)    | 8 (57%)        | 0.69   |
| Processed meats         | 9 (64%)     | 12 (86%)       | 0.38   |
| Red meat                | 9 (64%)     | 13 (92%)       | 0.16   |
| White meat              | 11 (79%)    | 14 (100%)      | 0.22   |
| Shellfish               | 6 (43%)     | 4 (29%)        | 0.69   |
| Fish                    | 7 (50%)     | 8 (57%)        | 1      |
| NSAIDs                  | 11 (79%)    | 7 (50%)        | 0.24   |
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*Our web collection on statistics for biologists contains articles on many of the points above.*

**Software and code**

Policy information about availability of computer code

| Data collection | No software was used for data collection. |

| Data analysis | Metagenomic sequence data were analyzed using Metaphlan2 (v2.7), Humann2 (v2.8.1). RNA-Seq data were analyzed using CIBERSORT. were Luminex assay results were read and quantified using a BioPlex multiplex plate reader and Microplate Manager software (v5). Statistical analysis was conducted using R v4.0.3 and R packages vegan (v2.5), DESeq (v1.30), Limma (v1.1.27). Custom code to run analyses and generate figures is available at github.com/cworby/UMB-study |

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Metagenomic sequence data are available from the Sequence Read Archive under Bioproject PRJNA400628. Questionnaire data, output files from Metaphlan2, Humann2 and StrainGE are available from github.com/cworby/UMB-study. PBMC RNASeq data are PBMC RNASeq data are available from dbGaP at http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs002728.v1.p1. Human genome hg19 used in PBMC RNAseq analysis available under Bioproject PRJNA31257.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample size calculation for longitudinal microbiome analyses is not straightforward, we note that each high-risk patient represents their own controlled experiment. No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous longitudinal microbiome studies which were able to detect significant effects, e.g. Dethlefsen & Relman PNAS 2011. 108 Suppl 1: p.4554-61; Turnbaugh et al., Nature, 2006. 444(7122): p. 1027-31. |
| Data exclusions | No data were excluded from our study. |
| Replication | Our study was an observational cohort study and no replication was performed, although we have described the recruitment process and sampling strategy sufficiently such that the study may be replicated. |
| Randomization | Our study was an observational study with no intervention and cohorts based on pre-determined criteria; as such, no randomization was required. Control participants were age-matched to rUTI participants, and few dietary differences existed between the cohorts based on survey responses. We adjusted for race in cohort comparisons of microbiome structure. |
| Blinding | Our study was an observational study with no intervention and cohorts based on pre-determined criteria; as such, blinding was not relevant. |

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | n/a |
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| Animals and other organisms | |
| Human research participants | |
| Clinical data | |
| Dual use research of concern | |

| Methods | n/a |
| n/a | Involved in the study |
| ChIP-seq | |
| Flow cytometry | |
| MRI-based neuroimaging | |

Human research participants

Policy information about studies involving human research participants

Population characteristics

Women from the St. Louis, MO area reporting three or more UTIs in the past 12 months were recruited into the rUTI study arm, while women with no history of UTI (at most one UTI ever) were recruited into the control arm via the Department of Urological Surgery at Barnes-Jewish Hospital in St. Louis, MO. We excluded women who: i) had inflammatory bowel disease (IBD) or urological developmental defects (e.g., ureteral reflux, kidney agenesis, etc.), ii) were pregnant, iii) take antibiotics as prophylaxis for rUTI, and iv) were younger than 18 years or older than 45 at the time of enrollment.

Recruitment

rUTI women were recruited based on clinical history via the Department of Urological Surgery, along with age-matched control women with no history of rUTI. Flyers were posted around Wash U Medical School, Wash U in St. Louis campus, and the Barnes-Jewish Hospital. Participants were remunerated midway through the study, and at the end of the study upon completion, via gift cards. Self selection biases may therefore exist; in particular we did not collect socio-economic data on participants. However, given age matching and the similarity in self-reported dietary habits between cohorts, we do not anticipate any such bias to have a significant impact on the composition of the gut microbiome.

Ethics oversight

This study was conducted with the approval and under the supervision of the Institutional Review Board of Washington University School of Medicine in St. Louis, MO

Note that full information on the approval of the study protocol must also be provided in the manuscript.