

**Title:** Redefining healthy urine: a cross-sectional exploratory metagenomic study of people with and without bladder dysfunction

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**Purpose:** To utilize the PathoScope platform to conduct species-level analyses of publicly available, 16S rRNA pyrosequenced asymptomatic urine data to determine relationships between microbiomes and clinical and functional phenotypes.

**Materials and Methods:** Reanalysis of previously reported cross-sectionally acquired urine samples from 47 asymptomatic subjects (23 controls and 24 subjects with neuropathic bladder (NB)). Urine was originally collected by the usual method of bladder drainage and analyzed with urinalysis, culture, and pyrosequencing. Urinalysis and culture values were stratified as follows: leukocyte esterase (LE) 0 or  $\geq 1$ , nitrite (+, -), pyuria  $< 5$  or  $\geq 5$  white blood cells/high power field (WBC/hpf), cloudy urine (+, -), and bacterial growth  $< 50,000$  or  $\geq 50,000$  colony forming units (cfu). PathoScope was used for next-generation sequencing alignment, bacterial classification, and characterization of microbial diversity.

**Results:** NB subjects were significantly more likely to have LE+, pyuria+, cloudy urine and bacterial growth. 23/47 samples had bacterial growth on culture while all samples had bacteria identified by pyrosequencing. The non-NB urine microbiomes had greater proportions of *Lactobacillus crispatus* (females) and *Staphylococcus haemolyticus* (males). The *Lactobacillus* community differed significantly amongst females depending on bladder function. Irrespective of gender, NB subjects had greater proportions of *Enterococcus faecalis*, *Proteus mirabilis*, and *Klebsiella pneumoniae*. In 4 NB subjects, *Actinobaculum* was detected by sequencing+PathoScope but not by cultivation, and in all cases was associated with pyuria.

**Conclusions:** Utilizing PathoScope plus 16S pyrosequencing, we were able to identify unique phenotype-dependent species-level microbes. Novel findings included an absence of *Lactobacillus crispatus* in female NB urine, and the presence of *Actinobaculum* in NB subjects only.