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Original Article

# Metabolic syndrome and the urinary microbiome of patients undergoing percutaneous nephrolithotomy

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**Abstract** *Objective:* To identify possible stone-promoting microbes, we compared the profiles of microbes grown from stones of patients with and without metabolic syndrome (MetS). The association between MetS and urinary stone disease is well established, but the exact pathophysiologic relationship remains unknown. Recent evidence suggests urinary tract dysbiosis may lead to increased nephrolithiasis risk.
*Methods:* At the time of percutaneous nephrolithotomy, bladder urine and stone fragments were collected from patients with and without MetS. Both sample types were subjected to expanded quantitative urine culture (EQUC) and 16 S ribosomal RNA gene sequencing.
*Results:* Fifty-seven patients included 12 controls (21.1%) and 45 MetS patients (78.9%). Both cohorts were similar with respect to demographics and non-MetS comorbidities. No controls had uric acid stone composition. By EQUC, bacteria were detected more frequently in MetS stones (42.2%) compared to controls (8.3%) ($p=0.041$). Bacteria also were more abundant in stones of MetS patients compared to controls. To validate our EQUC results, we performed 16 S ribosomal RNA gene sequencing. In 12/16 (75.0%) sequence-positive stones, EQUC reliably isolated at least one species of the sequenced genera. Bacteria were detected in both "infectious" and "non-infectious" stone compositions.
*Conclusion:* Bacteria are more common and more abundant in MetS stones than control stones. Our findings support a role for bacteria in urinary stone disease for patients with MetS regardless of stone composition.

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## 1. Introduction

Nephrolithiasis is highly prevalent across the globe with higher rates in North America and Europe, compared to Asia [1]. Men tend to have a higher prevalence of nephrolithiasis compared to women; however, this gender disparity is narrowing [2]. Recurrence of nephrolithiasis following the first episode is common [3]. Experts propose that this rising prevalence in urinary stone disease is related to the parallel rise in obesity around the world [4].

Metabolic syndrome (MetS) is the co-occurrence of certain metabolic abnormalities that confer an increased risk of cardiovascular disease and other health problems. There are multiple accepted definitions for MetS; however, all include measures of obesity, hypertension, dyslipidemia, and insulin resistance as major tenets [5]. MetS affects an estimated 35% of Americans and this prevalence is accelerating year after year [6]. With the spread of Western lifestyle and diet, the prevalence of MetS is expanding to a global population [7].

The abnormalities observed in MetS have a known association with nephrolithiasis, particularly uric acid nephrolithiasis [8]. Estimates suggest that up to 50% of stone-forming patients qualify for a MetS diagnosis with odds of developing nephrolithiasis increasing with greater numbers of MetS traits [9]. The pathophysiology of nephrolithiasis in MetS patients is incompletely understood, although prevailing theory considers it to be a multifactorial and complex interplay of metabolic disturbances. Insulin resistance results in low urinary pH through impairment of renal ammonium formation. This also may lead to hypocitraturia and an increase in urinary calcium excretion. Systemic inflammation in patients with MetS may act locally in the kidney to potentiate the development of urinary stones [10,11].

While the relationship between nephrolithiasis and MetS and the relationship between MetS and microbiome dysbiosis in the gut have been studied, the current theories of nephrolithiasis in MetS patients ignore the role of the urinary microbiome and its influence on stone formation [12]. Through the use of an enhanced culture technique and 16 S ribosomal RNA (rRNA) gene sequencing, bacteria have been identified in urinary stones of all compositions [13]. With this discovery, along with the complex physiologic disturbances in MetS patients, we hypothesize that the urinary microbiome plays a role in urinary stone formation in patients with MetS.

## 2. Materials (patients) and methods

### 2.1. Patient population and sample collection

Following Loyola University's institutional review board approval (LU208983), patients between the ages of 18 years and 90 years of age with nephrolithiasis undergoing percutaneous nephrolithotomy (PCNL) were prospectively enrolled from a single academic urology practice. All consecutive patients who met study criteria were enrolled from March 2017 to April 2019. Based on prior studies characterizing other urinary tract microbes and given widely recognized difficulties in performing power analysis for microbiome studies, a study size of 50 patients was determined to be sufficient [13,14].

The National Cholesterol Education Program Adult Treatment Panel III definition for MetS was utilized [5]. Patients meeting three of the following five criteria were included in the MetS cohort: waist circumference over 102 cm (men) or 89 cm (women), blood pressure over 130/85 mmHg or on treatment, fasting triglyceride level over 150 mg/dL, fasting high-density lipoprotein cholesterol level less than 40 mg/dL (men) or 50 mg/dL (women), and fasting blood glucose over 100 mg/dL. All patients that did not meet three of the five criteria were considered the control cohort. Patients were excluded if they had prior augmentation cystoplasty, intestinal urinary diversion, history of urologic malignancy (with the exception of National Comprehensive Cancer Network low-risk prostate cancer), were pregnant, or were unwilling to undergo required lab testing. Patients were not excluded based on prior antibiotic use, given the need for intraoperative antibiotic prophylaxis. Following enrollment, all patients underwent fasting blood draw for lipid panel. At follow-up, patients completed a 24-h urine chemistry profile performed by Quest Diagnostics (Valencia, CA, USA). Patients were also instructed to have serum biochemistry labs drawn at the follow-up.

On the day prior to PCNL, all patients underwent placement of a percutaneous nephroureteral catheter by interventional radiology, as per routine protocol at Loyola University Medical Center. Prior to all procedures, prophylactic intravenous antibiotics were administered, consistent with American Urological Association guidelines. Within 24 h, patients proceeded to the operating room for their stone removal procedure. At the time of PCNL, urine was collected from the bladder via transurethral catheterization. Stone removal occurred with or without stone fragmentation through a 30 Fr PCNL sheath under normal sterile operative technique. A portion of the stone fragment was sent for routine chemical analysis stone analysis by Louis C. Herring and Company Kidney Stone Analysis Laboratory (Orlando, FL, USA). A second portion was sent to the microbiology lab for immediate processing for expanded quantitative urine culture (EQUC) and 16 S rRNA gene sequencing, as described previously [13,14] and in the Supplemental Methods. Supplementary Table 1 summarizes the environmental and technical details of extraction and sequencing as recommended by the recently published consensus paper by Brubaker et al. [15].

### 2.2. 16 S rRNA gene sequence analysis

BaseSpace platform (Illumina, San Diego, CA, USA) was used to perform demultiplexing and trimming of sequence adapters and barcodes from raw sequences, and primers removed with Cutadapt program v1.13 [16]. In the quality trimming and filtering step, reads with maximum expected errors greater than 2 erroneous base calls were discarded as a quality filtering measure using "maxEE=c (2,2)" parameter (specified for both the forward and reverse reads separately). The trimmed and filtered reads were then analyzed by DADA2 software [17] as an R script (in R v.3.6) using its R package (dada2 v.1.14.1). DADA2 uses a parametric model to infer amplicon sequence variants (ASVs), which are true biological sequences from sequence reads. To obtain taxonomic classification of the ASVs, the SILVA database v.132 [18] was used. The resulting ASV table retained only high quality nonchimeric reads. Unless previously noted, default parameters were used for each software tool.

### 2.3. Statistical analysis

The baseline patient cohort was compared using descriptive statistics. Continuous variables were reported as mean±standard deviation, while significance was determined using a Student's *t*-test. Categorical variables were reported as percentages and analyzed using Chi-squared test or Fisher exact test where appropriate. Urinary stone chemical composition was averaged across the cohorts. Stone samples were categorized as "growth" or "no growth" based on presence or absence of bacterial colonies on any culture medium. The overall frequency of growth and the associated colony forming units per milliliter (CFU/mL) between each cohort were compared using Chi-squared and Student's *t*-test, respectively. Standard Chi-squared tests were applied to the relative abundance of bacterial taxon identified by 16 S rRNA gene sequencing. Those taxa present at a significantly higher proportion in urinary stones compared to their paired bladder urine samples were considered enriched, as previously described [13].

To determine whether the composition of the paired stone and urine samples differed, we performed hierarchical clustering, a form of beta diversity analysis. Hierarchical clustering groups samples that are similar in taxon composition, as measured by a chosen ecological distance [19]. The distance matrix was calculated using the Bray-Curtis dissimilarity index, which quantifies compositional dissimilarity between different groups. The Bray-Curtis dissimilarity is bounded between 0 and 1, where 0 means the two sites have the same composition (that is, they share all the taxa), and 1 means the two sites do not share any taxa [19]. Statistical analysis was performed using RStudio (version 1.1.456, RStudio, Inc., Boston, MA, USA).

### 2.4. Data availability

Sequences will be made publicly available prior to publication.

## 3. Results

Sixty-one patients were enrolled in the study. Adequate samples were collected from 57 patients, including 12 controls and 45 MetS patients; these 57 patients were included in the final analysis. Table 1 includes baseline patient demographics. Supplementary Table 2 includes 24-h urine collection and stone composition data. The two cohorts were demographically similar, except as it pertains to elements of MetS. The difference between stone composition for MetS and control cohorts was statistically significant ($p<0.001$), primarily because no one in the control cohort had a stone composed of uric acid. The 24-h urine chemistry did not differ significantly (Supplementary Table 2). Patients also were instructed to complete serum biochemistry labs to complete a standard metabolic stone disease work-up; however, not enough patients were able to complete this request to permit analysis.

EQUC detected bacteria in the urinary stones of 20 patients. This included one (8.3%) control patient and 19 (42.2%) MetS patients ($p=0.041$) (Fig. 1A). Similarly, EQUC detected bacteria in the bladder urine of two (16.7%) control patients compared to 10 (22.2%) MetS patients ($p=1.000$) (Fig. 1B). Thus, MetS patients were more likely to be culture-positive. Of the seven individuals with positive EQUC results for both sample types, there was 100% concordance between samples, where concordance is defined as the presence of at least one common bacterial taxon (Supplementary Table 3). Bacteria detected by EQUC in stone homogenates were from a variety of Gram-positive and Gram-negative genera (Supplementary Fig. 1) with the genera *Proteus* and *Staphylococcus* as the most commonly detected; members of the emerging uropathogenic genus *Aerococcus* also were frequently detected. Most EQUC-positive urinary stones were monomicrobial; three stones were polymicrobial (Fig. 1A).

To validate the EQUC results, we performed 16 S rRNA gene sequencing. The urinary stone homogenate was positive (defined as >2000 sequence reads) in 16 of 57 (28.1%) samples, including 2/12 (16.7%) control samples and 14/45 (31.1%) MetS samples ($p=0.71$). Corresponding bladder urine samples were positive in 14 of these 16 participants (87.5%). For all positive stone and urine samples, the average number of sequence reads was 41 429 with 24 genera represented. Fig. 2 shows the positive 16 S rRNA gene sequencing of stone homogenate samples with their corresponding positive bladder samples. *Proteus* was present at greater relative abundance in stone samples compared to paired bladder samples (Participants 5, 34, 52, and 55). Stone samples were statistically enriched for particular genera (higher proportion in stone compared to bladder) in 12 paired samples. In sequence-positive stone samples, EQUC reliably isolated at least one species of the sequenced genera in 12/16 (75.0%) stones (Table 2).

To further compare the paired samples, we performed a beta diversity analysis on the 16 S rRNA gene sequencing data using the Bray-Curtis dissimilarity index (Supplementary Fig. 2). The indices ranged from 0.041 to 0.993 (Table 2) with a mean of 0.522, meaning that some pairs differed almost completely (for example, Participants 5 and 21), whereas others were quite similar (particularly Participants 26 and 42).

**Table 1** Baseline patient demographics and medical characteristics comparing patients with MetS to controls.

| Baseline patient demographic and medical characteristic | Total | Control | MetS | *p*-Value |
|---|---|---|---|---|
| Samples collected, *n* | 57 | 12 | 45 | NA |
| Age, median (range), year | 62.3 (31.1–83.0) | 62.3 (42.0–69.9) | 62.8 (31.1–83.0) | 0.660 |
| Gender, *n* (%) | | | | 0.504 |
| Female | 36 (63.2) | 9 (75.0) | 27 (60.0) | |
| Male | 21 (36.8) | 3 (25.0) | 18 (40.0) | |
| Race or ethnicity, *n* (%) | | | | 0.484 |
| Caucasian | 47 (82.5) | 10 (83.3) | 37 (82.2) | |
| African American | 1 (1.8) | 1 (8.3) | 0 | |
| Hispanic | 6 (10.5) | 1 (8.3) | 5 (11.1) | |
| Asian | 2 (3.5) | 0 | 2 (4.4) | |
| Unknown | 1 (1.8) | 0 | 1 (2.2) | |
| Height, mean±SD, cm | 167.8±11.2 | 164.7±7.8 | 168.6±11.8 | 0.250 |
| Weight, mean±SD, kg | 95.4±27.0 | 78.5±31.9 | 99.8±24.0 | 0.011 |
| Body mass index, mean±SD, kg/m$^2$ | 33.9±9.5 | 28.9±11.7 | 35.2±8.5 | 0.036 |
| MetS criteria, *n* (%) | | | | |
| Blood pressure of >130/85 mmHg or one antihypertensive medication, *n* (%) | 46 (80.7) | 4 (33.3) | 42 (93.3) | <0.001 |
| Waist circumference over 89 cm (women) or 102 cm (men)[a,b] | 25 (73.5) | 3 (33.3) | 22 (88.0) | 0.004 |
| Fasting triglyceride level of >150 mg/dL[b] | 40 (71.4) | 2 (16.7) | 38 (86.3) | <0.001 |
| Fasting HDL level of <50 mg/dL (women) or <40 mg/dL (men) or dyslipidemia treatment[b] | 43 (76.8) | 4 (33.3) | 39 (88.6) | <0.001 |
| Fasting glucose level of >100 mg/dL or diabetes treatment | 38 (66.7) | 2 (16.7) | 36 (80.0) | <0.001 |
| Urologic history, *n* (%) | | | | |
| Indwelling Foley catheter or suprapubic tube | 0 (0) | 0 | 0 | NA |
| CIC | 0 (0) | 0 | 0 | NA |
| Prior indwelling ureteral stent or nephrostomy tube | 9 (15.8) | 1 (8.3) | 8 (17.8) | 0.669 |
| History of recurrent urinary tract infections | 13 (22.8) | 4 (33.3) | 9 (20.0) | 0.440 |
| Prior medical stone prevention | 13 (22.8) | 2 (16.7) | 11 (24.4) | 0.713 |
| Antibiotic use within 30 days (excluding prophylactic antibiotics) | 11 (19.3) | 1 (8.3) | 10 (22.2) | 0.426 |
| Periprocedural antibiotic duration prior to specimen collection, *n* (%) | | | | 0.058 |
| <3 h | 4 (7.0) | 2 (16.7) | 2 (4.4) | |
| 3–<6 h | 2 (3.5) | 1 (8.3) | 1 (2.2) | |
| 6–<12 h | 1 (1.8) | 1 (8.3) | 0 | |
| 12–<24 h | 38 (66.7) | 7 (58.3) | 31 (68.9) | |
| ≥24 h | 12 (21.1) | 1 (8.3) | 11 (24.4) | |

MetS, metabolic syndrome; CIC, clean intermittent catheterization; HDL, high-density lipoprotein; SD, standard deviation; NA, not available.

[a] 1 inch=2.54 cm.

[b] *n*=34 for waist circumference, *n*=56 for fasting triglyceride, and *n*=56 for fasting HDL.

## 4. Discussion

EQUC detected bacteria more frequently in urinary stones from patients with MetS compared to controls. These results were validated by 16 S rRNA gene sequencing, as it often detected the predominant microbe identified by EQUC, although the difference in detection by sequencing was not statistically significant. Both EQUC and 16 S rRNA gene sequencing detected bacteria in all stone compositions, including historically "non-infectious" such as calcium oxalate stone compositions. This also includes uric acid stones, which are more prevalent in MetS patients.

MetS has long been associated with urinary stone formation, but the pathophysiology is not well understood. Theories have mainly focused on urinary chemical composition disturbances, leading patients to increased stone susceptibility. This is most notably exemplified by the predisposition of patients with MetS toward uric acid nephrolithiasis [8]. For patients with MetS, insulin resistance results in an impairment of renal ammonium generation with resultant low urinary pH. This gouty diathesis increases risk of both uric acid and calcium urinary stones [20]. Furthermore, patients with MetS are known to have chronic inflammation, which plays a significant role in the development of atherosclerosis in this patient population [21]. This pro-inflammatory microenvironment may also exist in the collecting system, resulting in crystal aggregation and stone formation [11,23].

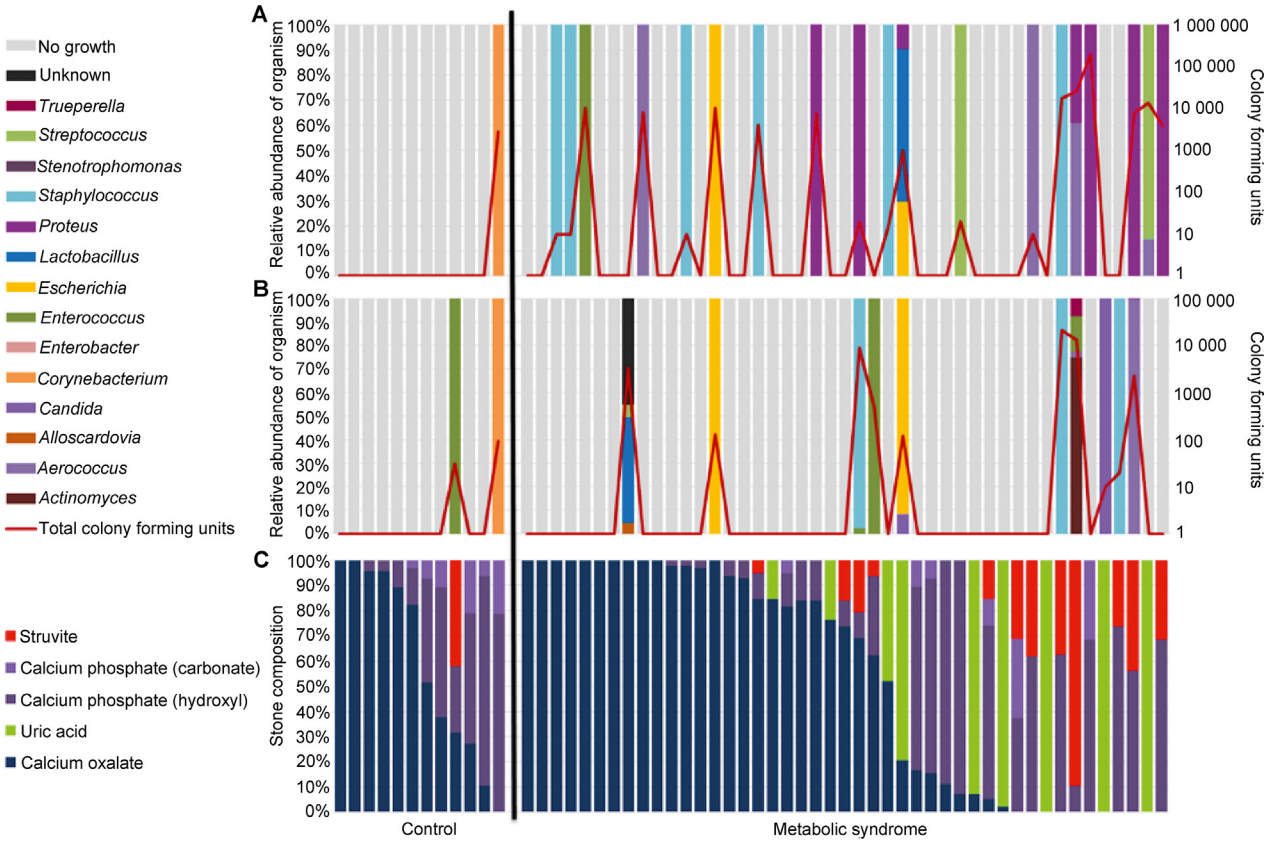


**Figure 1** Growth of bacteria on expanded quantitative urine culture. (A) Urinary stone; (B) Bladder urine; (C) Stone chemical composition of the corresponding stone. The solid black line marks the division of control and metabolic syndrome patients. Each column above or below corresponds to the same patient.

Notwithstanding these processes, recent evidence suggests that urinary bacteria may play a role in stone formation, outside of traditionally infectious stones [13,24–26]. Our study lends support to the notion that dysbiosis of the urinary microbiome may play a role or potentiate processes already at work in stone-forming

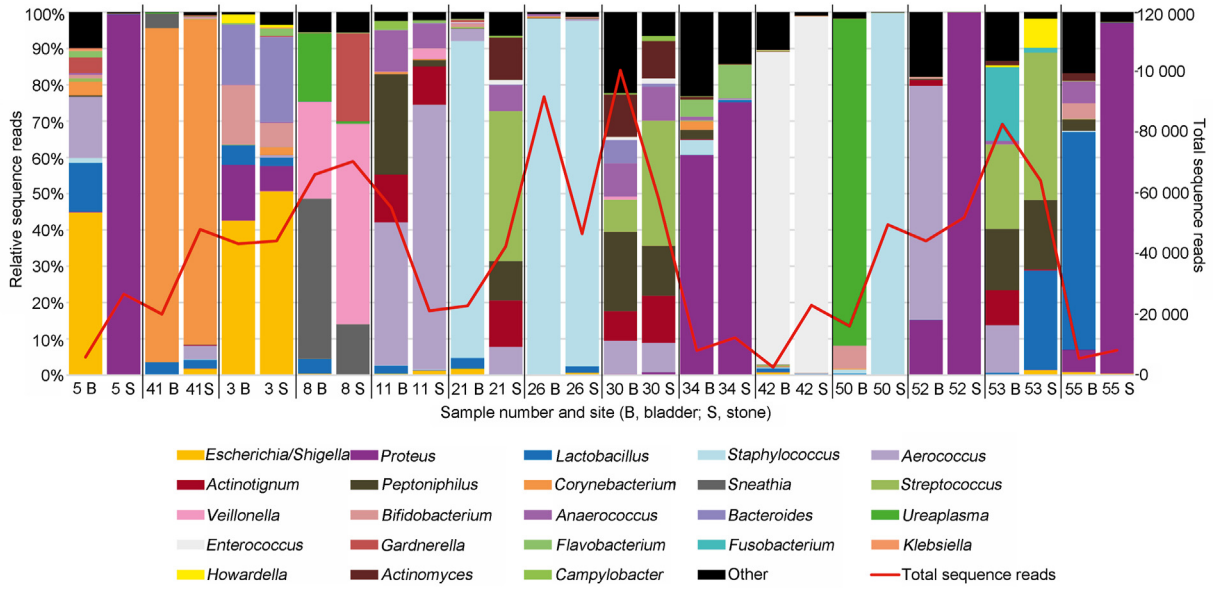


**Figure 2** 16 S rRNA gene sequencing of bladder and stone samples from controls and metabolic syndrome patients. The solid black vertical lines separate paired sampled.

**Table 2** Comparison of stone culture, stone sequencing, bladder culture, and bladder sequencing genera from the same study participant.

| Sample ID | Sample cohort | Stone culture genera | Stone sequence dominant genera | Bladder culture genera | Bladder sequence dominant genera | Bray-Curtis index between stone and bladder sequencing |
|---|---|---|---|---|---|---|
| 5 | Control | NA | *Proteus* | NA | *Escherichia*, *Aerococcus*, *Lactobacillus* | 0.993 |
| 41 | Control | ***Corynebacterium*** | ***Corynebacterium*** | ***Corynebacterium*** | ***Corynebacterium*** | 0.076 |
| 3 | MetS | ***Proteus*** | ***Escherichia***, *Bifidobacterium* | ***Escherichia*** | ***Escherichia***, ***Proteus***, *Bifidobacterium* | 0.410 |
| 8 | MetS | NA | *Veillonella* | NA | *Sneathia*, *Veillonella*, *Ureaplasma* | 0.528 |
| 11 | MetS | ***Aerococcus*** | ***Aerococcus*** | NA | ***Aerococcus***, *peptoniphilus*, *Anaerococcus* | 0.379 |
| 21 | MetS | ***Staphylococcus*** | *Streptococcus* | NA | ***Staphylococcus*** | 0.939 |
| 23 | MetS | ***Aerococcus*** | ***Aerococcus*** | NA | NA | NA |
| 26 | MetS | ***Staphylococcus*** | ***Staphylococcus*** | ***Staphylococcus*** | ***Staphylococcus*** | 0.041 |
| 30 | MetS | ***Aerococcus***, ***Proteus*** | *Streptococcus*, ***Aerococcus***, ***Proteus*** | *Actinomyces*, ***Aerococcus***, *Enterococcus* | *Peptoniphilus* | 0.326 |
| 34 | MetS | ***Proteus*** | ***Proteus*** | NA | ***Proteus*** | 0.201 |
| 40 | MetS | ***Proteus*** | ***Proteus***, *Klebsiella* | NA | NA | NA |
| 42 | MetS | ***Enterococcus*** | ***Enterococcus*** | NA | ***Enterococcus*** | 0.124 |
| 50 | MetS | NA | ***Staphylococcus*** | ***Staphylococcus*** | *Ureaplasma* | 0.987 |
| 52 | MetS | ***Proteus*** | ***Proteus*** | ***Aerococcus*** | ***Aerococcus***, ***Proteus*** | 0.848 |
| 53 | MetS | ***Aerococcus***, ***Streptococcus*** | ***Streptococcus*** | NA | ***Aerococcus***, ***Streptococcus***, *Fusobacterium* | 0.552 |
| 55 | MetS | ***Proteus*** | ***Proteus*** | NA | *Lactobacillus* | 0.905 |

MetS, metabolic syndrome; NA, not available.
Note: bold represents concordance between samples of a row.

patients with MetS. From stones obtained from many patients in our cohort, EQUC detected bacteria known to influence urinary stone formation, especially members of the genera *Proteus* and *Staphylococcus*. However, many of these stones were not of the classic "infectious" magnesium ammonium phosphate (struvite) stone composition. Many culture- and sequence-positive stones were discovered in purely calcium-based urinary stones (Fig. 1).

The urinary metabolite disturbances associated with MetS result in a low urinary pH, opposite the effects of bacterial-produced urease, which results in a high urinary pH. Bacteria may be bystanders to the metabolic dysbiosis in MetS stone-forming patients. However, their presence may also potentiate the increased risk of stone formation in MetS patients. This also may be true for non-MetS stone-forming patients. Stone-forming patients are already noted to have pro-inflammatory cytokines and chemokines in their urine [22]. Therefore, bacteria may act synergistically with this process, especially in MetS patients that already exist in a pro-inflammatory state [27].

Our study provides further information about the urinary microbiome in stone-forming patients. In many of our stone samples, bacteria were enriched within the urinary stones, demonstrating a higher concentration of bacteria within the stone sample than the surrounding urine. Using Bray-Curtis analysis, we showed that many samples were highly dissimilar comparing the microbial diversity of the stone homogenate and the bladder urine. This was most notable in samples that were *Proteus*-positive. The high concordance between our stone culture and stone sequencing suggests that the bacteria being sequenced are indeed present and enriched within the stone. It is important to recognize that our Bray-Curtis analysis demonstrated several highly similar diversity profiles between bladder and stone communities. In these samples, we cannot say that the bacteria detected in the stone sample are actually associated with the stone. Instead, it is formally possible that they are contaminants from residual urine within the stone sample. For these stone samples, it is unclear if these bacteria are truly stone-associated. However, we can say with confidence that stone samples showing bacterial enrichment are truly stone-associated.

In this study, we proceeded with sample collection using PCNL. Alternative methodology for sample collection

includes ureteroscopy. Our study group prefers PCNL for stone sample collection. It is our concern that stone samples obtained via ureteroscopy are subject to contamination by other urinary tract niches (ureter, bladder, and urethra). This may explain the different genera of bacteria detected in our study as compared to other recently published study that assessed the stone microbiome in patients undergoing ureteroscopy [13]. The present study offers validation to our methodology of sample collection, culturing, and sequencing. This methodology is in alignment with the recently published consensus on urolithiasis microbiome studies [28].

Our study does have certain inherent limitations. We were significantly limited by the sample size, mostly as we were unable to recruit control patients to allow for adequate comparison to our MetS patient population. Unfortunately, patients with stone burden large enough to require PCNL are more likely to exhibit MetS features. Further investigations into the role of MetS, the urinary microbiome, and stone formation would benefit from multi-institutional trials. Additionally, many patients had antibiotic exposure within the previous 30 days. This could be secondary to our institution being a tertiary referral center with many patients having multiple comorbidities, including frequent urinary tract infections requiring antibiotic use prior to planned surgery. All patients were exposed to antibiotics prior to nephroureteral catheter placement and PCNL based on American Urological Association guidelines. Bacteria may be eradicated by antibiotic exposure, leading to no growth on EQUC. Attempts at stone sample collection without antibiotics would be unethical, and thus were unavoidable. On the day prior to PCNL, patients underwent placement of a nephroureteral catheter with interventional radiology. The nephroureteral catheter traverses the skin and upper urinary tract on its way down to the bladder, possibly resulting in urine reflux and cross-contamination between bladder and upper tract urine. There may also be translocation of skin bacteria into the urinary tract along the catheter. These concerns regarding contamination may have confounded our results. Interestingly, our study did not demonstrate a significant difference in urine chemistries between the groups as we had predicted. This is likely due to the small sample size. Furthermore, serum biochemistry was not obtained, which could have provided further insight into metabolic disturbances in patients with MetS.

## 5. Conclusion

Our study characterizes the association amongst MetS, urinary microbiome, and urinary stone disease. In stones collected via PCNL, EQUC more frequently detected bacteria in MetS patients than controls with 16 S rRNA gene sequencing validating our EQUC results. Taken together, our observations support an association between bacteria and stone formation, regardless of stone composition; EQUC and 16 S rRNA gene sequencing detected bacteria in "infectious" stone compositions, as well as calcium-based and uric acid-based urinary stones. Many of these stone samples demonstrated enrichment on sequencing, suggesting true stone-association. However, due to a limited sample size, we were unable to offer adequate comparison between MetS patients and controls. Overall, we provided further validation to our methodology of sample collection by PCNL, processing, culturing, and sequencing consistent with the recently published consensus statement [15,28].

## Author contributions

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## Conflicts of interest

Wolfe AJ is a member of the Urobiome Therapeutics (Boston, MA, USA) advisory board and the Pathnostics Scientific (Irvine, CA, USA) advisory board. Wolfe AJ has research funding from the Craig H. Neilsen Foundation, Pathnostics (Irvine, CA, USA), and an anonymous donor. The other authors declared no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajur.2022.08.007.

## References

[1] Sorokin I, Mamoulakis C, Miyazawa K, Rodgers A, Talati J, Lotan Y. Epidemiology of stone disease across the world. World J Urol 2017;35:1301—20.
[2] Scales CD Jr, Smith AC, Hanley JM, Saigal CS; Urologic Diseases in America Project. Prevalence of kidney stones in the United States. Eur Urol 2012;62:160—5.
[3] Ziemba JB, Matlaga BR. Epidemiology and economics of nephrolithiasis. Investig Clin Urol 2017;58:299—306.
[4] DiBianco JM, Jarrett TW, Mufarrij P. Metabolic syndrome and nephrolithiasis risk: should the medical management of

nephrolithiasis include the treatment of metabolic syndrome? Rev Urol 2015;17:117—28.
[5] Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech 2009;2:231—7.
[6] Hirode G, Wong RJ. Trends in the prevalence of metabolic syndrome in the United States, 2011—2016. JAMA 2020;323:2526—8.
[7] Saklayen MG. The global epidemic of the metabolic syndrome. Curr Hypertens Rep 2018;20:12. https://doi.org/10.1007/s11906-018-0812-z.
[8] Kadlec AO, Greco K, Fridirici ZC, Hart ST, Vellos T, Turk TM. Metabolic syndrome and urinary stone composition: what factors matter most? Urology 2012;80:805—10.
[9] Wong Y, Cook P, Roderick P, Somani BK. Metabolic syndrome and kidney stone disease: a systematic review of literature. J Endourol 2016;30:246—53.
[10] Boyd C, Wood K, Whitaker D, Assimos DG. The influence of metabolic syndrome and its components on the development of nephrolithiasis. Asian J Urol 2018;5:215—22.
[11] Ramaswamy K, Shah O. Metabolic syndrome and nephrolithiasis. Transl Androl Urol 2014;3:285—95.
[12] Scheithauer TPM, Rampanelli E, Nieuwdorp M, Vallance BA, Verchere CB, van Raalte DH, et al. Gut microbiota as a trigger for metabolic inflammation in obesity and type 2 diabetes. Front Immunol 2020;11:571731. https://doi.org/10.3389/fimmu.2020.571731.
[13] Dornbier RA, Bajic P, Van Kuiken M, Jardaneh A, Lin H, Gao X, et al. The microbiome of calcium-based urinary stones. Urolithiasis 2020;48:191—9.
[14] Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J Clin Microbiol 2014;52:871—6.
[15] Brubaker L, Gourdine JF, Siddiqui NY, Holland A, Halverson T, Limeri R, et al. Forming consensus to advance urobiome research. mSystems 2021;6:e0137120. https://doi.org/10.1128/mSystems.01371-20.
[16] Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 2011;17:10—2.
[17] Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13:581—3.
[18] Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 2013;41:D590—6. https://doi.org/10.1093/nar/gks1219.
[19] Kindt R, Coe R. Tree Diversity Analysis: a manual and software for common statistical methods for ecological and biodiversity studies. Nairobi: World Agroforestry Centre (ICRAF); 2005. p. 161.
[20] Bray JR, Roger Bray J, Curtis JT. An ordination of the upland forest communities of Southern Wisconsin. Ecol Monogr 1957;27:325—49.
[21] Spatola L, Ferraro PM, Gambaro G, Badalamenti S, Dauriz M. Metabolic syndrome and uric acid nephrolithiasis: insulin resistance in focus. Metabolism 2018;83:225—33.
[22] Festa A, D'agostino R, Howard G, Mykkänen L, Tracy RP, Haffner SM. Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: the Insulin Resistance Atherosclerosis Study. Kidney Int 2000;58:1703—10.
[23] Suen JL, Liu CC, Lin YS, Tsai YF, Juo SHH, Chou YH. Urinary chemokines/cytokines are elevated in patients with urolithiasis. Urol Res 2010;38:81—7.
[24] Schwaderer AL, Wolfe AJ. The association between bacteria and urinary stones. Ann Transl Med 2017;5:32. https://doi.org/10.21037/atm.2016.11.73.
[25] Zampini A, Nguyen AH, Rose E, Monga M, Miller AW. Defining dysbiosis in patients with urolithiasis. Sci Rep 2019;9:5425. https://doi.org/10.1038/s41598-019-41977-6.
[26] Barr-Beare E, Saxena V, Hilt EE, Thomas-White K, Schober M, Li B, et al. The interaction between *Enterobacteriaceae* and calcium oxalate deposits. PLoS One 2015;10:e0139575. https://doi.org/10.1371/journal.pone.0139575.
[27] Eckel RH, Alberti K, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2010;375:181—3.
[28] Kachroo N, Lange D, Penniston KL, Stern J, Tasian G, Bajic P, et al. Standardization of microbiome studies for urolithiasis: an international consensus agreement. Nat Rev Urol 2021;18:303—11.