MP24-05: The Urine and Stone Microbiome in Kidney Stone Patients

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Abstract

Introduction/Objectives

The gut microbiota has been implicated in the pathophysiology of Urinary Stone Disease (USD), however, little is known about the microbiota of the urinary tract in USD, and no studies have directly compared urine to stone microbiota. Standard urine culture techniques detect known pathogenic urinary bacteria, but may not detect all bacteria involved in urolithiasis. Thus, the objective of the current study is to compare the urinary and stone microbiome through culture and microbial techniques.

Methods

Urine and stone samples were collected from USD patients and healthy controls. Samples were cultured on blood agar using conventional methods. DNA was extracted from urine, stones, along with blood agar cultures for microbial community profiling using high-throughput 16s rRNA sequencing. The resulting microbial profiles were used to compare 1) molecular vs. culturing techniques; 2) the kidney stone microbiome vs. urinary microbiome; and 3) the urinary microbiome between USD patients and healthy controls.

Results

The urine and stone microbiota demonstrated distinct yet overlapping microbiota, which were dominated by diverse bacteria from the *Bacteriodetes* and *Proteobacteria* phyla. There were distinct differences among *Alphaproteobacteria* taxa between the USD urinary microbiome and controls. The diversity of bacteria present in urine samples was higher in USD patients. Taxa from the *Bacteriodetes*, *Firmicutes*, and *Alphaproteobacteria* were different between the urinary and stone microbiome. When comparing DNA extracted from urine and stones to DNA extracted from cultures, there was a trend towards lower diversity when bacteria were cultured first. In particular, results suggest that culturing bacteria from the urine and stones may underrepresent *Alphaproteobacteria* diversity.

Conclusions

This is the first study to examine the urinary and stone microbiota through microbial profiling techniques, and demonstrates a distinct urinary microbiome in USD patients. Future work is needed to resolve the difference between culture and molecular techniques. These results have implications for perioperative screening and antibiotic prophylaxis, and the development of bacteriotherapies in USD.

Materials and Methods

Recruitment of Participants

- USD patients undergoing any procedure for treatment of Stone Disease
- Controls from Ophthalmology clinic, Clinical research unit or family of USD patients

Sample Collection and Processing

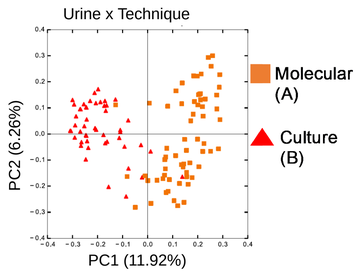
- Study subjects provided urine and stool sample. Stone sample collected during USD procedure.
- Stone and urine cultured on blood agar
- DNA extracted from stone, stone culture, urine, and urine culture

DNA Sequencing and Analysis

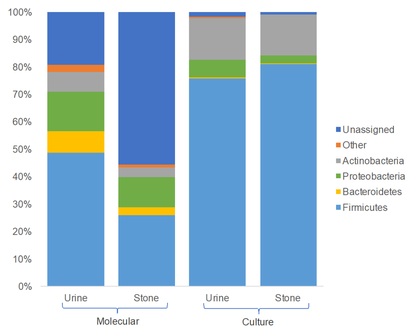
- Sequencing of V4 region of 16S rRNA performed at Argonne National Laboratory on Illumnia MiSeq
- OTUs assigned using a reference database
- DESeq2 algorithm for normalization
- B-diversity weighted and unweighted UniFrac distances calculated and analysis of similarity (ANOSIM) statistical analysis
- Differential abundance by Wald test
- P-values adjusted for false discoveries

Results

1. **Molecular vs. Culturing Techniques**

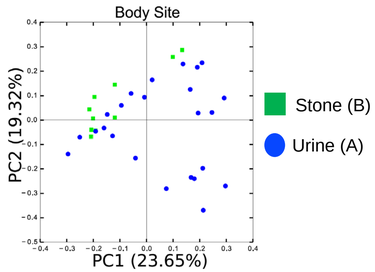


A) PCoA plot based on a weighted UniFrac analysis by technique.

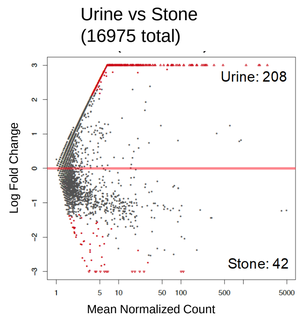


B) Phylum-level profile comparing molecular only vs. samples that were cultured prior to molecular analysis.

2. **Kidney Stone Microbiome vs. Urinary Microbiome (USD)**

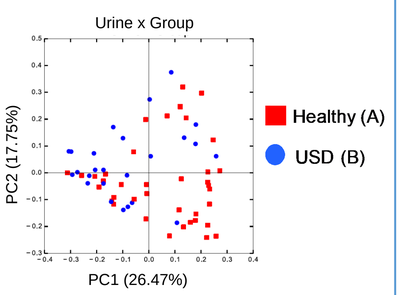


C) PCoA plot based on a weighted UniFrac analysis by body site.

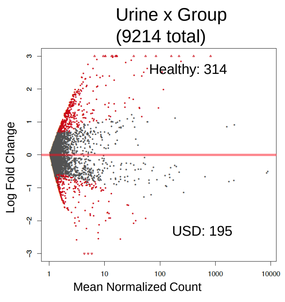


D) Differential abundance of OTUs by body site. Red dots: significantly different OTUs (FDR<0.05). Gray dots: non-significant OTUs.

3. **Urinary Microbiome USD Patients vs. Urinary Microbiome Healthy Controls**



E) PCoA plot based on a weighted UniFrac analysis by USD status



F) Differential abundance of OTUs by USD status. Red dots: significantly different OTUs (FDR<0.05). Gray dots: non-significant OTUs.

Conclusions

1. Urine and Stone microbiota are unique.
2. The urinary microbiome from USD patients is distinct from the urinary microbiome from healthy controls.
3. Conventional culture-based methods of bacterial analysis from urine and kidney stones produce bias on the taxa of bacteria detected.
4. Culturing prior to molecular analysis leads to greater taxonomic resolution but far fewer OTUs detected.
5. Culturing produces a heavy bias to the *Firmicutes* phylum.
6. These results strongly support moving towards a molecular means of bacterial analysis as opposed to culture-based analysis of urine and stones.

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