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

Graphical Abstract

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

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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.

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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.
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. 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
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
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. 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).

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%). 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.
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
left and right sides during bilateral stent placement, solidifying the true presence of a reproducible stent microbiota and corroborating previous findings from urinary catheters. 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. 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. 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. 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. 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. 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. In humans, Veillonella spp. are commensals of the oral cavity and gastrointestinal and urogenital tracts, with the potential to cause opportunistic infections, including UTI. Veillonella spp. are also commonly associated with a more-diverse urinary microbiota, an attribute often accompanied with urological disorders. 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. 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.

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. These genera have also been implicated in urogenital infections and disorders, such as pelvic inflammatory disease. Our findings add further credence to the hypothesis that the gut microbiota is a reservoir for the genito-urinary microbiota. 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

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

FDR, false discovery rate.
*Coefficient of association >0 are correlated with higher and <0 with lower relative abundance of the specified genus.*
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)
be explained. 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. 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. 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. 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 co-morbidities 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.
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.

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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.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial Strains** |        |            |
| *Escherichia coli* DH5α | ATCC   | ATCC 68233 |
| *Staphylococcus aureus* 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: GTGCCAGCMGCGCGGTAA | (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://benjneb.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 Reid64) | https://github.com/ggloor/CoDaSeq |
| ALDEEx v1.11.0 | (Fernandes et al.65) | https://bioconductor.org/packages/release/bioc/html/ALDEEx.html |
| Vegan v2.5-6 | (Oksanen et al.66) | https://cran.r-project.org/web/packages/vegan/vegan.pdf |
| MaAsLin2 v1.1.1 | (Mallick et al.67) | 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 DH5α, 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. 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 barcode primers (3.2 pMole/μL) were arrayed in 96-well plates such that each well contained a unique combination of left- and right-barcode primers (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 $2 \times 260$ cycles with the addition of 5% $\phi X-174$ at a cluster density of $\sim 1100$. 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. \(^6\) 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. \(^6\)

**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, \(^6\) and assigned taxonomy with the SILVA (v132) training set. \(^6\) Downstream analysis including PCA was performed conservatively in agreement with standards in the field, using CoDaSeq, ALDEEx2, MaAsLin2, Vegan and core R packages. \(^6\) 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. \(^6\) 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 $\leq 0.05$ as a cut-off.