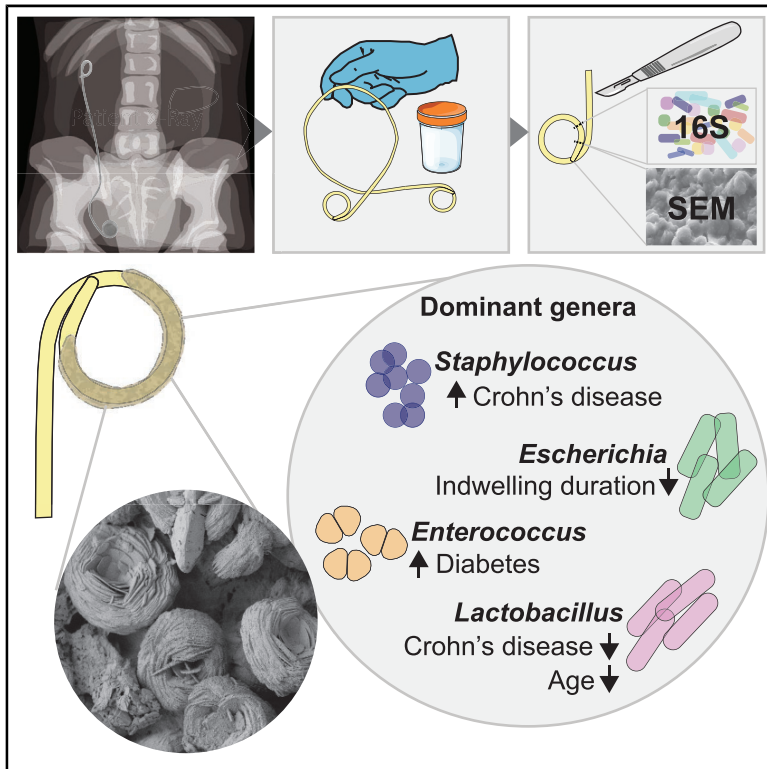


Ureteral Stent Microbiota Is Associated with Patient Comorbidities but Not Antibiotic Exposure

Graphical Abstract



Authors

Kait F. Al, John D. Denstedt, Brendan A. Daisley, ..., Gregor Reid, Hassan Razvi, Jeremy P. Burton

Correspondence

jeremy.burton@lawsonresearch.com

In Brief

Ureteral stent-associated bacteria and their impact on device encrustation are underexplored. Using microbiota sequencing and SEM/EDX, Al et al. identify a reproducible, temporally stable, patient-specific stent microbiota associated with patient comorbidities but not antibiotic exposure.

Highlights

- Ureteral stents harbor a reproducible and patient-specific microbiota
- Patient comorbidities (not UTIs or antibiotics) are correlated with the microbiota
- Urine is not an accurate biomarker of stent microbiota or encrustation
- Stent-specific prophylactic antibiotic administration may require recalibration



Article

Ureteral Stent Microbiota Is Associated with Patient Comorbidities but Not Antibiotic Exposure

Kait F. Al,^{1,2} John D. Denstedt,³ Brendan A. Daisley,^{1,2} Jennifer Bjazevic,³ Blayne K. Welk,³ Stephen E. Pautler,³ Gregory B. Gloor,⁴ Gregor Reid,^{1,2,3} Hassan Razvi,³ and Jeremy P. Burton^{1,2,3,5,6,*}

¹Centre for Human Microbiome and Probiotic Research, Lawson Health Research Institute, London, ON, Canada

²Department of Microbiology and Immunology, The University of Western Ontario, London, ON, Canada

³Division of Urology, Department of Surgery, The University of Western Ontario, London, ON, Canada

⁴Department of Biochemistry, The University of Western Ontario, London, ON, Canada

⁵Twitter: @our_microbiome

⁶Lead Contact

*Correspondence: jeremy.burton@lawsonresearch.com

<https://doi.org/10.1016/j.xcrm.2020.100094>

SUMMARY

Ureteral stents are commonly used to prevent urinary obstruction but can become colonized by bacteria and encrusted, leading to clinical complications. Despite recent discovery and characterization of the healthy urinary microbiota, stent-associated bacteria and their impact on encrustation are largely underexplored. We profile the microbiota of patients with typical short-term stents, as well as over 30 atypical cases (all with paired mid-stream urine) from 241 patients. Indwelling time, age, and various patient comorbidities correlate with alterations to the stent microbiota composition, whereas antibiotic exposure, urinary tract infection (UTI), and stent placement method do not. The stent microbiota most likely originates from adhesion of resident urinary microbes but subsequently diverges to a distinct, reproducible population, thereby negating the urine as a biomarker for stent encrustation or microbiota. Urological practice should reconsider standalone prophylactic antibiotics in favor of tailored therapies based on patient comorbidities in efforts to minimize bacterial burden, encrustation, and complications of ureteral stents.

INTRODUCTION

Ureteral stents are hollow conduits placed in the ureter from the renal pelvis to the bladder and are commonly used in urological practice to maintain urine drainage, which can be impeded by obstruction caused by urolithiasis, stricture, or malignancy. Due to constant contact with the urine, deposition of urinary crystals and formation of bacterial biofilms on stents are common.^{1,2} The formation of these encrustations can lead to complications, including infection, failure of the stent to drain urine, more frequent device exchanges, and subsequent difficulty with removal.¹ Indwelling ureteral stents have been associated with the development of urinary tract infections (UTIs) and, in more severe cases, pyelonephritis or urosepsis, which may be related to single species or polymicrobial biofilms attached to the stent.³

The urinary tract harbors a unique microbiota that is distinct from that of the gut in composition and is of much lower abundance.^{4,5} Based on recent evidence, it is likely that the different sites and tissues throughout this system have different microbiotas.^{5–7} The biofilms that form on urinary devices, such as stents and catheters, may originate from this microbiota or contamination during insertion of the device.^{8,9} Regardless of their origin,

the development of biofilms on these devices illustrates that even very low numbers of bacteria can quickly take advantage of the niche-altering foreign material to expand their populations. Previous studies in stent patients have identified bacterial colonization rates from 70% to 90%.^{10,11} Bacteriuria can be common in upward of 20% of patients with stents, and *Escherichia coli* is often the most commonly cultured and identified organism.¹⁰ Bacterial isolates derived from stent biofilms of clinical origin often demonstrate resistance to multiple antibiotics, and antibiotic prophylaxis or concomitant antibiotic administration does not appear to reduce the incidence of stent-related symptoms or UTI incidence or severity.^{12–15} Due to these findings, the use of antibiotic prophylaxis for stents is controversial, and it is unclear how these compounds may impact the urinary microbiota during stenting.

The purpose of this study was to elucidate how the urinary microbiota and other host factors impact bacterial colonization and encrustation of indwelling ureteral stents. Specific interest was taken in determining whether the urine microbiota accurately recapitulated the adhered stent microbiota, allowing it to act as a “biomarker” of potential device infection or encrustation. As such, we utilized 16S rRNA gene sequencing and scanning electron microscopy (SEM) to characterize the urine and



Table 1. Demographic and Clinical Characteristics of Study Participants

| Participant Characteristic | N = 241 (%) | |
|--|--|------------|
| Age | 59.01 ± 13.84 (range 22–90) | |
| Gender | 122 females (50.6), 119 males (49.4) | |
| Indwelling time | 22.79 ± 34.61 days (range 2–394) | |
| Body mass index | 31.04 ± 7.64 (range 17.00–60.00) | |
| Reason for stent placement: | uroolithiasis | 219 (90.9) |
| | stricture | 5 (2.1) |
| | mass | 10 (4.1) |
| | other | 7 (2.9) |
| Stent placement method: | retrograde | 219 (90.9) |
| | antegrade | 22 (9.1) |
| Patients with bilateral stents | 11 | |
| Patients with multiple sequential stents over time | 11 (9 patients with 2 devices and 2 patients with 3 devices) | |
| Time between sequential stent placements | 63.5 ± 28.6 days (range 25–105) | |
| Use of antibiotics within the last 30 days from stent collection | 225 (93.4) | |
| Previous history of UTI | 99 (41.1) | |
| UTI within 7 days of stent placement or while indwelling | 37 (15.4) | |
| Diabetes | 54 (22.4) | |
| Hyperlipidemia | 99 (41.1) | |
| Hyperuricemia | 16 (6.6) | |
| Hypertension | 122 (50.6) | |
| Irritable bowel syndrome | 17 (7.1) | |
| Inflammatory bowel disease | | 28 (11.6) |
| | Crohn's disease | 8 (3.3) |
| | Ulcerative colitis | 20 (8.3) |
| Pulmonary disease | 72 (29.9) | |

stent microbiota from 241 patients that were sampled from a single urology center. The large sample size and complementary nature of the samples provide a high-resolution insight into bacterial attachment to ureteral stents under different clinical scenarios.

RESULTS

Study Participant Demographics

Two hundred and forty-one participants were recruited from a single center over approximately 1 year (Figure S1). Patient demographic characteristics are summarized in Table 1. The majority of samples were collected from typical stenting events, where one double-J stent links between one of the kidneys and the bladder. However, cases of bilateral (stents between both the left and right kidney to the bladder that indwell at the same time), longitudinal (multiple consecutive devices in the same patient over time that were collected independently), antegrade (placed downward from the kidney percutaneously rather than upward from the urethra), uncommonly long indwelling times, and various encrustation levels were also examined. The majority of study participants had an indwelling stent placed for treatment related to stone disease, though in 22 participants, the stents were necessitated for other reasons, including radiation-

induced ureteral stricture and the presence of retroperitoneal masses.

The Stent Microbiota Is Dominated by Urinary Bacteria

Microbiota sequencing was performed on 1-cm-long slices of both proximal and distal ends of the stents and bacterial pellets from mid-stream urine samples (Figure S1). Stringent bioinformatic filtering was performed on sequenced reads such that 711 samples and 43 amplicon sequence variants (SVs) were maintained for downstream analysis. The most abundant SVs in stent and urine samples corresponded to the bacterial genera *Staphylococcus*, *Enterococcus*, *Lactobacillus*, and *Escherichia* (Table S1). The clinical samples (not including positive and negative controls) contained an average of 13.5 SVs, ranging from 3 to 31. There was a positive correlation between read count and observed SVs (Figure S2A). Urine samples had significantly more SVs observed (Figure S2B) and higher total read count compared to stent samples (Figure S2C).

The sequence counts were center log ratio (CLR) transformed, generating samplewise Aitchison distances.¹⁶ A heatmap representing the relative abundance of CLR-transformed samples was generated based on the Aitchison distance average linkage clustering (Figure 1). The differences in microbiota composition at the genus level were not driven by gender or sample type (urine or stent). This was confirmed with a Benjamini-Hochberg-corrected

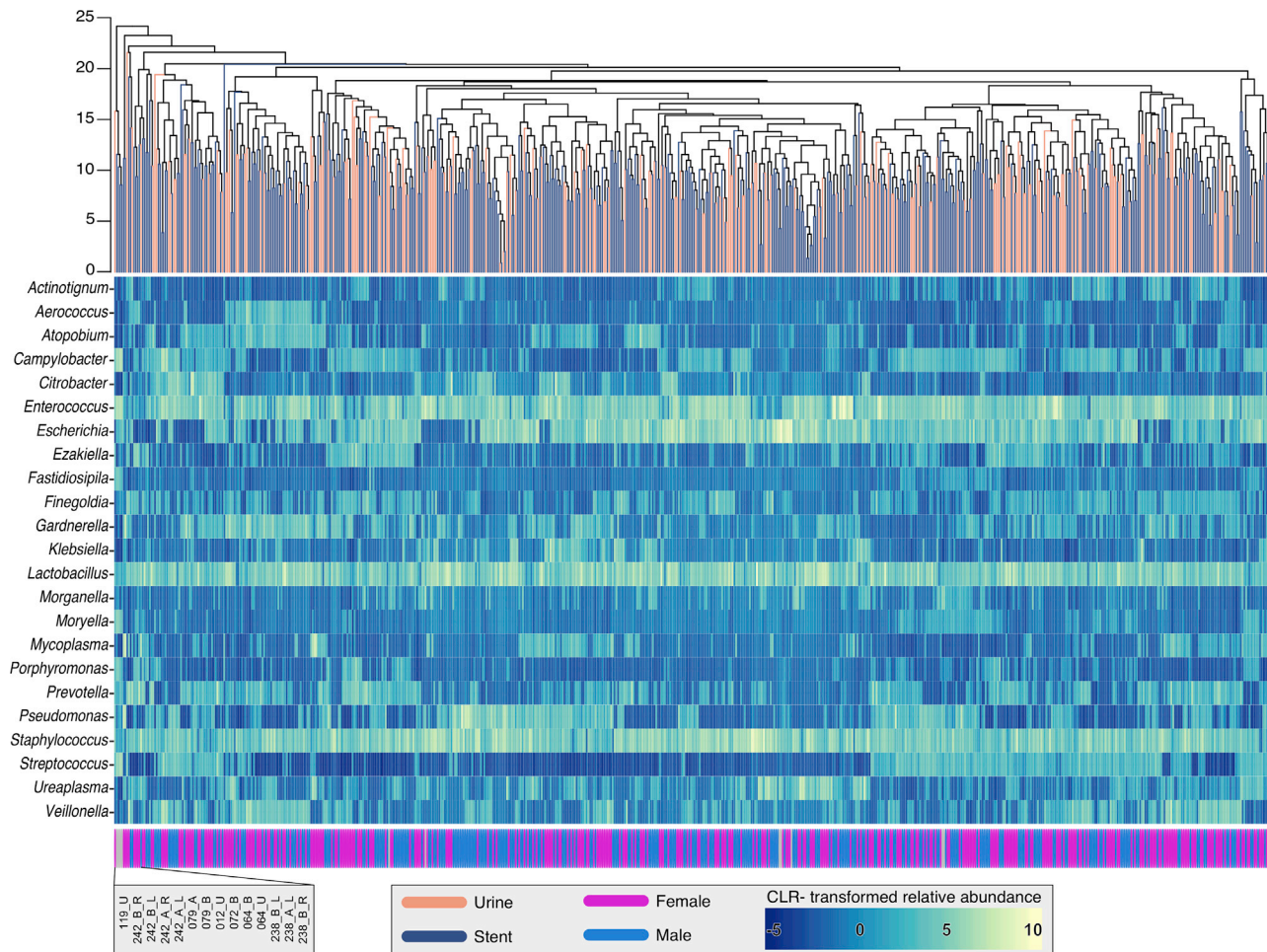


Figure 1. The Stent Microbiota Is Dominated by Urinary Bacteria

Samples are plotted left to right and ordered by the dendrogram. The dendrogram was generated from CLR-transformed read counts grouped by genera, based on the average linkage clustering of per-sample Aitchison distance. Branches of the dendrogram are colored by sample type (stents are navy; urine is orange). The heatmap represents the relative abundance of genera within samples (more abundant genera are lighter in color). Color coding below the heatmap corresponds to patient gender (females are pink; males are blue). An excerpt from the fourteen leftmost branches of the tree illustrates that, in general, samples from the same individual group nearby on the dendrogram (see also [Figure S4A](#)). n = 667; 213 urine and 454 stent samples from 241 patients.

Welch's t test and principal-component analysis (PCA) performed on the log-ratio transformed data at SV level ([Figure S3](#)), where all samples ([Figure S3A](#)) and gender subsets ([Figures S3B](#) and [S3C](#)) did not separate by sample type. Furthermore, samples were more similar within participants than between participants ([Figure S4A](#)). These findings demonstrate that the same microbes dominate both stent and urine samples from a single patient, and therefore, stent microbiota is likely to be urinary derived.

Within Patients, the Stent Microbiota Is Stable and Reproducible

Although sample types were dominated by similar organisms, stent samples were further compared based on curl position to determine whether the two curls (proximal curl in the kidney and distal in the bladder) had a distinct microbial profile compared to that of the patient's urine ([Figure S4B](#)). Specifically,

beta diversity was measured by Aitchison distance to evaluate the distance between proximal and distal curls from each stent, between the urine and the stent curls of the same patient, and finally between the urine and stent curls from all other patients ([Figure S4B](#)). Within participants, stent curls had significantly shorter distances between proximal and distal curls versus the distance between stent curls to the urine, although distance between urine and stent curls from other participants was the greatest. Thus, microbiota composition of the stent curls was more similar to each other than either curl to the urine, indicating the presence of a patient- and stent-specific microbiota that does not directly reflect the composition of the urine but likely derives from it.

From the devices recovered from participants with multiple sequentially placed stents, many of the same organisms were detected within the same individual over time ([Figures 2](#) and [S5](#)). Upon PCA, samples from the same individual generally

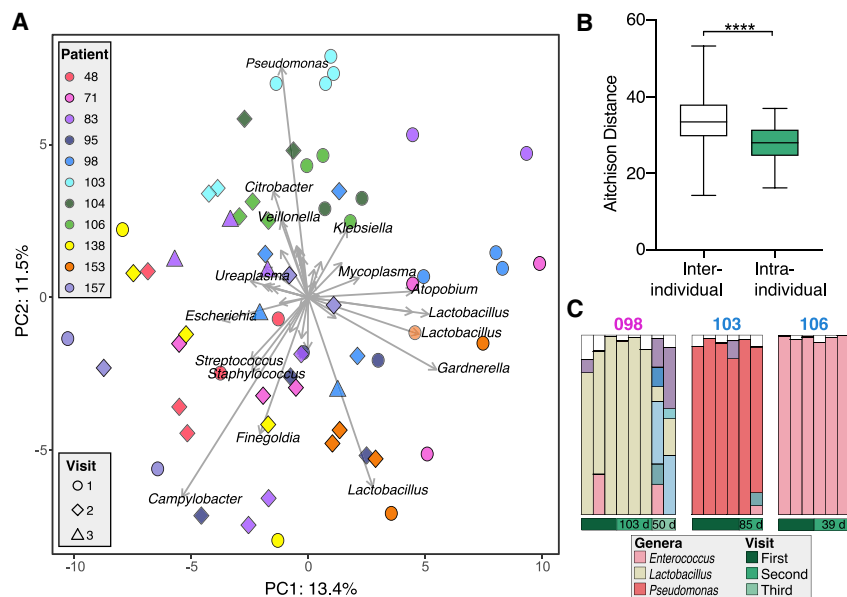


Figure 2. Principal-Component Analysis of Longitudinal Samples

(A) PCA was performed on CLR-transformed Aitchison distances of longitudinally collected samples. Each colored point represents a sample. Distance between samples on the plot represents differences in microbial community composition, with 24.9% of total variance being explained by the first two components shown. Strength and association for genera (sequence variants) are depicted by the length and direction of the gray arrows, respectively. Points are colored by participant and shaped by visit number. $n = 64$.

(B) Aitchison distance was greater between inter-individual samples of the same type ($n = 1,254$) than between samples from the same participant of the same type at different visits ($n = 37$; Bonferroni-corrected Mann-Whitney U test; $p < 0.0001$). Boxplot whiskers represent minimum and maximum.

(C) Representative relative abundance bar plot of three longitudinal stent patients. Each vertical bar represents the relative SV abundance within a single sample. Samples are grouped by participant. Relative abundance of SVs is colored by genera, with common genera shown in the legend. Days between sample collections are listed in the green visit code.

cluster together (Figure 2A). Distance between samples from the same participants at different time points was shorter than between samples from different participants (Figure 2B). There were no significant effects of visit number on the samples (Benjamini-Hochberg-corrected Wilcoxon rank sum test; data not shown). Thus, on a per-patient basis, the stent microbiota is a reproducible community over time, even over the course of up to 150 days.

The microbiota of bilateral stents did not differ significantly, as determined from eleven subjects (Figures 3 and S6). Within patients, both proximal and distal ends of bilateral stents clustered separately from the urine (Figure 3A). Intraindividual samples were closer together than interindividual samples (Figure 3B). There was greatest spread between stent and urine samples from the same individual, and the distance between stent samples was the shortest (Figure 3B), indicating the presence of a distinct stent-specific microbiota.

Microbiota Variation of Ureteral Stents Correlates with Patient Attributes

To determine whether patient and sample attributes (metadata) correlated with microbiota variation, CLR-transformed sample-wise Aitchison distances were evaluated.¹⁷ With this approach, several metadata factors were determined to be microbiota confounders, including stent indwelling time and patient comorbidities (Table S2). These confounders were subsequently adjusted for, and several statistically significant associations of microbiota variation remained between metadata characteristics and taxonomic features as determined using a general linear model, including patient age, body mass index, stent indwelling time, pulmonary disease, hypertension, diabetes, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and hyperlipidemia (Table 2).¹⁸

To determine whether the degree of encrustation correlated with microbial composition, stents were categorized based on visible encrustation level (Table S3). There was a correlation between the degree of stent encrustation and the amount of time stents were indwelling (Figure S7A). Shannon's index of alpha diversity was negatively correlated with degree of stent encrustation (Figure S7B) and was lower in grade-3 encrusted stents compared to grade 0 (Figure S7C). This suggests that the longer a stent is indwelling, the more likely it will be to become encrusted and dominated by a less diverse microbial community.

Ten study participants had stents indwelling for greater than 2 months; these participants were determined to be outliers, having stents significantly longer than the average indwelling time of 23 days (ROUT method of outlier detection; $Q = 0.1\%$).¹⁹ The microbiota of these patients was not significantly different when compared to all other samples or to samples from the ten participants with the shortest indwelling durations (Figure S8). However, as indwelling time increased, relative abundance of the genera *Finegoldia* and *Porphyromonas* increased, whereas *Enterococcus* and *Escherichia* decreased (Table 2).

Antibiotic exposure was widespread among participants; about 93% had been exposed to antibiotics within 30 days of sample collection (Table 1). However, the microbiota of the few participants without recent antibiotic exposure was not significantly different than the majority (Table 2; Figure 4A). These participants also did not differ by encrustation grade or Shannon's index of alpha diversity (Figures 4B and 4C).

Stents were evaluated based on their placement method. The majority (90.9%; Table 1) of stents were placed in a retrograde manner; however, the microbiota of the stents placed antegrade (i.e., during percutaneous nephrolithotomy or nephroscopy) was not significantly different than those placed retrograde

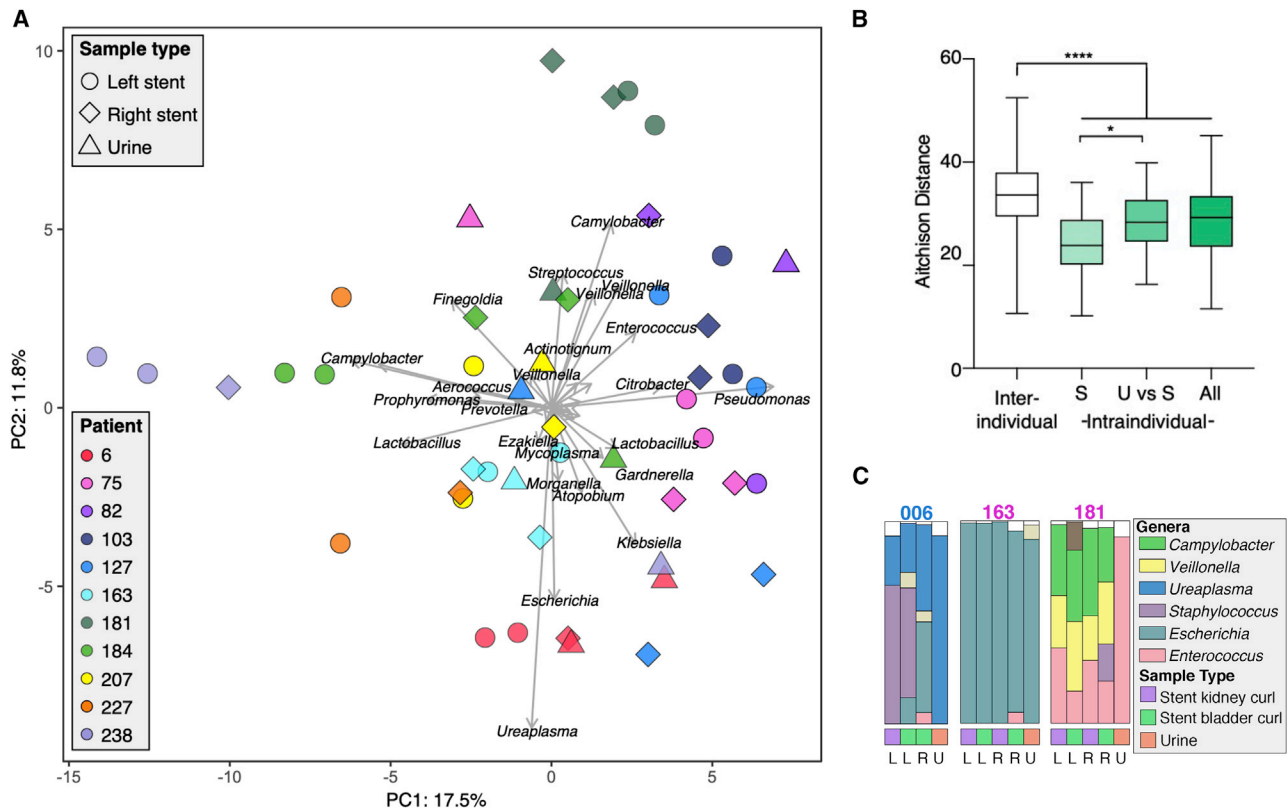


Figure 3. Microbial Communities of Bilateral Stents

(A) PCA was performed on CLR-transformed Aitchison distances of samples from patients with bilateral indwelling stents. Each colored point represents a sample. Distance between samples on the plot represents differences in microbial community composition, with 29.3% of total variance being explained by the first two components shown. Strength and association for genera (sequence variants) are depicted by the length and direction of the gray arrows, respectively. Points are colored by participant and shaped by sample type, which include both proximal and distal stent ends ($n = 55$).

(B) Aitchison distance was compared between interindividual samples and intraindividual samples. S, distance between stent samples from the same participant ($n = 154$); U versus S, distance between urine and stent samples from the same participant ($n = 39$); all, all samples from a single participant ($n = 234$). All intraindividual comparisons had significantly shorter distances than the distance between samples from different individuals ($n = 2,736$; Bonferroni-corrected Dunn's tests; $p < 0.0001$). In intraindividual comparisons, the distance was shortest between stent samples and furthest from urine to stent samples ($p = 0.022$). Boxplot whiskers represent minimum and maximum.

(C) Representative relative abundance bar plot of three bilateral stent patients. Each vertical bar represents the relative SV abundance within a single sample. Samples are grouped by participant. Relative abundance of SVs is colored by genera, with common genera shown in the legend. Sample type is color coded. Stents from the left side are denoted by "L" and from the right side by "R"; urine is denoted by "U."

(Table 2; Figure 4D). These participants also did not differ by encrustation grade or Shannon's index of alpha diversity (Figures 4E and 4F).

About 15% of patients had culture-confirmed UTIs within 7 days of stent placement or throughout the stent indwelling period (Table 1). The microbiota and degree of stent encrustation in these patients was not significantly different than those without UTIs (Figures 4G and 4H); however, Shannon's index of alpha diversity was lower for patients with UTIs (Figure 4I).

SEM/EDX Confirm the Presence of Urinary Crystals and Bacterial Biofilms

SEM imaging of stent samples revealed characteristic crystal phases and the presence of bacterial biofilms (Figure 5). Where bacteria-like structures were visualized, their morphology showed concordance with the genera that were present in the sample based on microbiota sequencing (Figure 5, samples

014 and 195). The predominant substances on the stent surfaces consisted of organic deposits and crystals. X-ray diffraction of crystalline structures confirmed the presence of calcium oxalate monohydrate in oval and multiple-twinning morphologies, calcium oxalate dihydrate, calcium phosphate (Figure 5), uric acid, and struvite (not shown).

DISCUSSION

This study characterized the urinary and device-adhered microbiota of ureteral stent patients. Importantly, we identified several patient factors and comorbidities that correlated with stent microbiota composition and demonstrated that gender, antibiotic exposure, and stent placement method did not have any significant associations with the urinary or stent microbiota. Our findings also demonstrate consistency in stent microbial community over time in patients with multiple stent placements and in both

Table 2. Significant Correlations between Metadata Attributes and the Microbiota after Adjusting for Confounders

| Metadata | Genus | Coefficient ^a | FDR |
|-----------------------|-----------------------|--------------------------|-------|
| Age | <i>Campylobacter</i> | 0.250 | 0.034 |
| | <i>Lactobacilli</i> | -0.370 | 0.002 |
| | <i>Veillonella</i> | 0.457 | 0.002 |
| Body mass index | <i>Actinotignum</i> | 0.499 | 0.048 |
| | <i>Morganella</i> | 0.546 | 0.049 |
| Stent indwelling time | <i>Enterococcus</i> | -0.292 | 0.008 |
| | <i>Escherichia</i> | -0.309 | 0.034 |
| | <i>Fingoldia</i> | 0.202 | 0.034 |
| | <i>Porphyromonas</i> | 0.245 | 0.001 |
| Pulmonary disease | <i>Campylobacter</i> | 0.627 | 0.004 |
| | <i>Ezakiella</i> | 0.498 | 0.034 |
| Hypertension | <i>Campylobacter</i> | -0.595 | 0.013 |
| | <i>Klebsiella</i> | 0.592 | 0.034 |
| | <i>Moryella</i> | 0.349 | 0.035 |
| Diabetes | <i>Citrobacter</i> | -1.653 | 0.002 |
| | <i>Enterococcus</i> | 1.086 | 0.034 |
| IBS | <i>Prevotella</i> | 0.046 | 0.001 |
| | <i>Veillonella</i> | 0.041 | 0.020 |
| Crohn's disease | <i>Lactobacillus</i> | -0.112 | 0.023 |
| | <i>Staphylococcus</i> | 0.009 | 0.061 |
| Ulcerative colitis | <i>Veillonella</i> | 0.036 | 0.020 |
| Hyperlipidemia | <i>Aerococcus</i> | -0.424 | 0.049 |
| | <i>Ureaplasma</i> | -0.816 | 0.003 |

FDR, false discovery rate.

^aCoefficients of association >0 are correlated with higher and <0 with lower relative abundance of the specified genus.

left and right sides during bilateral stent placement, solidifying the true presence of a reproducible stent microbiota and corroborating previous findings from urinary catheters.⁸ Interestingly, intraindividual microbiotas of proximal and distal stent ends were more similar than either stent end compared to the urine, indicating that, although the same genera may be present in the urine, it is not proportionally representative of the bacterial community colonizing the stent.

Previous culture-based studies have shown that removed stents are frequently culture positive despite patients exhibiting a culture-negative urine profile.^{1,20} Corroborating these findings, we demonstrated that urinary and stent microbiotas were dominated by similar bacterial genera; however, when investigating the patterns on a per-patient basis, both proximal and distal ends of the stent, as well as left and right bilateral stents, were more similar to each other in microbiota composition than to the urine. Additionally, culture-confirmed UTI was not associated with increased encrustation level in this cohort, although a caveat to this analysis was that only 15% of patients had confirmed UTI. These findings illustrate that urine is not an accurate biomarker of stent encrustation or representative of the stent-adhered microbiota. Instead, the degree of stent encrustation was positively correlated with indwelling time and negatively correlated with microbial diversity, indicating that the longer a

stent is indwelling, the greater the likelihood of it becoming encrusted and colonized with a less-diverse microbial community.

The urinary microbiome may extend as far as the renal collecting system. This renal microbiota may contribute to the microbial community of the proximal stent curl, or bacteria residing in the bladder could adhere to the proximal stent curl during retrograde insertion.^{6,21} Bacteria are also thought to ascend from the distal curl during movement of the stent while indwelling or by utilizing active motility, a process that can occur quite rapidly.^{11,22-26} Our findings support these previous studies and suggest that the stent-associated microbiota is derived from the urinary bladder, based on the fact that no difference was observed in microbial composition between antegrade or retrograde placement method (though only 9% of stents were inserted in an antegrade fashion). Further validation is provided by the stent-associated microbiota being dominated by common urinary bacteria, which is unlikely to have originated from skin or gut contamination during placement.²⁷ Taken together, our findings suggest that, although the urinary microbiota may originally seed onto the stent, the stent microbial community is shaped and enriched for competitively adherent bacteria and eventually diverges significantly from the urine.

A previous microbiota study of stent encrustations demonstrated a lack of association between “urotype” and patient conditions, including age, gender, BMI, diabetes, urinary crystals, and other factors.²⁸ The current study differed by utilizing a non-partisan analysis method, whereby arbitrary community groups or “urotypes” were not used and instead the entire dataset was tested against all patient and sample characteristics. With this approach, confounders were adjusted for and significant associations between eight metadata features and genus-level microbiota changes were established.

In concordance with previous studies, age was determined to be significantly associated with increased *Veillonella* spp. and decreased *Lactobacillus* spp.^{29,30} In humans, *Veillonella* spp. are commensals of the oral cavity and gastrointestinal and urogenital tracts, with the potential to cause opportunistic infections, including UTI.³¹⁻³⁵ *Veillonella* spp. are also commonly associated with a more-diverse urinary microbiota, an attribute often accompanied with urological disorders.^{36,37} In contrast, *Lactobacillus* spp. are commensals, with a robust body of evidence detailing their beneficial effects in the healthy urinary tract of both men and women.^{4,27,38} It is unclear what effect the aging process does to alter the urinary microbiota; however, the observed decrease in protective urinary lactobacilli may account for common stent-associated UTI and encrustation in older populations.^{39,40}

Patients with IBS and IBD had increased stent and urinary presence of *Prevotella* and *Veillonella* species and decreased lactobacilli. These findings are consistent with previous literature on the gut microbiota in these conditions.⁴¹⁻⁴³ These genera have also been implicated in urogenital infections and disorders, such as pelvic inflammatory disease.^{44,45} Our findings add further credence to the hypothesis that the gut microbiota is a reservoir for the genito-urinary microbiota.^{46,47} In the same manner that gut colonization with uropathogenic *Escherichia coli* (UPEC) increases the risk of UPEC UTI, the concurrence of inflammatory urinary tract symptoms in patients with IBS may

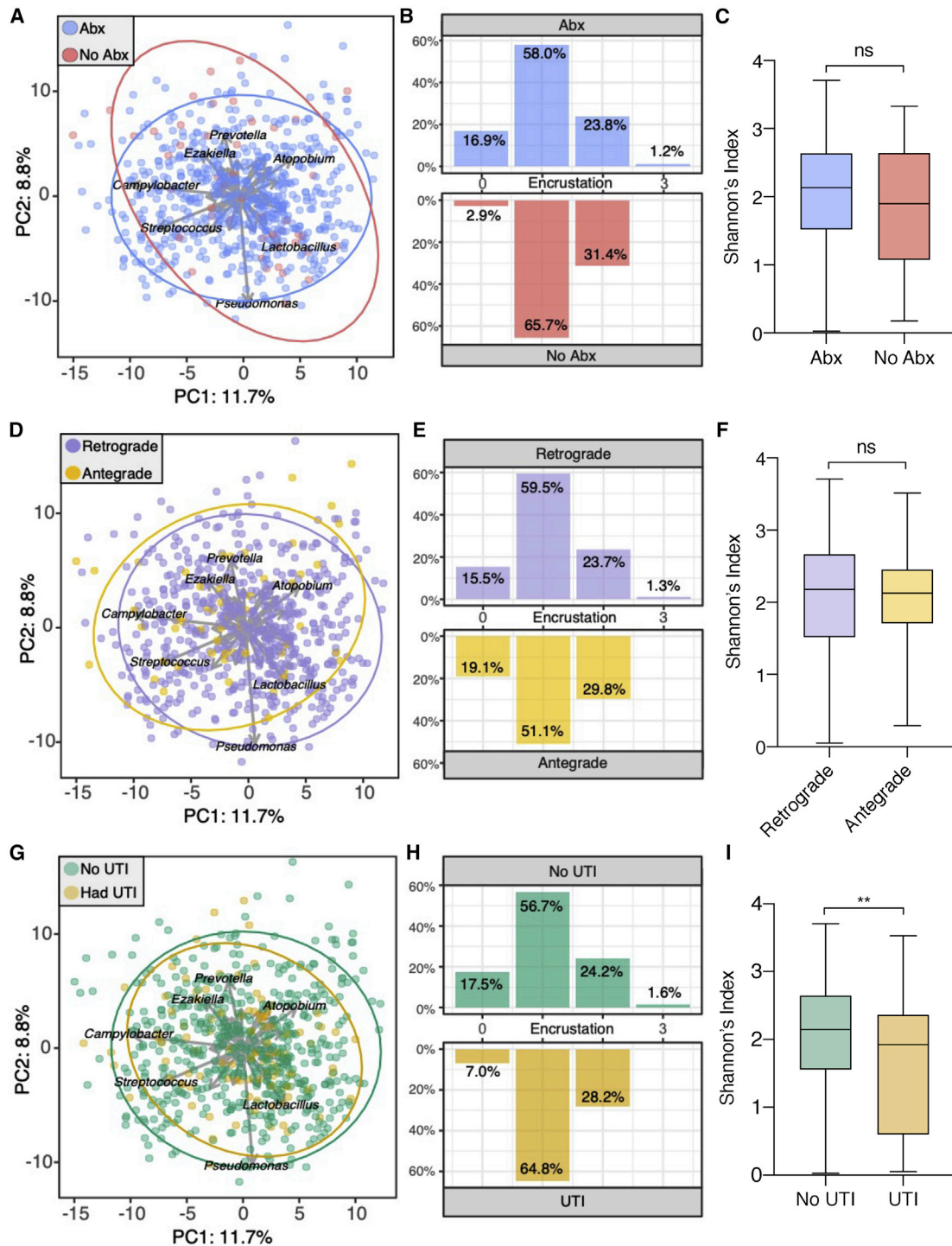


Figure 4. Stent Microbiota and Encrustation Are Unchanged by Antibiotic Exposure, Device Placement Method, and UTI

PCA was performed on CLR-transformed Aitchison distances. Each colored point represents a sample. Distance between samples on the plot represents differences in microbial community composition, with 20.5% of total variance being explained by the first two components shown. Strength and association for genera (sequence variants) are depicted by the length and direction of the gray arrows, respectively.

(A, D, and G) Samples are colored based on (A) whether the study participant had exposure to antibiotics within the last 30 days prior to sample collection (blue) or not (pink), (D) whether the stents were placed in a retrograde (purple) or antegrade (orange) manner, and (G) whether the participant had a UTI within 7 days of stent placement or throughout the indwelling period (orange) or not (green). Ellipses represent the 95% confidence interval.

(legend continued on next page)

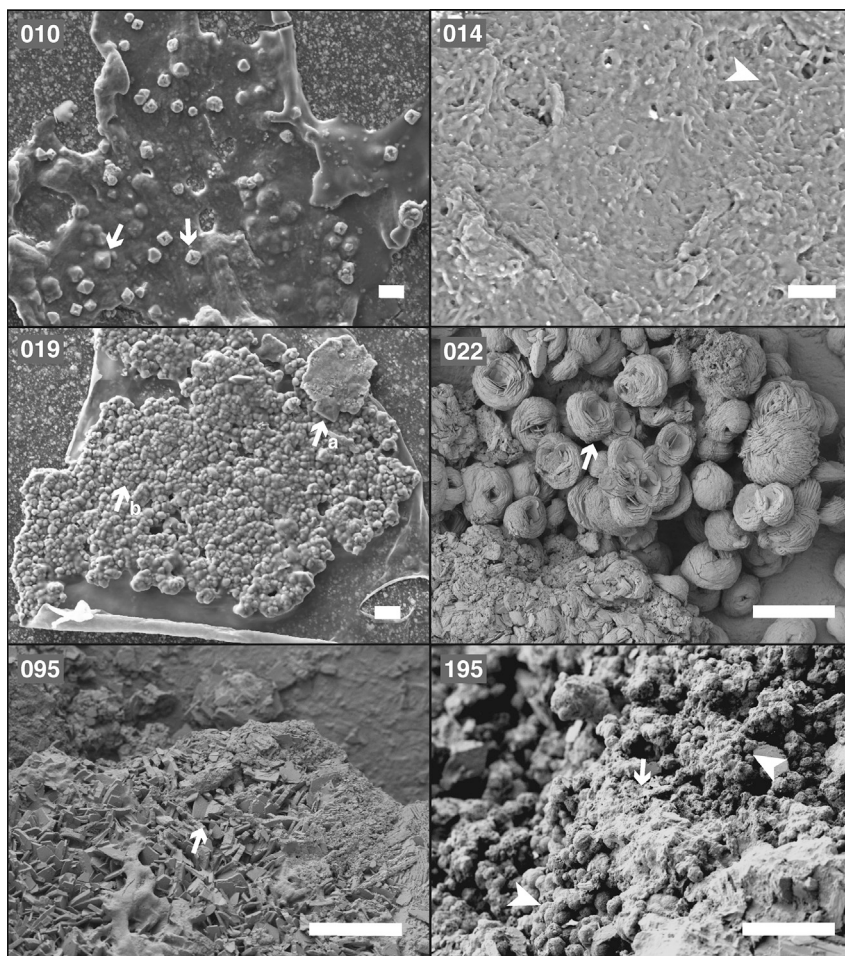


Figure 5. SEM Confirms the Presence of Urinary Crystals and Bacterial Biofilms

Representative scanning electron micrographs of stent encrustations illustrating typical bacterial biofilms and crystal morphologies. Based on microbiota sequencing, bacteria visible (white arrowheads) likely correspond to the genera (014) *Lactobacillus* and (195) *Enterococcus*. X-ray diffraction of crystalline microstructures (white arrows) correspond to calcium oxalate dihydrate (010 and 019a), calcium oxalate monohydrate in oval (022) and multiple-twinning (095) morphologies, and calcium phosphate (019b and 195). Scale bars represent 20 μ m.

In addition to many other maladies, these treatments have shown efficacy against IBS/IBD symptoms, urogenital infections in the elderly, and recurrent UTI.^{53–56}

The majority of stents imaged by SEM revealed encrustations composed of organic material and urinary crystals, although bacteria were only visualized in a small number of cases. This was expected due to the low bacterial load present in urinary samples, as well as the high proportion of urolithiasis patients among the study participants.^{57,58} If these organisms were involved in crystal deposition on the biomaterial, the urologist should ensure device removal within 3 weeks, given the positive correlation between indwelling time and stent encrustation.

Due to the low bacterial biomass nature of the samples collected, this study utilized

be explained.^{46,48–51} A limitation of the current study was that lower urinary tract symptoms and extended quantitative urine culture were not evaluated in the stent patient population.⁵² Future studies should look to correlate the urinary and stent microbiota with device encrustation, patient outcomes, and further serum and urinary parameters throughout the indwelling period if urological patients with IBS/IBD experience increased stent-associated complications in addition to the documented urinary tract symptoms.

Of the various comorbidities that significantly correlated with the stent microbiota, it was notable that they originated from distant sites (pancreas, respiratory tract, liver, and gastrointestinal tract), suggesting some common physiological denominator. Potentially, it is the gastrointestinal tract that is altered by these conditions, with systemic consequences of bacterial translocation. For this reason, it is feasible that microbiota-based treatment, including oral consumption of probiotic lactobacilli, or even fecal microbiota transplantation could be of therapeutic potential to stent patients.

stringent pre-sequencing processing methods in addition to the application of conservative bioinformatic cutoffs and analysis tools in order to minimize contamination effects.^{59,60} In future studies, quantification of total 16S rRNA gene copies by qPCR or the use of extended quantitative urine culture may complement and validate microbiota analysis of urinary and ureteral stent samples.^{28,52} Nevertheless, the detection of reproducible, patient-specific, stent microbiota signatures provides confidence that our findings are not due to contamination.

In summary, this study has characterized the urinary and stent microbiota of ureteral stent patients from a single center over a 1-year period, uncovering the importance of patient characteristics in explaining microbiota variation. Actions taken by the physician, such as antibiotic exposure and stent placement method, had no association with the microbiota in these samples but comorbidities and patient age did. The stent microbiota appears to originate from patient-specific adhesion of urinary microbes and subsequently diverges to a distinct reproducible population,

(B, E, and H) The degree of stent encrustation was compared between groups of interest. Groups were not significantly different by two-tailed Mann-Whitney U test.

(C, F, and I) Shannon's index of alpha diversity was not significantly different between antibiotic (C) or placement (F) groups, but patients with a UTI had lower diversity than those without (I); two-tailed Mann-Whitney U test; $p = 0.002$. Boxplot whiskers represent minimum and maximum.

thereby negating the urine as an accurate biomarker for stent encrustation or microbiota status. These findings suggest that timely stent removal is likely the most important action to be taken by the treating urologist in preventing encrustation and that stent-specific antibiotic administration practices need recalibration. Elderly patients or those diagnosed with pulmonary disease, hypertension, diabetes, or IBS/IBD may need closer evaluation to minimize stent- and microbiota-associated complications.

Limitations of Study

These data were derived at a single center from a heterogeneous patient population. Thus, the identified metadata factors associated with microbiota variability in this study may be cohort specific. For this reason, the subgroup analyses should be confirmed in a larger study population. Additionally, no standardized method exists for determining stent encrustation; the approach taken in this study, which was developed and validated internally, should be taken into consideration when comparing this work with future studies of the ureteral stent microbiota.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- [KEY RESOURCES TABLE](#)
- [RESOURCE AVAILABILITY](#)
 - Lead Contact
 - Materials Availability
 - Data and Code Availability
- [EXPERIMENTAL MODEL AND SUBJECT DETAILS](#)
- [METHOD DETAILS](#)
 - Sample processing and DNA extraction
 - 16S rRNA gene sequencing
 - SEM and X-ray diffraction spectroscopy
- [QUANTIFICATION AND STATISTICAL ANALYSIS](#)

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.xcrm.2020.100094>.

ACKNOWLEDGMENTS

We thank Dr. Todd Simpson from the Western University Nanofabrication facility for SEM and X-ray diffraction spectroscopy analysis. We also thank Linda Nott and Dr. Patricia Rosas-Arellano for logistical support and sample collection. K.F.A. was supported by an Ontario Graduate Scholarship. This work was supported by the W. Garfield Weston Foundation.

AUTHOR CONTRIBUTIONS

J.P.B. and H.R. designed the study. J.D.D., B.K.W., S.E.P., and H.R. acquired clinical samples. K.F.A. performed experiments. K.F.A., B.A.D., and G.B.G. performed data analysis. K.F.A. and J.P.B. wrote the paper. All authors contributed to the editing and revision of the paper and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: February 28, 2020

Revised: May 29, 2020

Accepted: August 21, 2020

Published: September 22, 2020

REFERENCES

1. Riedl, C.R., Plas, E., Hübner, W.A., Zimmerl, H., Ulrich, W., and Pflüger, H. (1999). Bacterial colonization of ureteral stents. *Eur. Urol.* 36, 53–59.
2. Zumstein, V., Betschart, P., Albrich, W.C., Buhmann, M.T., Ren, Q., Schmid, H.P., and Abt, D. (2017). Biofilm formation on ureteral stents - Incidence, clinical impact, and prevention. *Swiss Med. Wkly.* 147, w14408.
3. Lange, D., Bidnur, S., Hoag, N., and Chew, B.H. (2015). Ureteral stent-associated complications—where we are and where we are going. *Nat. Rev. Urol.* 12, 17–25.
4. Whiteside, S.A., Razvi, H., Dave, S., Reid, G., and Burton, J.P. (2015). The microbiome of the urinary tract—a role beyond infection. *Nat. Rev. Urol.* 12, 81–90.
5. Wolfe, A.J., Toh, E., Shibata, N., Rong, R., Kenton, K., Fitzgerald, M., Mueller, E.R., Schreckenberger, P., Dong, Q., Nelson, D.E., and Brubaker, L. (2012). Evidence of uncultivated bacteria in the adult female bladder. *J. Clin. Microbiol.* 50, 1376–1383.
6. Barr-Beare, E., Saxena, V., Hilt, E.E., Thomas-White, K., Schober, M., Li, B., Becknell, B., Hains, D.S., Wolfe, A.J., and Schwaderer, A.L. (2015). The interaction between enterobacteriaceae and calcium oxalate deposits. *PLoS ONE* 10, e0139575.
7. Cavarretta, I., Ferrarese, R., Cazzaniga, W., Saita, D., Lucianò, R., Ceresola, E.R., Locatelli, I., Visconti, L., Lavorgna, G., Briganti, A., et al. (2017). The microbiome of the prostate tumor microenvironment. *Eur. Urol.* 72, 625–631.
8. Bossa, L., Kline, K., McDougald, D., Lee, B.B., and Rice, S.A. (2017). Urinary catheter-associated microbiota change in accordance with treatment and infection status. *PLoS ONE* 12, e0177633.
9. Frank, D.N., Wilson, S.S., St Amand, A.L., and Pace, N.R. (2009). Culture-independent microbiological analysis of foley urinary catheter biofilms. *PLoS ONE* 4, e7811.
10. Rahman, M.A., Alam, M.M., Shamsuzzaman, S.M., and Haque, M.E. (2010). Evaluation of bacterial colonization and bacteriuria secondary to internal ureteral stent. *Mymensingh Med. J.* 19, 366–371.
11. Reid, G., Denstedt, J.D., Kang, Y.S., Lam, D., and Nause, C. (1992). Microbial adhesion and biofilm formation on ureteral stents in vitro and in vivo. *J. Urol.* 148, 1592–1594.
12. Chatterjee, S., Maiti, P., Dey, R., Kundu, A., and Dey, R. (2014). Biofilms on indwelling urologic devices: microbes and antimicrobial management prospect. *Ann. Med. Health Sci. Res.* 4, 100–104.
13. Chew, B.H., and Lange, D. (2009). Ureteral stent symptoms and associated infections: a biomaterials perspective. *Nat. Rev. Urol.* 6, 440–448.
14. Moltzahn, F., Haeni, K., Birkhäuser, F.D., Roth, B., Thalman, G.N., and Zehnder, P. (2013). Peri-interventional antibiotic prophylaxis only vs continuous low-dose antibiotic treatment in patients with JJ stents: a prospective randomised trial analysing the effect on urinary tract infections and stent-related symptoms. *BJU Int.* 111, 289–295.
15. Paz, A., Amiel, G.E., Pick, N., Moskovitz, B., Nativ, O., and Potasman, I. (2005). Febrile complications following insertion of 100 double-J ureteral stents. *J. Endourol.* 19, 147–150.
16. Gloor, G.B., Macklaim, J.M., Pawlowsky-Glahn, V., and Egozcue, J.J. (2017). Microbiome datasets are compositional: and this is not optional. *Front. Microbiol.* 8, 2224.

17. Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., et al. (2019). Vegan: Community ecology package. R package version 2.5-6. <https://cran.r-project.org/web/packages/vegan/index.html>.
18. Morgan, X.C., Tickle, T.L., Sokol, H., Gevers, D., Devaney, K.L., Ward, D.V., Reyes, J.A., Shah, S.A., LeLeiko, N., Snapper, S.B., et al. (2012). Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol.* **13**, R79.
19. Motulsky, H.J., and Brown, R.E. (2006). Detecting outliers when fitting data with nonlinear regression - a new method based on robust nonlinear regression and the false discovery rate. *BMC Bioinformatics* **7**, 123.
20. Kehinde, E.O., Rotimi, V.O., Al-Hunayan, A., Abdul-Halim, H., Boland, F., and Al-Awadi, K.A. (2004). Bacteriology of urinary tract infection associated with indwelling J ureteral stents. *J. Endourol.* **18**, 891–896.
21. Tavichakorntrakool, R., Boonsiri, P., Prasongwatana, V., Lulitanond, A., Wongkham, C., and Thongboonkerd, V. (2017). Differential colony size, cell length, and cellular proteome of *Escherichia coli* isolated from urine vs. stone nidus of kidney stone patients. *Clin. Chim. Acta* **466**, 112–119.
22. Chew, B.H., Knudsen, B.E., Nott, L., Pautler, S.E., Razvi, H., Amann, J., and Denstedt, J.D. (2007). Pilot study of ureteral movement in stented patients: first step in understanding dynamic ureteral anatomy to improve stent comfort. *J. Endourol.* **21**, 1069–1075.
23. Lane, M.C., Alteri, C.J., Smith, S.N., and Mobley, H.L. (2007). Expression of flagella is coincident with uropathogenic *Escherichia coli* ascension to the upper urinary tract. *Proc. Natl. Acad. Sci. USA* **104**, 16669–16674.
24. Nickel, J.C., Downey, J., and Costerton, J.W. (1992). Movement of *Pseudomonas aeruginosa* along catheter surfaces. A mechanism in pathogenesis of catheter-associated infection. *Urology* **39**, 93–98.
25. Siitonen, A., and Nurminen, M. (1992). Bacterial motility is a colonization factor in experimental urinary tract infection. *Infect. Immun.* **60**, 3918–3920.
26. Tenke, P., Köves, B., Nagy, K., Hultgren, S.J., Mendling, W., Wullt, B., Grabe, M., Wagenlehner, F.M., Cek, M., Pickard, R., et al. (2012). Update on biofilm infections in the urinary tract. *World J. Urol.* **30**, 51–57.
27. Gottschick, C., Deng, Z.L., Vital, M., Masur, C., Abels, C., Pieper, D.H., and Wagner-Döbler, I. (2017). The urinary microbiota of men and women and its changes in women during bacterial vaginosis and antibiotic treatment. *Microbiome* **5**, 99.
28. Buhmann, M.T., Abt, D., Nolte, O., Neu, T.R., Stempel, S., Albrich, W.C., Betschart, P., Zumstein, V., Neels, A., Maniura-Weber, K., and Ren, Q. (2019). Encrustations on ureteral stents from patients without urinary tract infection reveal distinct urotypes and a low bacterial load. *Microbiome* **7**, 60.
29. Liu, F., Ling, Z., Xiao, Y., Yang, Q., Zheng, L., Jiang, P., Li, L., and Wang, W. (2017). Characterization of the urinary microbiota of elderly women and the effects of type 2 diabetes and urinary tract infections on the microbiota. *Oncotarget* **8**, 100678–100690.
30. Rowe, T.A., and Juthani-Mehta, M. (2013). Urinary tract infection in older adults. *Aging Health* **9**. <https://doi.org/10.2217/ahe.13.38>.
31. Aujoulat, F., Bouvet, P., Jumas-Bilak, E., Jean-Pierre, H., and Marchandin, H. (2014). *Veillonella seminalis* sp. nov., a novel anaerobic Gram-stain-negative coccus from human clinical samples, and emended description of the genus *Veillonella*. *Int. J. Syst. Evol. Microbiol.* **64**, 3526–3531.
32. Berenger, B.M., Chui, L., Borkent, A., and Lee, M.C. (2015). Anaerobic urinary tract infection caused by *Veillonella parvula* identified using cystine-lactose-electrolyte deficient media and matrix-assisted laser desorption/ionization-time of flight mass spectrometry. *IDCases* **2**, 44–46.
33. Mashima, I., and Nakazawa, F. (2014). The influence of oral *Veillonella* species on biofilms formed by *Streptococcus* species. *Anaerobe* **28**, 54–61.
34. Scheiman, J., Luber, J.M., Chavkin, T.A., MacDonald, T., Tung, A., Pham, L.D., Wibowo, M.C., Wurth, R.C., Punthambaker, S., Tierney, B.T., et al. (2019). Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. *Nat. Med.* **25**, 1104–1109.
35. van de Wijkert, J.H., Borgdorff, H., Verhelst, R., Crucitti, T., Francis, S., Verstraelen, H., and Jaspers, V. (2014). The vaginal microbiota: what have we learned after a decade of molecular characterization? *PLoS ONE* **9**, e105998.
36. Pearce, M.M., Zilliox, M.J., Rosenfeld, A.B., Thomas-White, K.J., Richter, H.E., Nager, C.W., Visco, A.G., Nygaard, I.E., Barber, M.D., Schaffer, J., et al. (2015). The female urinary microbiome in urgency urinary incontinence. *Am. J. Obstet. Gynecol.* **213**, 347.e1–347.e11.
37. Thomas-White, K.J., Kliethermes, S., Rickey, L., Lukacz, E.S., Richter, H.E., Moalli, P., Zimmern, P., Norton, P., Kusek, J.W., Wolfe, A.J., et al. (2017). Evaluation of the urinary microbiota of women with uncomplicated stress urinary incontinence. *Am. J. Obstet. Gynecol.* **216**, 55.e1–55.e16.
38. Aragón, I.M., Herrera-Imbroda, B., Queipo-Ortuño, M.I., Castillo, E., Del Moral, J.S., Gómez-Millán, J., Yucel, G., and Lara, M.F. (2018). The urinary tract microbiome in health and disease. *Eur. Urol. Focus* **4**, 128–138.
39. Akay, A.F., Aflay, U., Gedik, A., Sahin, H., and Bircan, M.K. (2007). Risk factors for lower urinary tract infection and bacterial stent colonization in patients with a double J ureteral stent. *Int. Urol. Nephrol.* **39**, 95–98.
40. Altunal, N., Willke, A., and Hamzaoglu, O. (2017). Ureteral stent infections: a prospective study. *Braz. J. Infect. Dis.* **21**, 361–364.
41. Lee, K.J., and Tack, J. (2010). Altered intestinal microbiota in irritable bowel syndrome. *Neurogastroenterol. Motil.* **22**, 493–498.
42. Sha, S., Xu, B., Wang, X., Zhang, Y., Wang, H., Kong, X., Zhu, H., and Wu, K. (2013). The biodiversity and composition of the dominant fecal microbiota in patients with inflammatory bowel disease. *Diagn. Microbiol. Infect. Dis.* **75**, 245–251.
43. Shankar, V., Agans, R., Holmes, B., Raymer, M., and Paliy, O. (2013). Do gut microbial communities differ in pediatric IBS and health? *Gut Microbes* **4**, 347–352.
44. Brook, I. (2004). Urinary tract and genito-urinary suppurative infections due to anaerobic bacteria. *Int. J. Urol.* **11**, 133–141.
45. Haggerty, C.L., and Taylor, B.D. (2011). *Mycoplasma genitalium*: an emerging cause of pelvic inflammatory disease. *Infect. Dis. Obstet. Gynecol.* **2011**, 959816.
46. Magruder, M., Sholi, A.N., Gong, C., Zhang, L., Edusei, E., Huang, J., Al-Bakry, S., Satlin, M.J., Westblade, L.F., Crawford, C., et al. (2019). Gut uropathogen abundance is a risk factor for development of bacteriuria and urinary tract infection. *Nat. Commun.* **10**, 5521.
47. Yamamoto, S., Tsukamoto, T., Terai, A., Kurazono, H., Takeda, Y., and Yoshida, O. (1997). Genetic evidence supporting the fecal-perineal-urethral hypothesis in cystitis caused by *Escherichia coli*. *J. Urol.* **157**, 1127–1129.
48. Matsumoto, S., Hashizume, K., Wada, N., Hori, J., Tamaki, G., Kita, M., Iwata, T., and Kakizaki, H. (2013). Relationship between overactive bladder and irritable bowel syndrome: a large-scale internet survey in Japan using the overactive bladder symptom score and Rome III criteria. *BJU Int.* **111**, 647–652.
49. Moreno, E., Andreu, A., Pérez, T., Sabaté, M., Johnson, J.R., and Prats, G. (2006). Relationship between *Escherichia coli* strains causing urinary tract infection in women and the dominant faecal flora of the same hosts. *Epidemiol. Infect.* **134**, 1015–1023.
50. Moreno, E., Andreu, A., Pigrau, C., Kuskowski, M.A., Johnson, J.R., and Prats, G. (2008). Relationship between *Escherichia coli* strains causing acute cystitis in women and the fecal *E. coli* population of the host. *J. Clin. Microbiol.* **46**, 2529–2534.
51. Zingone, F., Iovino, P., Santonicola, A., Gallotta, S., and Ciacci, C. (2017). High risk of lower urinary tract symptoms in patients with irritable bowel syndrome. *Tech. Coloproctol.* **21**, 433–438.
52. Hilt, E.E., McKinley, K., Pearce, M.M., Rosenfeld, A.B., Zilliox, M.J., Mueller, E.R., Brubaker, L., Gai, X., Wolfe, A.J., and Schreckenberger, P.C. (2014). Urine is not sterile: use of enhanced urine culture techniques to

- detect resident bacterial flora in the adult female bladder. *J. Clin. Microbiol.* **52**, 871–876.
53. Ducrotté, P., Sawant, P., and Jayanthi, V. (2012). Clinical trial: *Lactobacillus plantarum* 299v (DSM 9843) improves symptoms of irritable bowel syndrome. *World J. Gastroenterol.* **18**, 4012–4018.
 54. Hocquart, M., Pham, T., Kuete, E., Tomei, E., Lagier, J.C., and Raoult, D. (2019). Successful fecal microbiota transplantation in a patient suffering from irritable bowel syndrome and recurrent urinary tract infections. *Open Forum Infect. Dis.* **6**, ofz398.
 55. Kim, J.M., and Park, Y.J. (2017). Probiotics in the prevention and treatment of postmenopausal vaginal infections: Review article. *J. Menopausal Med.* **23**, 139–145.
 56. Tariq, R., Pardi, D.S., Tosh, P.K., Walker, R.C., Razonable, R.R., and Khanna, S. (2017). Fecal microbiota transplantation for recurrent *Clostridium difficile* infection reduces recurrent urinary tract infection frequency. *Clin. Infect. Dis.* **65**, 1745–1747.
 57. Dyer, R., and Nordin, B.E. (1967). Urinary crystals and their relation to stone formation. *Nature* **215**, 751–752.
 58. Lewis, D.A., Brown, R., Williams, J., White, P., Jacobson, S.K., Marchesi, J.R., and Drake, M.J. (2013). The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. *Front. Cell. Infect. Microbiol.* **3**, 41.
 59. Karstens, L., Asquith, M., Davin, S., Fair, D., Gregory, W.T., Wolfe, A.J., Braun, J., and McWeeney, S. (2019). Controlling for contaminants in low-biomass 16s rRNA gene sequencing experiments. *mSystems* **4**, e00290-19.
 60. Minich, J.J., Sanders, J.G., Amir, A., Humphrey, G., Gilbert, J.A., and Knight, R. (2019). Quantifying and understanding well-to-well contamination in microbiome research. *mSystems* **4**, e00186-19.
 61. Caporaso, J., et al. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* **6**, 1621–1624.
 62. Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J., and Holmes, S.P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**, 581–583.
 63. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590–D596.
 64. Gloor, G.B., and Reid, G. (2016). Compositional analysis: a valid approach to analyze microbiome high-throughput sequencing data. *Can. J. Microbiol.* **62**, 692–703.
 65. Fernandes, A.D., Macklaim, J.M., Linn, T.G., Reid, G., and Gloor, G.B. (2013). ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-seq. *PLoS ONE* **8**, e67019.
 66. Mallick, H., McIver, L.J., Rahnavard, A., Ma, S., Zhang, Y., Nguyen, L.H., Tickle, T.L., Weingart, G., Ren, B., Schwager, E., et al. (2020). Maaslin2: Multivariable association in population-scale meta-omics studies. <http://huttenhower.sph.harvard.edu/maaslin>.
 67. Parada, A.E., Needham, D.M., and Fuhrman, J.A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* **18**, 1403–1414.
 68. R Core Team (2019). R: A language and environment for statistical computing (R Foundation for Statistical Computing).
 69. Salonen, A., Lahti, L., Salojärvi, J., Holtrop, G., Korpela, K., Duncan, S.H., Date, P., Farquharson, F., Johnstone, A.M., Lopley, G.E., et al. (2014). Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *ISME J.* **8**, 2218–2230.

STAR★METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|-----------------------------------|---|
| Bacterial Strains | | |
| <i>Escherichia coli</i> DH5 α | ATCC | ATCC 68233 |
| <i>Staphylococcus aureus</i> Newman | ATCC | ATCC 25904 |
| Biological Samples | | |
| Healthy adult human urine (5 – 50 mL) | This paper | N/A |
| Healthy adult ureteral stents | This paper | N/A |
| Chemicals, Peptide, and Recombinant Proteins | | |
| LB broth | BD Difco | Catalog No. B244601 |
| RNase AWAY | ThermoScientific | Catalog No. 7003PK |
| Nuclease free water | Ambion | Catalog No. AM9932 |
| GoTaq hot start colorless Master Mix | Promega | Catalog No. M5133 |
| Critical Commercial Assays | | |
| DNEasy PowerSoil HTP 96 kit | QIAGEN | Catalog No. 12955-4 |
| Quanti-iT PicoGreen dsDNA assay | Invitrogen | Catalog No. P11496 |
| QIAquick PCR Purification kit | QIAGEN | Catalog No. 28106 |
| MiSeq Reagent Kit v3 (600-cycle) | Illumina | Catalog No. MS-102-3003 |
| Deposited Data | | |
| Raw data | This paper | 16S rRNA sequence data (NCBI) BioProject ID #PRJNA601180 |
| Oligonucleotides | | |
| 16S rRNA forward primer 515F: GTGCCAGCMGCCGCGGTAA | (Caporaso et al. ⁶¹) | N/A |
| 16S rRNA reverse primer 806R: GGACTACHVGGGTWTCTAAT | (Caporaso et al. ⁶¹) | N/A |
| Software and Algorithms | | |
| DADA2 v1.14 | (Callahan et al. ⁶²) | https://benjjneb.github.io/dada2/ |
| SILVA Database v132 | (Quast et al. ⁶³) | https://www.arb-silva.de |
| R v3.6.1 | R Core Team | https://www.r-project.org |
| CoDaSeq v0.99.4 | (Gloor and Reid ⁶⁴) | https://github.com/ggloor/CoDaSeq |
| ALDEx2 v1.11.0 | (Fernandes et al. ⁶⁵) | https://bioconductor.org/packages/release/bioc/html/ALDEx2.html |
| Vegan v2.5-6 | (Oksanen et al. ¹⁷) | https://cran.r-project.org/web/packages/vegan/vegan.pdf |
| MaAsLin2 v1.1.1 | (Mallick et al. ⁶⁶) | http://huttenhower.sph.harvard.edu/maaslin |
| GraphPad Prism v8.3.1 | Graph Pad Software | N/A |

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jeremy Burton (Jeremy.Burton@lawsonresearch.com).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

16S rRNA gene sequencing data generated in this study is available through the NCBI Sequence Read Archive: BioProject ID #PRJNA601180.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Two hundred and forty-one ureteral double-J stent patients (122 females, 119 males) were recruited from the Urology Department at St. Joseph's Hospital in London, Ontario. Patients ranged in age from 22-90 years (average 59). Ethical approval for the study was granted by the Health Sciences Research Ethics Board at the University of Western Ontario (REB #107941) in London, Ontario. Written consent was obtained from all the study participants at the time of study inclusion and the methods were carried out in accordance with the approved guidelines. Inclusion and exclusion criteria for the participants are provided in [Table S4](#). All patients that met the inclusion criteria were recruited to the study during regularly scheduled clinic appointments.

METHOD DETAILS

Sample processing and DNA extraction

Upon recruitment, patients were asked about relevant demographic and medical history including antibiotic usage and their history of urinary tract infections. Following enrolment, participants provided a mid-stream urine sample. Stents were collected during cystoscopy (either in-clinic or OR) and placed by the surgeon into a sterile urine collection cup ([Figure S1](#)). Urine samples were processed within 6-hours of their collection. The entire volume of urine (5-50 mL) was centrifuged for 10 minutes at 5,000 x g, after which the supernatant was decanted off and the pellet was stored dry at -20°C until DNA extraction. The urine volume that resulted in the pellet for 16S rRNA gene sequencing was recorded to identify confounding factors in the downstream sequencing analysis associated with processing conditions.

Within 6 hours of their collection, stents were frozen at -20°C and stored until DNA extraction. Bacterial culture was not undertaken on stent encrustations in order to preserve as much of the biomaterial as possible for 16S rRNA gene sequencing and SEM/EDX. Instead, a qualitative grade for the degree of device encrustation was determined ([Table S3](#)). Both proximal and distal ends of each stent were graded by a single evaluator twice: prior to and following frozen storage. After frozen storage, the evaluator was blinded to the grading from the first evaluation. Grades at both time points were identical for all samples.

On the day of DNA extraction, the stents were thawed and processed in a sterile biosafety hood. A scalpel sterilized with RNase AWAY was utilized to slice two x 1 cm segments from both the proximal and distal curls of the stents ([Figure S1](#)). One segment from each curl was reserved for 16S rRNA gene sequencing. Potential external contamination which may have occurred during device removal or frozen storage was mitigated by rinsing: tweezers sterilized with RNase AWAY were used to hold the stent segment over a sterile reservoir while 1 mL of nuclease free water was gently rinsed over the external surface. The rinsed segment was then sliced open lengthwise to expose the interior lumen and directly transferred into the bead plate of the DNeasy PowerSoil HTP 96 Kit utilized for DNA extraction. The second 1 cm cut segment was reserved for SEM: both the internal lumen and exterior of the stent were of interest for imaging so the stent cut was sliced lengthwise and both halves were transferred to separate sterile 1.5 mL Eppendorf tubes for SEM preparation.

For DNA extraction, frozen urine pellets were thawed and suspended in 100 μL of nuclease-free water, then pipetted into individual wells of the PowerSoil HTP bead plate with PCR-grade filter tips. Two wells in every plate were left empty and acted as negative controls. Two positive controls, or spikes, were added to each plate and were 100 μL of pure bacterial culture: Spike 1 was *Escherichia coli* strain DH5 α , and Spike 2 was *Staphylococcus aureus* strain Newman. For preparation of the spikes, a single colony of the bacteria was inoculated into 10 mL of Luria-Bertani (LB) broth and grown overnight at 37°C . One hundred 100 μL aliquots of the overnight cultures were portioned into 1.5 mL Eppendorf tubes and frozen at -80°C . For each DNA extraction plate, a single tube of both spikes was thawed and pipetted directly into the PowerSoil HTP bead plate with PCR-grade filter tips. DNA was isolated from urine and stent samples using the DNeasy PowerSoil HTP 96 Kit according to the manufacturer's instructions. DNA was stored at -20°C until PCR amplification.

16S rRNA gene sequencing

PCR amplification was completed using the Earth Microbiome universal primers, 515F and 806R, which are specific for the V4 variable region of the 16S rRNA gene.⁶⁷ Primers and barcode sequences are listed in [Table S5](#). PCR reagent set-up was performed using a Biomek® 3000 Laboratory Automation Workstation (Beckman-Coulter, Mississauga, ON, CAN). Ten μL of each left and right-barcoded primers (3.2 pMole/ μL) were arrayed in 96-well plates such that each well contained a unique combination of left- and right-barcodes (up to a maximum of 576 unique combinations). Two μL of DNA template was added to the primer plate, followed by 20 μL of Promega GoTaq hot-start colorless master mix. The reaction was briefly mixed by pipetting, then plates were sealed with foil plate covers and centrifuged for 2 minutes at room temperature at 2250 x g.

Amplification was carried out using an Eppendorf thermal cycler (Eppendorf, Mississauga, ON, CAN), where the lid temperature was maintained at 104°C . An initial warm-up of 95°C for 4 minutes was utilized to activate the GoTaq, followed by 25 cycles of 1 minute each of 95°C , 52°C , and 72°C .

Due to the total number of samples exceeding the number of unique barcode combinations, two Illumina MiSeq runs were completed to accommodate the sequencing of all the samples (Illumina Inc., San Diego, CA, USA). In order to identify potential batch effects between the two sequencing runs, several samples and controls were sequenced on both runs. In total, accounting for doubly sequenced samples, 822 samples were sequenced across 9 PCR plates (5 x 96-well plates containing 438 samples on the first

MiSeq run, 4 plates containing 384 samples on the second). Sequencing was carried out at the London Regional Genomics Centre (<http://www.lrgc.ca>; London, ON, CAN). Amplicons were quantified using pico green and pooled at equimolar concentrations before cleanup. Using the 600-cycle MiSeq Reagent Kit, paired-end sequencing was carried out as 2 × 260 cycles with the addition of 5% φX-174 at a cluster density of ~1100. Data was exported as raw fastq files (uploaded to NCBI Sequence Read Archive, BioProject ID #PRJNA601180).

From the two sequencing runs, run 1 contained 438 samples and yielded a total of 16,211,576 reads, ranging from 419 to 358,493 reads per sample. Run 2, containing 384 samples, yielded a total of 10,424,180 reads, ranging from 168 to 400,010 reads per sample. An average of 20.8% and 18.8% of reads were removed from each sample in Runs 1 and 2, respectively, following quality filtering performed utilizing the DADA2 pipeline.⁶² The remaining filtered reads from the two runs (14,477,624 and 9,697,990) were then merged by amplicon sequence variants (SVs). SVs that were only detected in one of the two runs were removed. Samples and SVs were then further pruned such that the final dataset utilized in all downstream analyses retained samples with greater than 1,000 filtered reads, SVs present at 1% relative abundance in any sample, and SVs with greater than 10,000 total reads across all samples in both runs. This cleaning reduced the dimensions of the dataset from 460 SVs and 822 samples down to 43 SVs and 710 samples. The remaining 43 SVs were assigned taxonomy with the SILVA (v132) training set, and a further 5 SVs were removed due to their alignment to human mitochondrial sequences.⁶³

SEM and X-ray diffraction spectroscopy

One-centimeter stent cuts were cut open lengthwise with a sterile razor and mounted upon aluminum stubs such that one half exposed the inner lumen, and the other half exposed the external surface. They were then gently rinsed with DI water to remove salt precipitation prior to SEM and X-ray diffraction spectroscopy analysis.

QUANTIFICATION AND STATISTICAL ANALYSIS

Raw 16S rRNA gene sequencing reads were demultiplexed and quality filtered utilizing the DADA2 pipeline,⁶² and assigned taxonomy with the SILVA (v132) training set.⁶³ Downstream analysis including PCA was performed conservatively in agreement with standards in the field, using CoDaSeq, ALDEx2, MaAsLin2, Vegan and core R packages.^{16–18,64–66,68} For subgroup comparisons (Figures 2, 3, and 4), all pairwise distances were incorporated in the analysis in an effort to avoid artificially minimized data variance through averaging, and the appropriate false-discovery rate corrections were employed.^{64,69} P values, sample numbers, and names of statistical tests are provided in the main text and figure legends for Figures 2, 3, 4, and S2–S4. Determination of data stratification and statistical tests were performed in GraphPad Prism (v8.3.1) and R (Method Details). All tests of statistical significance used a p value ≤ 0.05 as a cut-off.