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COMPARTMENT-SPECIFIC URINARY MICROBIAL PROFILES AND THEIR ASSOCIATION WITH EARLY POSTOPERATIVE SYSTEMIC INFLAMMATION AFTER ENDOUROLOGICAL SURGERY: A PROSPECTIVE OBSERVATIONAL COHORT STUDY

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ABSTRACT

Systemic inflammation may follow endourological surgery despite a negative preoperative midstream urine (MSU) culture. MSU may not reflect microbial findings in upper urinary tract or on indwelling devices. We compared microbial findings across urinary compartments and examined their association with postoperative systemic inflammatory response syndrome (SIRS). This prospective single-centre cohort included 120 adults undergoing retrograde intrarenal surgery (RIRS, n = 89) or robot-assisted ureteroplasty (n = 31) at Tikrit General Hospital, Iraq, between June 2024 and December 2025. Preoperative MSU, intraoperative renal-pelvic urine, and ureteral stents retrieved on postoperative day 14 ± 2 underwent culture; stents were sonicated for biofilm recovery. The primary outcome was SIRS within 48 hours. All 14 participants with SIRS and 14 matched controls underwent 16S rRNA gene (V3–V4) sequencing. Because stents were sampled after the outcome window, their findings were analysed retrospectively. SIRS occurred in 14/120 participants (11.7%). Stent sonication was positive in 50/120 (41.7%), including 12/14 (85.7%) participants with SIRS and 38/106 (35.8%) without (odds ratio [OR] 10.7, 95% confidence interval [CI] 2.3–50.5; Fisher p < 0.001). Pelvic-urine cultures were positive in 9/14 versus 19/106 (OR 8.2, 95% CI 2.5–27.4), while MSU cultures were positive in 3/14 versus 6/106 (OR 4.5, 95% CI 0.99–20.8). Shannon diversity was highest in stent biofilm (3.42), followed by pelvic urine (2.15) and MSU (1.12). Findings suggest compartment-specific microbial differences and an association between stent positivity and earlier SIRS. Temporal limitations, few events, and outcome-selected sequencing preclude causal or predictive conclusions. Prospective studies with pre-outcome sampling are warranted.

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Introduction

Infectious and inflammatory complications remain important concerns after endourological surgery, and preoperative midstream urine (MSU) culture is the standard screening test. A negative MSU, however, reflects only the culturable organisms in voided or bladder urine under routine conditions. Sampling site, growth requirements, prior antimicrobial exposure and device-associated biofilm may all produce discordance between MSU and the microbial communities of the upper tract [1–3].

Biofilms are structured microbial communities embedded in an extracellular matrix and readily form on urinary devices, including ureteral stents [4,5]. Their composition may differ from that of planktonic organisms in urine. Culture, including expanded quantitative urine culture (EQUC), shows which organisms are recoverable under the conditions used, whereas 16S rRNA amplicon sequencing characterises bacterial DNA without establishing viability or pathogenicity [6–8]. Sonication improves recovery of organisms from implanted devices [9].

It is unclear whether microbial measurements from different urinary compartments carry concordant information, and how discordance relates to postoperative inflammation. We therefore compared culture findings from preoperative MSU, intraoperative renal-pelvic urine and stent-associated material, and used 16S rRNA sequencing in a small, outcome-selected subgroup to describe compartment-specific diversity and composition. The primary outcome was SIRS within 48 hours of surgery. Because stents were sampled on day 14, all analyses involving stent findings are reported as retrospective associations.

Materials and Methods

Design, setting and approval

This prospective observational cohort study was conducted at Tikrit General Hospital (Salah Al-Din Health Directorate, Tikrit, Iraq) from June 2024 to December 2025, and is reported according to the STROBE statement [10]. The protocol was approved by the Institutional Review Board of the Salah Al-Din Health Directorate (SD-REC-2024-082) and registered in the Iraqi Clinical Trials Registry (ICTR20240612-TGH). All participants gave written informed consent. The study followed the Declaration of Helsinki.

Participants

We enrolled consecutive adults (≥ 18 years) undergoing elective RIRS or robot-assisted ureteroplasty with ureteral stent placement for unilateral obstructive uropathy due to urolithiasis or ureteral stricture, who consented and agreed to planned stent removal about 14 days after surgery.

Exclusion criteria were: symptomatic urinary tract infection, or a positive preoperative MSU that prompted treatment before surgery, systemic antibiotic use in the preceding 14 days, chronic instrumentation (indwelling catheter or stent for more than 30 days), severe immunocompromise, active urogenital malignancy or pregnancy, and congenital urinary tract anomalies. Low-level or asymptomatic MSU growth that did not prompt pre-operative treatment was not an exclusion criterion, so MSU positivity was possible within the cohort.

Of 152 patients assessed, 32 were excluded (positive MSU or active infection, $n = 9$, antibiotics within 14 days, $n = 8$, chronic instrumentation, $n = 6$, immunocompromise, malignancy or pregnancy, $n = 5$, congenital anomaly, $n = 4$), leaving 120 participants (RIRS 89, ureteroplasty 31). All 120 were followed to stent retrieval and included in all analyses (Figure 1).

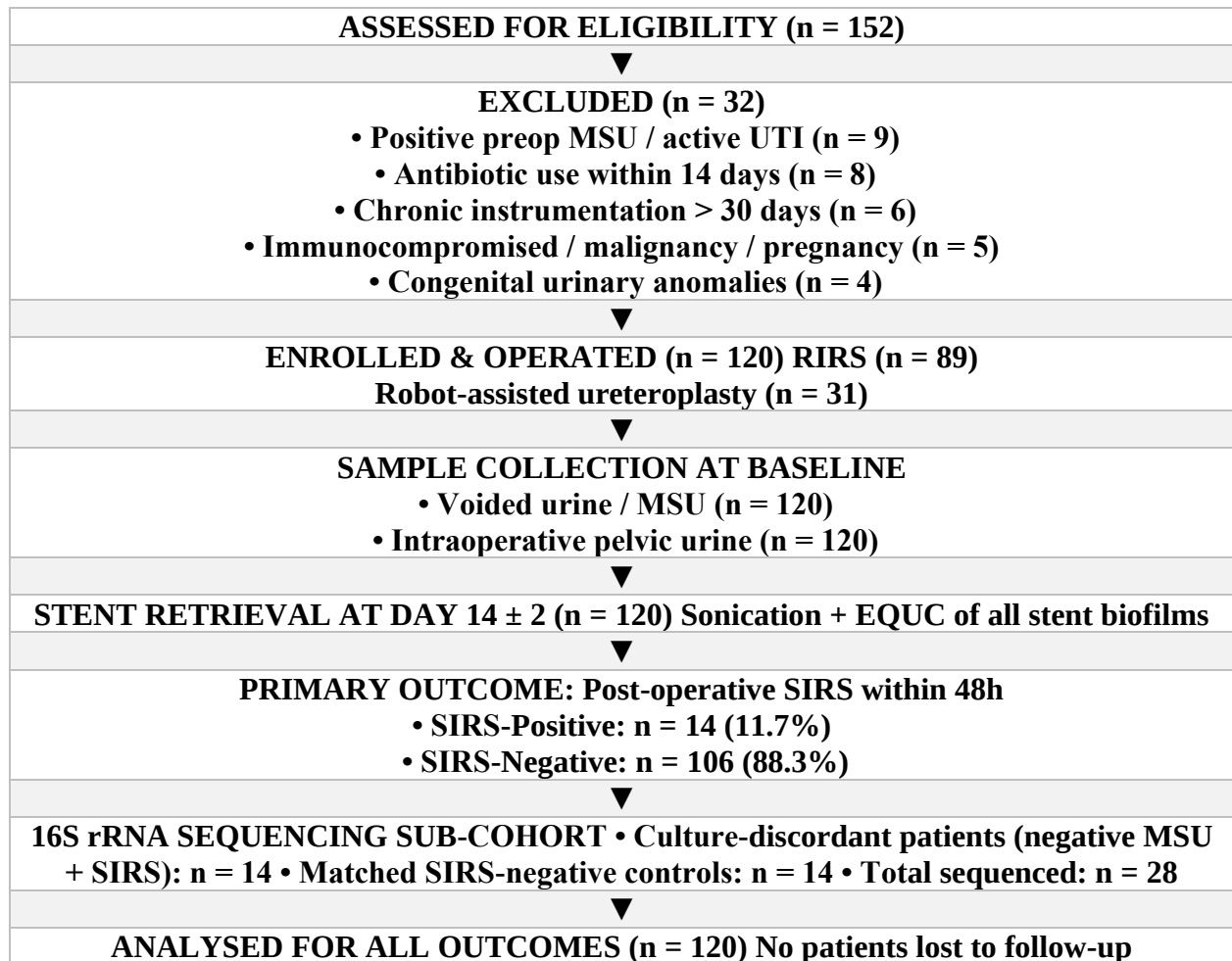


Figure 1. Participant flow.

Of 152 patients assessed, 32 were excluded and 120 were enrolled (RIRS 89, ureteroplasty 31). MSU and pelvic urine collected perioperatively. All participants contributed MSU and pelvic-urine samples, and stents were retrieved at day 14 ± 2. The sequencing subgroup comprised 14 SIRS-positive participants and 14 matched SIRS-negative controls. RIRS, retrograde intrarenal surgery, MSU, midstream urine, SIRS, systemic inflammatory response syndrome, EQUC, expanded quantitative urine culture.

Sampling

A. MSU: A clean-catch MSU was collected on the day of surgery before antibiotic prophylaxis.

B. Prophylaxis: Intravenous cefazolin 2 g, or ciprofloxacin 400 mg in beta-lactam allergy, was given within 60 minutes before incision.

C. Renal-pelvic urine: After prophylaxis and before ureteroscopic instrumentation, urine was collected through a sterile 5-Fr open-ended ureteral catheter, the first 2 mL were discarded and the next 5 mL collected in a sterile DNA-free container. Samples were transported on ice and processed within 60 minutes. Because pelvic urine was collected after prophylaxis whereas MSU was not, culture yields are not strictly comparable.

D. Stent biofilm: At planned removal on postoperative day 14 ± 2, the stent was retrieved with sterile cystoscopic forceps and rinsed three times in sterile phosphate-buffered saline. Proximal and distal 2-cm segments were cut with a sterile scalpel, immersed in 10 mL sterile Ringer's solution and sonicated (40 kHz, 300 W, 5 minutes) followed by 30 seconds of vortexing. The sonicate was

centrifuged at $3,000 \times g$ for 15 minutes before culture and DNA extraction. Stents pass through the urethra and bladder at removal, so contamination cannot be excluded.

Culture

Expanded quantitative urine culture (EQUC) was used to investigate culturable microorganisms in preoperative midstream urine (MSU), intraoperative renal-pelvic urine, and ureteral-stent sonicate. MSU and renal-pelvic urine were collected as described in Section 2.3. For stent-associated culture, the retrieved stent was rinsed three times with sterile phosphate-buffered saline. The proximal and distal 2-cm segments were immersed in 10 mL of sterile Ringer's solution and subjected to sonication at 40 kHz and 300 W for 5 minutes, followed by vortexing for 30 seconds. The resulting suspension was centrifuged at $3,000 \times g$ for 15 minutes before culture.

Culture results were recorded separately for each specimen type. The positivity criteria and quantitative culture results were used to classify specimens as culture-positive or culture-negative. Because renal-pelvic urine was collected after perioperative antibiotic prophylaxis, whereas MSU was collected before prophylaxis, differences in culture yield between these specimen types were interpreted cautiously. Stent-associated culture findings were analysed as retrospective associations with postoperative systemic inflammatory response syndrome (SIRS), because stents were retrieved on postoperative day 14 ± 2 , after the primary outcome assessment window.

16S rRNA gene sequencing

16S rRNA gene sequencing was performed on samples from 28 participants: all 14 participants who developed postoperative systemic inflammatory response syndrome (SIRS) and 14 participants without SIRS selected as controls. Controls were matched to SIRS cases by age (± 5 years), procedure type, and stone size (± 5 mm). These matching variables were selected because of their potential relevance to infection risk. Because participants were selected according to postoperative outcome, the sequencing analysis represented an outcome-selected exploratory subgroup and was not intended to represent the full study cohort.

Microbial DNA was extracted using the DNeasy PowerUrine Kit, incorporating bead beating. The V3–V4 region of the bacterial 16S rRNA gene was amplified using primers 341F and 806R. Amplicons were sequenced on an Illumina MiSeq platform using paired-end 300-bp reads (2×300 bp). Sequence data were processed using QIIME 2 (version 2024.2) [11]. DADA2 was used for sequence processing and amplicon sequence variant (ASV) inference [12], and taxonomic classification was performed against the SILVA version 138 reference database [13].

Extraction and sequencing control negative samples were processed together with study samples in order to detect possible contamination. ASVs detected in negative controls were evaluated using a prevalence-based contaminant-filtering approach informed by the *decontam* method [14]. The contaminant-classification criterion and removal rule must be specified according to the analysis actually performed. Samples were standardized to 10,000 sequencing reads at diversity analysis stage. Samples containing less than 10,000 reads could be included only if they satisfied the minimum requirement, the total number of excluded samples should also be reported.

Alpha diversity was evaluated using the Shannon diversity index and observed ASV richness. Beta diversity was assessed using weighted and unweighted UniFrac distance matrices. Functional potential was inferred from 16S rRNA gene sequencing profiles using PICRUSt2 [15]. Differences in predicted pathway abundance were evaluated using the statistical procedures applied in the original analysis, with P values adjusted for multiple comparisons using the Benjamini–Hochberg procedure. These functional predictions were interpreted as inferred microbial functional potential

rather than direct measurements of gene expression or metabolic activity.

Quality control results of sequencing, which include per sample counts and the samples retained after quality steps, should be reported together with final findings. Versions of bioinformatics tools and PICRUSt2, together with all statistical techniques and parameters used in diversity and pathway analysis, should be indicated to ensure reproducibility of the results.

Outcome

The primary outcome was SIRS within 48 hours of surgery, defined as at least two of: temperature $>38^{\circ}\text{C}$ or $<36^{\circ}\text{C}$, heart rate $>90/\text{min}$, respiratory rate $>20/\text{min}$, white-cell count $>12,000/\text{mm}^3$ or $<4,000/\text{mm}^3$ [16]. SIRS is a non-specific systemic inflammatory endpoint and is not equivalent to Sepsis-3 sepsis, for which organ dysfunction must be documented [17]. The USSQ was collected as a separate secondary endpoint and will be reported elsewhere.

Sample Size and Statistical Analysis

A total of 120 participants were enrolled in this prospective cohort study. The rationale for the initial sample size was derived from an expected incidence rate for postoperative systemic inflammatory response syndrome (SIRS) of twelve percent, an odds ratio goal of 4.0, a two-tailed significance level of 0.05, and a power level of eighty percent. The sample size cannot be independently checked using just these parameters due to the lack of data on the exposure prevalence and the calculation procedure in the available methodological documents. Thus, it may not be appropriate to keep the required minimum sample size indicated in the source, which is 96 subjects, until everything has been verified. The sample-size justification applies to the primary association and does not establish adequate power for subgroup analyses or exploratory microbiome comparisons.

Continuous variables were summarized as medians and interquartile ranges (IQRs) and compared between independent groups using the Mann–Whitney U test. Categorical variables were compared using Fisher’s exact test. Proportions were reported with Wilson 95% confidence intervals, and crude odds ratios (ORs) with Woolf 95% confidence intervals. Given the small number of observed SIRS events ($n = 14$), multivariable modelling with multiple covariates was not undertaken because of the risk of overfitting and unstable estimates [18,19]. Accordingly, the clinical associations are reported as unadjusted estimates and should not be interpreted as independent effects after adjustment for potential confounders.

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated descriptively for day-14 stent culture in relation to postoperative SIRS status. Because stents were retrieved after the 48-hour primary outcome-assessment period, these measures describe the observed association between culture status and an earlier clinical outcome, they do not establish prospective diagnostic or predictive performance. Receiver operating characteristic analysis was not performed because the culture result was binary and provided a single operating point.

For comparisons of alpha diversity among midstream urine, renal-pelvic urine, and stent-associated samples obtained from the same participants, the Friedman test was used as the omnibus non-parametric test for repeated measures. Where the omnibus comparison indicated a difference, paired Wilcoxon signed-rank tests were used for post hoc pairwise comparisons, with Holm adjustment applied to the resulting P values. These analyses were restricted to participants with the required paired specimen data. The originally specified independent-samples Kruskal–Wallis/Dunn approach was not retained for these within-participant comparisons. This revised procedure must be

applied to the data before the corresponding results are reported.

All clinical comparisons were considered exploratory, and no multiplicity correction was applied across the clinical outcome analyses. The Benjamini–Hochberg procedure was used for pathway-level tests in the functional-prediction analysis. Clinical statistical analyses were performed using IBM SPSS Statistics version 28. Sequencing and microbiome analyses were conducted using the bioinformatics workflow.

Results

Participants

Baseline characteristics are shown in Table 1. Operative time was longer in participants with SIRS (median 68 vs 50 minutes, $p = 0.03$). With nine comparisons and 14 events, absence of a significant difference does not demonstrate comparability of the groups. SIRS occurred in 11/89 RIRS participants (12.4%) and 3/31 ureteroplasty participants (9.7%, $p = 1.0$).

Table 1. Baseline characteristics by SIRS status

Characteristic	All (n = 120)	SIRS (n = 14)	No SIRS (n = 106)	p
Age, median (IQR), years	47 (35–61)	54 (42–67)	46 (34–60)	0.18
Male sex, n (%)	78 (65.0)	9 (64.3)	69 (65.1)	0.95
BMI, median (IQR), kg/m ²	27.4 (24.1–31.2)	29.1 (25.3–33.6)	27.2 (24.0–31.0)	0.21
Stone size, median (IQR), mm	14.2 (8–22)	17.8 (12–26)	13.8 (8–21)	0.09
Hydronephrosis grade ≥ II, n (%)	64 (53.3)	10 (71.4)	54 (50.9)	0.14
Diabetes mellitus, n (%)	28 (23.3)	5 (35.7)	23 (21.7)	0.28
Prior ureteral stenting, n (%)	34 (28.3)	6 (42.9)	28 (26.4)	0.21
Operative time, median (IQR), min	52 (38–74)	68 (55–88)	50 (36–71)	0.03
RIRS, n (%)	89 (74.2)	11 (78.6)	78 (73.6)	0.68

IQR, interquartile range, RIRS, retrograde intrarenal surgery, SIRS, systemic inflammatory response syndrome. Mann–Whitney U (continuous) and Fisher’s exact test (categorical).

Culture findings and association with SIRS

Postoperative systemic inflammatory response syndrome (SIRS) occurred in 14 of 120 participants (11.7%, 95% CI, 7.1–18.6). Stent sonication cultures were positive in 50 participants (41.7%, 95% CI, 33.2–50.6), including 12 of 14 participants with SIRS (85.7%, 95% CI, 60.1–96.0) and 38 of 106 participants without SIRS (35.8%, 95% CI, 27.4–45.3). Stent culture positivity was associated with SIRS status (crude OR, 10.74, 95% CI, 2.28–50.52).

Renal-pelvic urine cultures were positive in 28 of 120 participants (23.3%), including 9 of 14 participants with SIRS and 19 of 106 without SIRS. Pelvic-urine culture positivity was associated with SIRS status (crude OR, 8.24, 95% CI, 2.48–27.38). Midstream urine (MSU) cultures were positive in 9 of 120 participants (7.5%), including 3 of 14 participants with SIRS and 6 of 106 without SIRS. The association between MSU culture positivity and SIRS was imprecisely estimated (crude OR, 4.55, 95% CI, 0.99–20.77), with the confidence interval including the null value.

Descriptive classification indices for the three specimen types are presented in Table 2. Stent sonication culture had a sensitivity of 85.7%, specificity of 64.2%, positive predictive value of 24.0%, and negative predictive value of 97.1% in relation to SIRS status. Pelvic-urine culture had a sensitivity of 64.3%, specificity of 82.1%, positive predictive value of 32.1%, and negative predictive value of 94.6%. MSU culture had a sensitivity of 21.4%, specificity of 94.3%, positive predictive value of 33.3%, and negative predictive value of 90.1%.

These classification indices are descriptive and should not be interpreted as evidence of prospective diagnostic or predictive performance. SIRS was assessed during the first 48 hours after surgery, whereas ureteral stents were retrieved approximately 14 days postoperatively. Consequently, the organisms recovered from stent sonication cannot be assumed to have caused the earlier SIRS episodes, and the high negative predictive value of stent culture should be interpreted in the context of the low SIRS prevalence. Differences in culture positivity across specimen types should also be interpreted cautiously because specimen collection times and perioperative antibiotic exposure differed.

Table 2. Association between culture positivity and SIRS (retrospective, day-14 stent sampling followed the outcome)

Measure	Stent sonication	Renal-pelvic urine	MSU
Culture-positive, total	50/120 (41.7%)	28/120 (23.3%)	9/120 (7.5%)
Positive with SIRS	12/14 (85.7%)	9/14 (64.3%)	3/14 (21.4%)
Positive without SIRS	38/106 (35.8%)	19/106 (17.9%)	6/106 (5.7%)
Crude OR	10.74	8.24	4.55
Woolf 95% CI for OR	2.28–50.52	2.48–27.38	0.99–20.77
Sensitivity	85.7%	64.3%	21.4%
Specificity	64.2%	82.1%	94.3%
Positive predictive value	24.0%	32.1%	33.3%
Negative predictive value	97.1%	94.6%	90.1%

MSU, midstream urine, OR, odds ratio, CI, confidence interval. The OR confidence intervals use the Woolf log-odds method. Classification indices use SIRS status as the reference outcome and are descriptive, not validated prospective diagnostic performance measures.

Microbial Diversity and Community Composition in the Sequencing Subgroup

Among the 28 sequenced participants, alpha-diversity measures showed a descriptive increase across specimen compartments, from midstream urine (MSU) to renal-pelvic urine and stent-associated biofilm (Table 3). Mean Shannon diversity increased from 1.12 ± 0.45 in MSU to 2.15 ± 0.82 in renal-pelvic urine and 3.42 ± 0.95 in stent biofilm. Observed ASV richness followed a similar pattern, increasing from 18 ± 6 in MSU to 42 ± 12 in renal-pelvic urine and 88 ± 24 in stent biofilm.

Beta-diversity ordination indicated compositional separation between stent-associated microbial communities and those detected in urine specimens (Figure 2). Proteobacteria and Firmicutes were prominent across all three specimen compartments, while Actinobacteria showed a higher relative abundance in stent-associated samples. Of the 2,980 detected amplicon sequence variants (ASVs), 1,847 had a reported relative abundance below 1%, this finding indicates that a substantial proportion of detected ASVs were individually low-abundance features, although the

interpretation depends on how relative abundance was defined and summarized.

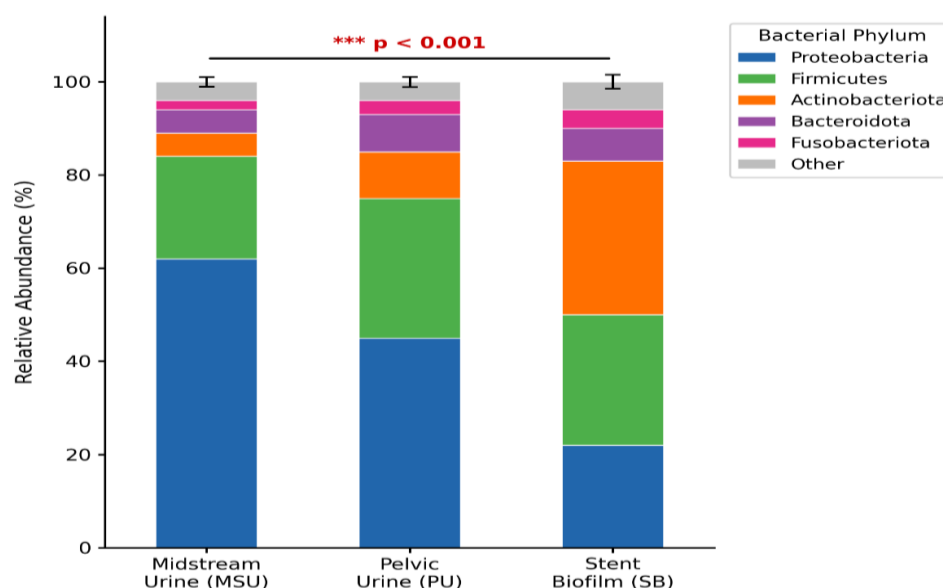


Figure 2. Relative abundance of major bacterial phyla by compartment in the sequencing subgroup (n = 28 participants per compartment). Error bars: [state SD or SEM].

These findings describe differences in microbial diversity and composition across specimen compartments in the sequencing subgroup. They do not, by themselves, establish statistically significant differences between compartments or between participants with and without SIRS. The sequencing subgroup was small and selected according to outcome status, limiting generalizability to the full cohort. In addition, 16S rRNA gene sequencing detects bacterial DNA and cannot distinguish viable organisms from non-viable cells. Accordingly, the observed compositional patterns should't be interpreted as direct evidence of active infection or microbial causation of SIRS.

Table 3. Alpha diversity by compartment (n = 28 participants, mean $\pm$ SD)

Specimen compartment	Shannon index, mean $\pm$ SD	Observed ASVs, mean $\pm$ SD
Midstream urine (MSU)	1.12 $\pm$ 0.45	18 $\pm$ 6
Renal-pelvic urine	2.15 $\pm$ 0.82	42 $\pm$ 12
Stent-associated biofilm	3.42 $\pm$ 0.95	88 $\pm$ 24

ASV, amplicon sequence variant, SD, standard deviation

Predicted functional profiles (exploratory)

Functional predictions based on the application of PICRUSt2 have shown several KEGG pathways to be more abundant in stent-supported patients with SIRS as compared to patients without SIRS (Table 4). The SIRS/control ratios ranged from 1.98 for lipopolysaccharide biosynthesis to 3.12 for bacterial chemotaxis. The exploration further revealed pathways related to *Vibrio cholerae* biofilm formation, ABC transporters, and quorum sensing. However, the results should be regarded as exploratory, as the comparison involved only 14 participants per group and was based on functional profiles obtained through prediction rather than measurement.

The reported ratios and adjusted *p* values require verification against the original analysis

output, including confirmation of the effect-size calculation and differential-abundance test. The findings should therefore be interpreted as hypotheses for further investigation, not as evidence of increased pathway activity or a demonstrated mechanism underlying SIRS.

Table 4. Exploratory differences in PICRUSt2-predicted KEGG pathway abundance in stent-associated samples from participants with and without SIRS (n = 14 per group)

Predicted KEGG pathway	Reported SIRS/control ratio	Reported FDR-adjusted <i>p</i> value
Bacterial chemotaxis	3.12	<0.001
Biofilm formation (<i>Vibrio cholerae</i> reference map)	2.85	0.002
ABC transporters	2.44	0.005
Quorum sensing	2.15	0.008
Lipopolysaccharide biosynthesis	1.98	0.012

Abbreviations: FDR, false discovery rate, KEGG, Kyoto Encyclopedia of Genes and Genomes, SIRS, systemic inflammatory response syndrome.

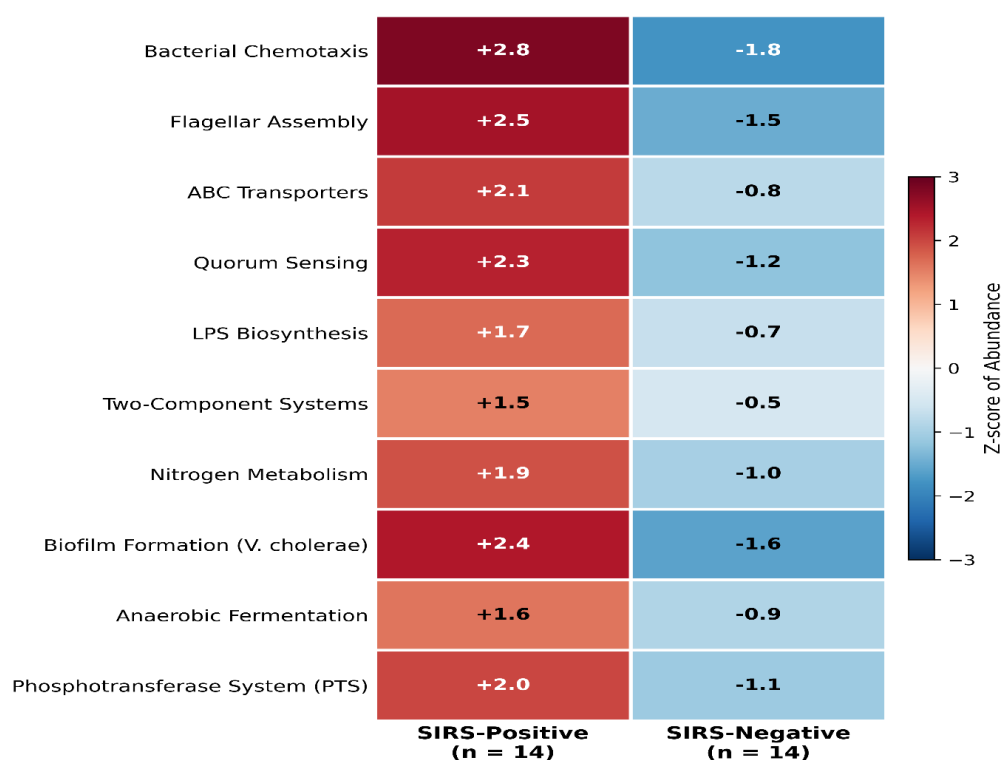


Figure 3. Heatmap of PICRUSt2-predicted KEGG pathway abundances in ureteral stent biofilm samples from SIRS-positive participants and matched SIRS-negative controls (n = 14 per group). Pathway relative abundances are Z-score normalised for visualisation. Colours indicate relatively higher or lower predicted pathway abundance and do not represent measured gene expression, pathway activation, or metabolic activity. Differential pathway analysis used Benjamini–Hochberg correction for multiple comparisons, with an FDR threshold of 5%, as reported in Table 4. PICRUSt2-derived functional profiles are computational predictions based on 16S rRNA gene sequencing.

Discussion

In this prospective cohort, microbial findings differed across bladder urine, renal-pelvic urine, and ureteral stent material, while day-14 stent culture positivity was associated with SIRS recorded within 48 hours of surgery. The principal contribution is a compartment-focused description and a hypothesis-generating association, the findings do not establish that stent biofilm caused SIRS or that stent culture can predict its occurrence. The observed increase in microbial diversity from MSU to pelvic urine and stent biofilm is consistent with evidence that bladder urine may not fully represent upper urinary tract or device-associated microbial communities [3,6,7]. However, higher diversity on stents may also reflect biofilm structure, longer dwell time, contamination during retrieval through the lower urinary tract, or residual contamination in low-biomass sequencing [14]. Moreover, pelvic urine was collected after antibiotic prophylaxis, whereas MSU was collected beforehand, potentially affecting culture-based comparisons. Stent colonisation is common and often asymptomatic [20–22], and the 41.7% positivity observed in this study is consistent with that literature. Consequently, stent positivity may reflect colonisation rather than clinically significant infection, which is relevant when interpreting the reported specificity of 64.2% and positive predictive value of 24.0%. The high negative predictive value of 97.1% should also be interpreted cautiously because it is influenced by the low prevalence of SIRS and does not establish a validated rule-out test. The fact that stents were extracted on postop day 14 implies the organisms detected might have existed preoperatively, been acquired perioperatively, or impacted by postoperative processes and antibiotics. Thus, no conclusions on the order of events can be made, nor are the authors able to predict effects in the future. A prediction study should collect evidence in advance of the events predicted, make use of a prior specified model, and should be validated internally and externally. SIRS is also a nonspecific postoperative occurrence and may happen because of irrigation effects, anaesthesia or surgical stress even in the absence of infection [16,17]. The fact that there were only few cases (14) makes it difficult to sustain statistically reliable results and adjusted predictions are unstable [18,19].

The uncertainty related to the stent-associated odds ratio is reflected in the large confidence interval (2.3–50.5), and the difference in operative time between the groups may have influenced this association. Functional data obtained from PICRUST2 analysis should be viewed as predictions based on taxonomic composition, and any claims about upregulation, virulence, or antimicrobial resistance should be based on direct evidence. Future research using methods such as shotgun metagenomics, metatranscriptomics, susceptibility testing, or metabolomics could test these hypotheses [15]. From a pragmatic point of view, the current findings provide no grounds for changing antibiotic prophylaxis or treating patients based on day-14 stent positivity only. They provide more relevance for future studies employing compartment-specific microbial sampling utilizing samples taken before an actual clinical event with a focus on larger prospective studies assessing temporal relations and clinical significance.

Limitations

There are a few limitations of this study. One is the single-center study design with the use of two procedures involving different stenting practices that could have an effect on the generalizability of the findings. Use of ureteral stents occurred outside of the postoperative outcome time frame which prevents inferences about causality and future predictions. Only 14 SIRS events occurred which borders on fractions that are too small to produce reliable results limiting the ability for adjusted analyses. Factors that could have confounded results such as irrigation pressure, type of

access sheaths utilized, composition of the stones, stent type and material, and use of antibiotics after the surgery were not considered. Another limitation is that the sequencing subgroup was very small with only 28 matching subjects based on only three characteristics. The fact that sequencing was done with 16S rRNA gene doesn't prove that the organisms present in the sample are alive. Also, while renal-pelvic urine samples were taken after the use of prophylactic antibiotics, MSU samples were collected before the use of antibiotics which might affect the comparison between the two types of samples.

Seventh, it is possible that contamination was introduced through the stent retrieval via the urethra and bladder, however, no retrieval-negative control was utilized. Eighth, microbiological sampling at the time of SIRS was not measured from this dataset, SIRS may occur due to non-infectious causes. Lastly, the baseline microbiological results were permitted in the MSU positive cases where treatment is not indicated, this should be kept in mind during interpretation of the MSU results. Such limitations should be taken into consideration, and the results should be validated in larger multicenter studies with standardized sampling.

Future directions

Larger, preferably multicentre cohorts should collect microbial samples before the outcome of interest, prespecify endpoints with adequate event numbers, document antibiotic exposure and stent characteristics, include rigorous negative controls for low-biomass sequencing, and validate any prediction model independently. Functional hypotheses should be tested with direct molecular methods, and biofilm-directed interventions evaluated in prospective trials before any change to routine care.

Conclusion

In this single-centre cohort, microbial findings differed across urinary compartments, and stent culture positivity at planned day-14 removal was associated with SIRS recorded within 48 hours after surgery. Because sampling followed the outcome and only 14 events occurred, these results cannot establish causality or predictive value. Prospective studies with pre-outcome sampling and independent validation are needed to determine whether compartment-specific microbiology has clinical utility.

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