INTRODUCTION

Clinical urine specimens are usually considered to be sterile when they do not yield urapathogens using standard clinical cultivation procedures. Our aim was to test if the adult female bladder might support bacteria that are not identified by these routine procedures. An additional aim was to identify and recommend the appropriate urine collection method for the study of bacterial communities in the female bladder.

METHODS

Study design and patients: Following IRB approval for all phases of this project, participants gave verbal and written consent to collect and analyze their urine for research purposes. Eligibility required absence of known urinary tract infection (UTI) or current use of antibiotics or antifungals. Urine samples were assessed in all participants with a validated symptoms questionnaire, the Pelvic Floor Distress Inventory (PFDI). Urine was collected from 2 groups of women. The control group, composed of patients undergoing surgery for benign gynecologic conditions, reported no urinary symptoms. The treatment group, composed of patients undergoing surgery for treatment of common urogynecologic conditions including pelvic organ prolapse (POP) and/or urinary incontinence (UI) who reported at least one PFDI symptom. Metadata, including age, race/ethnicity and BMI were abstracted from the electronic medical record for descriptive purposes only.

Microbial DNA isolation: Within 4 hours of collection, the urine samples were centrifuged at 5000 g for 10 minutes and the resulting pellets were re-suspended in DNA stabilization buffer. Swabs were washed with sterile phosphate buffered saline supplemented with DNA stabilization buffer. All samples were frozen at -80°C until microbial DNA extraction and sequence analysis. Genomic DNA (gDNA) was isolated from the samples and reagent only control samples in a GX-3 Microplate Extractor. DNA was isolated from urine using the Qiagen DNeasy tissue extraction kit with the Gram-positive extraction buffer. Urine samples were centrifuged at 5000 g for 10 minutes and from the supernatant suspensions, Genomic DNA (gDNA) was isolated using the Qiagen DNeasy tissue extraction kit with the Gram-positive extraction buffer. DNA was isolated from urine using the QIAamp DNA Mini Kit.

Sequencing and analysis: Genomic DNA (gDNA) was isolated from the samples and reagent only control samples in a GX-3 Microplate Extractor. DNA was isolated from urine using the Qiagen DNeasy tissue extraction kit with the Gram-positive extraction buffer. DNA was isolated from urine using the QIAamp DNA Mini Kit. Microbial DNA was isolated from 2/23 SPA samples, indicating the bladders of some women may not contain bacteria.

RESULTS

Microscopy: Microscopic evaluation of voided urine samples (fig. 1) revealed the presence of bacterial colonies, insect debris, and superficial contamination. Microscopic examination of TUC samples (fig. 2) revealed bacterial colonies, insect debris, and superficial contamination. Microscopic examination of SPA samples (fig. 3) revealed bacterial colonies, insect debris, and superficial contamination.

Culture: Cultivation of voided urine samples (fig. 1) revealed bacterial colonies, insect debris, and superficial contamination. Cultivation of TUC samples (fig. 2) revealed bacterial colonies, insect debris, and superficial contamination. Cultivation of SPA samples (fig. 3) revealed bacterial colonies, insect debris, and superficial contamination.

Microbial DNA isolation: Genomic DNA (gDNA) was isolated from the samples and reagent only control samples in a GX-3 Microplate Extractor. DNA was isolated from urine using the Qiagen DNeasy tissue extraction kit with the Gram-positive extraction buffer. DNA was isolated from urine using the QIAamp DNA Mini Kit.

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C O N C L U S I O N S

While our data provide evidence for the presence of bacteria in the female bladder, we found that some of the bacteria detected in the samples do not appear to be culturable. Further research is needed to determine the significance of these findings.