ARTICLE

Clinical Research

Prospective examination of the changes in the urinary microbiome induced by transrectal biopsy of the prostate using 16S rRNA gene analysis

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Abstract

Objectives To prospectively examine the changes in microbiota within the urinary tract after transrectal prostate biopsy.
Materials and methods Data, urine, and fecal samples prospectively collected from 30 patients before and after transrectal biopsy of the prostate. DNA was extracted from urine collected after a prostate massage before and after prostate biopsy, and from fecal samples collected before the biopsy. We sequenced DNA using the bacterial 16S rRNA high-throughput next-generation sequencing and analyzed changes in microbial profiles for taxonomy comparison between samples.
Results Pre-biopsy urinary microbial profiles contained *Lactobacillus* and *Staphylococcus* bacteria. Post-biopsy urinary microbial profiles included lower levels of *Lactobacillus* and higher levels of *Prevotella* bacteria. *Bacteroides* bacteria were predominant in fecal samples. We identified two clustering patterns containing both pre- and post-biopsy urine samples. Cluster 1 had a urine cluster pattern that was distinct from fecal, whereas cluster 2 was similar to fecal. We observed two different modes of microbial changes, 11 patients had both of their urine (pre and post) samples associated with a particular cluster group, whereas others ($n = 15$) had movement between clusters 1 and 2 following the biopsy procedure. Four patient's post-biopsy urine microbial profiles clustered very tightly to the fecal microbial profile.
Conclusions We describe two models of change in the urinary tract microbiota after prostate biopsy using 16S RNA gene analysis. Further research to determine what controls changes in the urinary microbiota after prostate biopsy can help us understand why some patients are more susceptible to develop post-biopsy infections.

Introduction

Infections are a serious and common complication of prostate biopsy. The known infectious complication rates associated with prostate cancer diagnosis range from 0.1% to 7%, depending on the antimicrobial agent used [1]. Even with prophylactic antibiotics, 5% of men undergoing prostate biopsy will develop asymptomatic bacteriuria and 2–3% will develop symptomatic urinary tract infection [2]. Also, the overall risk of post prostate biopsy infections has risen over the past decade [2, 3]. The American Urological Association introduced the directive to compile a White Paper on incidence, prevention, and treatment of complications related to prostate needle biopsy. This paper brought to the community's attention the escalating danger of post prostate biopsy infections, and the need for more intensive research to understand the mechanism of these infections and help decrease their incidence in the urologic population.

Our understanding of the microbial ecology of the prostate continues to be limited. New evidence indicates that the human urinary tract contains microbial communities; however, the role of these communities in urinary health has not been elucidated. The recent detection of bacterial DNA [4, 5], and live bacteria in urine from patients with negative urine cultures based on standard urine culture protcols [6, 7] indicates that the urinary tract possesses its

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unique microbiota. Research into the roles of these bacterial communities in urinary health and disease requires sensitive and specific detection and classification. Despite the significant advances in clinical microbiology, many urinary bacteria cannot be cultured under in-vitro laboratory conditions. Thus, culture-independent methods for bacterial detection, such as high-throughput sequencing of the 16S ribosomal RNA (rRNA) gene, have emerged as the predominant research technique, especially as they become increasingly accessible due to declining sequencing cost and improved bioinformatics tools. 16S rRNA gene sequencing is commonly used to identify, classify, and provide the relative abundance of microbes within complex biological mixtures. The 16S rRNA gene is a highly conserved component of the transcriptional machinery of prokaryotic DNA and thus is highly suited as a target gene for sequencing DNA in samples containing up to thousands of different species. Universal PCR primers can be designed to target the conserved regions of 16S making it possible to amplify the gene in a wide range of various microorganisms from a single sample. Conveniently, the 16S rRNA gene consists of both conserved and variable regions. Although the conserved region makes universal amplification possible, sequencing the variable regions allows discrimination between specific different microorganisms [8].

In this study, we utilized 16S rRNA gene sequencing to characterize the microbial environment of the lower rectum and urogenital tract from rectal swabs and first voided urine, respectively, obtained after a prostate massage from men before and 2 weeks after prostate biopsy. Our goal was to identify the differences in the frequency and abundance of bacteria present in the urinary tract before and after a prostate biopsy that may have happened through bacterial introduction.

Methods

Patient recruitment and biopsy method

The Experimental group ($n = 40$) consists of patients scheduled to undergo magnetic resonance imaging (MRI)-targeted prostate biopsy during the management of suspected prostate cancer. Patients were used as their control because we obtained the specimens before and after the biopsy. Patients enrolled were white, non-Hispanic males, > 18 years of age, residing in small metro and rural communities, and were able to provide informed consent. We excluded patients who received antibiotics within 30 days before the biopsy and administered the pre-biopsy antibiotics after collecting the urine and fecal samples for this study. Prophylactic antibiotics consisted of intravenous cephalosporin. The MRI-targeted biopsy of the prostate

included 2–4 targeted biopsy of the index lesion in addition to mapping biopsies of the rest of the prostate. No alcohol or other antiseptics were used to clean the biopsy needle in between obtaining individual cores.

Sample collection and processing

Under institutional review board approved protocol, and after an informed consent, a rectal swab and first voided urine were collected after digital rectal examination and prostate massage immediately before and 2 weeks after transrectal ultrasound-guided prostate biopsy. We collected urine after the prostate massage to collect expressed prostate secretions and obtained a similar sample when patients presented for follow-up on pathology results within 2 weeks after the biopsy date. The rectal swab was collected to allow for comparing the results of examining the urine microbial species with the structure of the rectal bacterial communities.

Rectal swabs processing

We gathered rectal swabs by inserting a sterile Dacron swab into the rectum (to a depth of < 1 inch); the swab is then rotated for 360 degrees while scrapping the rectal wall for no more than 10 s to avoid discomfort to the patient. Once collected, the swab tip was snapped off into a plastic tube containing 1 mL of sterile saline. The tube was tightly capped and placed into refrigeration upon completion of the urological examination to store the specimen until transferred from the clinic to the research laboratory.

Urine samples processing

First voided urine samples after digital rectal examination were placed in a sterile 15 mL conical, tightly capped, and put into refrigeration after collection then transferred from the clinic to the research laboratory.

Data collection

We recorded patients and disease characteristics in a protected database. No identifiers remained on samples transferred to the laboratory or collaborators at the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana, IL.

Next-generation sequencing (NGS) of bacterial phylotypes

We defined bacterial phylotype profiles from urine and rectal samples. DNA extraction was performed on frozen urine and fecal samples using the MoBio Ultraclean Soil Kit
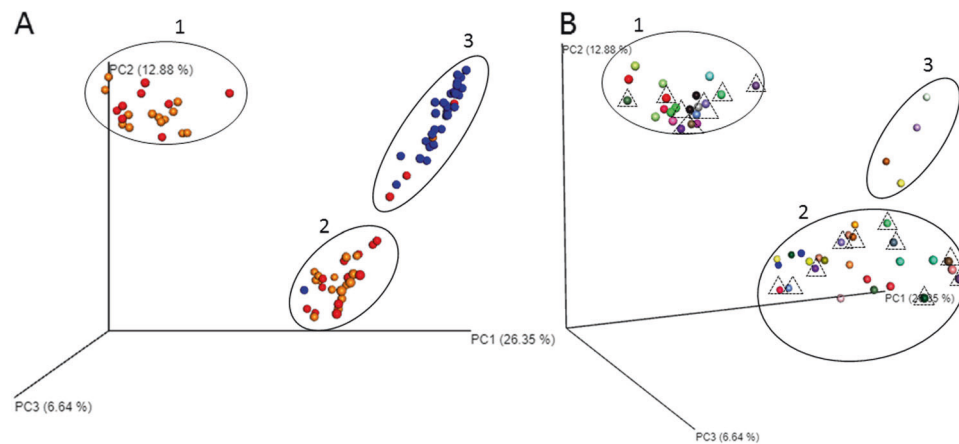
Fig. 1 Urine samples, pre (orange) and post (red), and fecal samples (blue) were collected from patients receiving prostate biopsies. Sample bacterial community profiling by principal component analysis (PCOA) was conducted to determine variables driving community similarities or differences. **a** PCOA clustering of samples by sample type and time of collection. **b** PCOA clustering of only urine samples (pre- and post) by patient ID. Dominant clustering groups are circled and numbered. Triangles in (**b**) indicate patients that shifted clustering groups from pre vs post collection

(Qiagen, Valencia, CA) using an MP FastPrep (MP Biomedicals, Solon, OH) for 40 s for rapid cell lysis. Extracted DNA was quantified using High Sensitivity Qubit (Thermofisher, Carlsbad, CA) and 15 ng of DNA was PCR amplified using specific barcoded primers around the V3–V5 16S rRNA, using protocols established by Jumpstart Consortium Human Microbiome Project Data Generation Working Group. Amplified samples were then sequenced using the 16S rRNA high-throughput next-generation Illumina MiSeq (250 PE, San Diego, CA) sequencing platform. A total of 52,100,366 sequence reads were generated and suitable for further analysis. Sequence reads were aligned using the Illinois-Mayo Taxon Operations for RNA Dataset Organization (IM_TORNADO), and paired-end reads were used for determination of operational taxonomical units (OTUs), as previously described [9]. OTUs were clustered at 97% sequence similarity and classified using NCBI and the RDP reference databases. The OTUs were then analyzed using QIIME software for taxonomy comparison between biological samples [10].

Statistical analyses

After sequence processing, rarefaction analysis was performed to ensure evenness among the sequences analyzed. Samples that did not fit our minimum rarefaction level of 10,000 sequences per sample were dropped from further study resulting in the removal of 10 patients from our analyses. At 10,000 sequences per sample, rarefaction curves plateaued demonstrating that sufficient sequencing was conducted to characterize bacterial communities within the samples (figure - supplement). Specific investigations on the remaining 30 patients included, alpha-diversity, beta-

diversity was accessed using both weighted and unweighted UNIFRAC values and taxonomical summary. We measured differences between sample alpha-diversity by Shannon index scores and by Mann–Whitney U-test, and we set a significance level of $p < 0.05$ as our threshold for significance. To analyze microbial community types within the urine samples collected pre- and post-biopsy and rectal swabs collected before the prostate biopsy, we performed nonmetric dimensional scaling (NMDS). Principal component analysis (PCoA) is a type of NMDS that groups samples based on their bacterial community similarities and separates them by their differences.

Results

Our goal was to identify alterations in the urinary bacterial species present before and after prostate biopsy and compare these with bacterial species that we found in rectal swabs within our study population. Forty patients were enrolled, but the complete set of samples was available for 30 patients in the final analysis. Patients were all white, 37.8% were smokers, 18.4% were diabetic, and 45% had a prostate cancer diagnosis after the biopsy. Only one patient developed a post-biopsy infection.

Alteration of microbial community dynamics induced by prostate biopsy

Our analysis of the urine samples from patients undergoing prostate biopsies indicated two distinctive clusters both containing urine samples collected pre-or post-biopsy evident by distribution of orange (pre) and red (post) sample
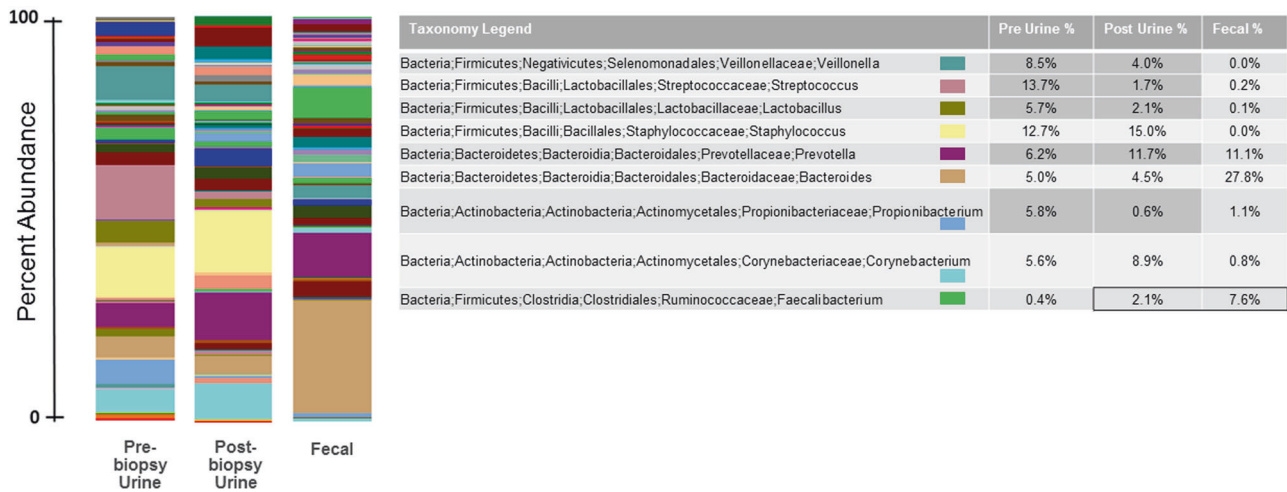
Taxonomy Legend		Pre Urine %	Post Urine %	Fecal %
Bacteria;Firmicutes;Negativicutes;Selenomonadales;Veillonellaceae;Veillonella		8.5%	4.0%	0.0%
Bacteria;Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcus		13.7%	1.7%	0.2%
Bacteria;Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus		5.7%	2.1%	0.1%
Bacteria;Firmicutes;Bacilli;Bacillales;Staphylococcaceae;Staphylococcus		12.7%	15.0%	0.0%
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;Prevotella		6.2%	11.7%	11.1%
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides		5.0%	4.5%	27.8%
Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Propionibacteriaceae;Propionibacterium		5.8%	0.6%	1.1%
Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Corynebacteriaceae;Corynebacterium		5.6%	8.9%	0.8%
Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Faecalibacterium		0.4%	2.1%	7.6%

Fig. 2 Level 6 taxonomic summary of relevant abundance of bacterial species within each sample type. Most abundant species are highlighted in the taxonomical legend and percent of abundance was reported for each sample type. Bordered box highlights bacterial species normally found only in fecal samples but elevated in post-urine samples

types in both cluster 1 and cluster 2 (Fig. 1a). Fecal samples primarily dominated the cluster group 3, although we did have some post-biopsy urine samples cluster with fecal samples. Cluster 1 had a pattern that was very distinct from the fecal samples, whereas cluster 2 was spatial closer to the fecal samples, indicating similarity of urine and fecal microbial profiles in a subset of patients. To determine if the time of urine sample collection (pre vs. post) influenced bacterial community types, we then classified community types within each patient and found three different patterns of urine sample clustering (Fig. 1b). About one-third of our patients ($n = 11$) had both of their urine samples associated with a particular cluster group (cluster $2 = 8$ patients, cluster $1 = 3$ patients), whereas other patients ($n = 15$) had unidirectional movement from cluster 1 to cluster 2 following the biopsy procedure, indicated by triangles. Additionally, we detected that some post-biopsy urine samples (cluster 3) did fall within the fecal cluster group, unlike pre-biopsy urine samples, indicating that some patients ($n = 4$) may have had an introduction of fecal bacterial species to the urogenital canal due to the biopsy procedure. The only patient who developed a post-biopsy infection fell within this group.

Bacterial taxonomical diversity

Our analysis showed that urine bacterial abundance profiles were altered between pre- and post-biopsy urine samples (Fig. 2). Examination of genera identified within each sample type recognized that the phylotypes that were different comparing pre- and post-biopsy urine samples were *Veilonella*, *Streptococcus*, *Lactobacillus*, *Prevotella*, and *Propionibacterium* (gray boxes). We also found an increase of *Faecalibacetium*, which was abundant in fecal samples,
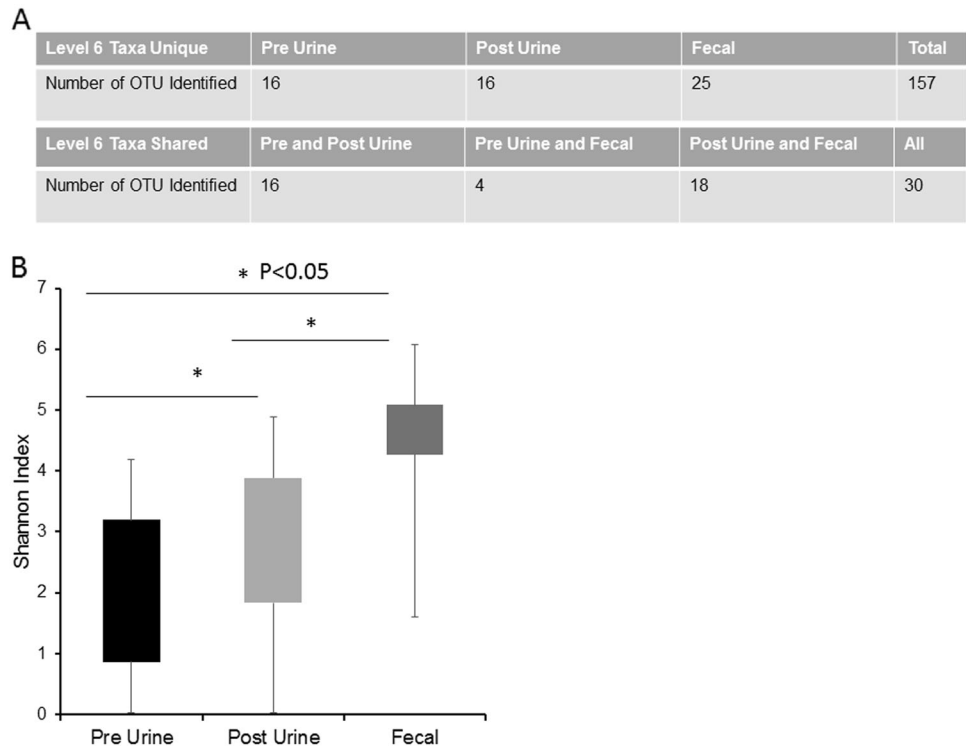
in post-biopsy urine samples (bordered box). Further analysis of bacterial diversity was performed to detect unique and shared bacterial species across sample type (Fig. 3). Out of the 157 total OTUs identified, we found 16 unique OTU among both pre- and post-biopsy urine samples whereas fecal samples had 25 different OTU. Comparison of shared OTU between samples recognized, 16 shared OTU among pre- and post-biopsy samples, 4 shared OTU among pre-biopsy urine and fecal samples, and 18 shared OTU among post-biopsy urine and fecal samples (Fig. 3a). Measuring the degree of diversity between sample types, we found that there was a significant difference in diversity indices among each sample type comparison (Fig. 3b). Together these data indicate that post-biopsy urine samples were more similar to fecal samples than pre-biopsy urine samples. This similarity between post-biopsy urine and fecal samples suggest that the puncture of the rectal wall during the prostate biopsy could lead to the introduction of the rectal bacterial species to the urinary tract (Fig. 3a; comparison of pre-urine/fecal with post-urine/fecal). The introduction of these bacterial species from the rectal wall could then increase the susceptibility of these patients to the development of post-biopsy urinary tract infections and sepsis, and necessitate tailored antibiotic treatment.

Discussion

We report here for the first time from a prospective study on the changes in genitourinary microbial environment induced by transrectal ultrasound-guided prostate biopsy using NGS of fecal and urinary samples. We found that there were two unique clustering patterns in the urine samples of our patient population before prostate biopsy. These clustering patterns

Fig. 3 Diversity analysis of operational taxonomical unit (OTU) identified in each sample type. **a** Identification of unique (top) and shared (bottom) OTU among sample types. **b** Shannon index analysis of sample diversity comparison among sample types. Significance is denoted by $*p < 0.05$

A

Level 6 Taxa Unique	Pre Urine	Post Urine	Fecal	Total
Number of OTU Identified	16	16	25	157

Level 6 Taxa Shared	Pre and Post Urine	Pre Urine and Fecal	Post Urine and Fecal	All
Number of OTU Identified	16	4	18	30

B

are altered after the biopsy due to a shift of the taxonomical abundance levels between pre- and post-biopsy urine samples, and this alteration may be due to the increased bacterial diversity in urine samples collected post-biopsy through the introduction of bacterial genera similar to those identified in fecal samples. Overall, these data support fecal bacterial introduction into the prostate during a prostate biopsy in many, but not all, patients and that we need further analysis to understand the factors that govern the introduction of microbes during urologic surgical procedures. Our results as well suggest a potential role for more advanced bacterial detection methods in developing a better understanding of the factors that determine changes in urinary microbial environment induced by invasive procedures.

Our results overlap with recent research using NGS though there were differences in the quantities of specific strains between the groups, which is what we expect since microbial community features are strongly related to the local environment and patients samples collected from different areas may have different microbiotas [11]. Additionally, age-related changes due to lifestyle, comorbidities, medications, and the reduction in immune function have been shown to change both gut and urinary microbiotas [9]. It is interesting to notice the change in urinary microbial features after the biopsy and compare the percentages of the predominant bacteria in the urine to fecal microbial elements. Specifically, *bacteroidales prevotella* bacteria, the second most dominant bacteria in the fecal microbial environment, doubled its predominance in urinary post-

biopsy samples, whereas *Bacteroidales bacteroides*, although paramount in the urinary microbial climate, maintained the same levels in post-biopsy urine when compared with pre-biopsy urinary specimens. Both *prevotella* and *bacteroides* bacteria have been shown before to be well represented in the fecal microbiota of healthy American individuals indicating the validity of our analysis [9]. Anaerobes have also been shown to increase in urinary samples after urologic procedures (endoscopic) but not after prostate biopsy. Mohanty evaluated 300 patients undergoing transurethral instrumentation (TUI) [12]. The authors cultured urine from patients for aerobic and anaerobic organism preoperatively and postoperatively. They then documented an increase in the incidence of anaerobic UTI from 2% preoperatively to 14% postoperatively following TUI. The authors concluded that TUI increases the frequency of anaerobic UTI. As far as prostate biopsy is concerned, Shivde et al. studied the prevalence of aerobic and anaerobic bacteria in patients undergoing transrectal ultrasound-guided biopsies of the prostate and detected post-procedure bacteriuria due to anaerobic bacteria in only 4.3% of the post-biopsy mid-stream urine samples [13]. Our study is the first to use high-throughput sequencing to characterize the changes in urinary microbial features after prostate biopsy and document increased levels of anaerobic bacteria. The superior sensitivity of our methods could explain the difference in our results when compared with those of Shivde et al. Environmental and patient-specific characteristics could also account for these differences.

It is important to remember that we performed our study on healthy individuals from the United States Midwest region who did not develop a post-biopsy infection. We are therefore characterizing the changes in the nondiseased microbial environment with this urologic procedure. The relative health of the study population decreases the impact of comorbid urinary conditions or blood profiles on the microbiota clusters. The importance of this work is that it is using a cutting-edge method to characterize the process of bacterial introduction, which is considered the primary mechanism behind post-transrectal ultrasound biopsy sepsis. So far, the evidence supporting such introduction has depended mainly on studies that showed an increased rate of bacteremia (16–75%) and bacteriuria (36–53%) immediately post-procedure in the absence of prophylactic antibiotics [14]. It is also important to realize that although *Escherichia coli* (*E. coli*) is the most common cause of post-biopsy infections that organism is very rare in the lower gastro-intestinal tract. In fact, the large intestine contains a luxuriant microflora typically with total concentrations of 10 bacteria/g [9] of stool with anaerobes such as *Bacteroides*, anaerobic streptococci, and clostridia outnumber facultative anaerobes such as *E. coli* by a factor of 1000 [15]. These facts explain why, in this study, *E. coli* was not detected in the fecal microbial environment and was not a member of the process of introduction that we have shed light on with this experiment. It is plausible that patients who develop post-biopsy infection are influenced by factors that select low levels of *E. coli* to move across the rectal wall similar to the mechanisms that increase the susceptibility of certain individuals to recurrent infections by *E. coli* that ascends through the urethra into the bladder aided by special surface proteins [16]. It is also possible that these individuals have an altered fecal microbiota to start with higher levels of *E. coli*. Therefore, modifications in the technique of prostate biopsy could decrease the risk of infection but may not abolish it entirely in patients who are predisposed to develop this complication after prostate biopsy.

Limitations of this study include the geographic location and homogeneity of study participants. However, previous studies did show a standard pattern of fecal microbiota shared among inhabitants of sizeable geographic area (countries) not living very close to each other [17]. The sample size for this study is also small, which may affect the generalizability of the results, but our sample size is acceptable given the nature of the homogeneous population of our patients for this exploratory research and comparable to the published research on the subject [11, 18]. In addition, a single dose of prophylactic antibiotics was given to all patients before performing the biopsy. Since even a single dose of antibiotics could alter the urinary microbiome for a duration of a few weeks, and since long-term follow-up data on the microbiome of the study cohort is not available to us, there is no way to rule out that the effect seen is a result of the antibiotic treatment alone. Therefore, we recommend that future study designs in the same field include serial sample collections to investigate whether the changes we saw in the microbiome are long lasting and thus more significant. Finally, the same population has been used for a different paper that utilizes similar analysis to look at the difference in the microbiome between patients who received the diagnosis of prostate cancer and those who did not. We made sure there is no overlap in the results presented in these two papers. Future research should aim at understanding the factors that control bacterial introduction with the aid of NGS in patients who developed infections after ultrasound-guided biopsy of the prostate to compare with the current results of healthy patients. Such research would also examine the durability of the change in the urinary microbial environment induced by prostate biopsy to identify an "at risk" period within which a repeat biopsy could increase the risk for biopsy-induced infections.

Conclusion

This study characterizes changes in the urinary microbiome after biopsy of the prostate. It also supports fecal bacterial introduction into the urinary tract during prostate biopsy in some patients.

Compliance with ethical standards

References

1. Liss MA, Chang A, Santos R, Nakama-Peeples A, Peterson EM, Osann K, et al. Prevalence and significance of fluoroquinolone resistant *Escherichia coli* in patients undergoing transrectal ultrasound guided prostate needle biopsy. J Urol. 2011;185:1283–8.

2. Zani EL, Clark OA, Rodrigues Netto N, Jr. Antibiotic prophylaxis for transrectal prostate biopsy. Cochrane Database Syst Rev. 2011;CD006576.

3. Nam RK, Saskin R, Lee Y, Liu Y, Law C, Klotz LH, et al. Increasing hospital admission rates for urological complications after transrectal ultrasound guided prostate biopsy. J Urol. 2010;183:963–8.

4. Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, Fitzgerald M, et al. Evidence of uncultivated bacteria in the adult female bladder. J Clin Microbiol. 2012;50:1376–83.

5. Brubaker L, Nager CW, Richter HE, Visco A, Nygaard I, Barber MD, et al. Urinary bacteria in adult women with urgency urinary incontinence. Int Urogynecol J. 2014;25:1179–84.

6. 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.

7. Khasriya R, Sathiananthamoorthy S, Ismail S, Kelsey M, Wilson M, Rohn JL, et al. Spectrum of bacterial colonization associated with urothelial cells from patients with chronic lower urinary tract symptoms. J Clin Microbiol. 2013;51:2054–62.

8. Riesenfeld CS, Schloss PD, Handelsman J. Metagenomics: genomic analysis of microbial communities. Annu Rev Genet. 2004;38:525–52.

9. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011;334:105–8.

10. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335–6.

11. Shrestha E, White JR, Yu SH, Kulac I, Ertunc O, De Marzo AM, et al. Profiling the urinary microbiome in men with positive versus negative biopsies for prostate cancer. J Urol. 2017;199:161–71.

12. Mohanty NK, Jolly BB. Incidence of anaerobic bacterial infection following transurethral instrumentation. Indian J Pathol Microbiol. 1996;39:33–6.

13. Shivde SR, Cooke RP, O'Neill WA, Cowie AG, Lawrence WT, Watson GM. Trimethoprim versus gentamicin for the prevention of bacteriuria following transrectal biopsy of the prostate–do patients need additional anaerobic cover? Urol Int. 2002;69:106–10.

14. Duplessis CA, Bavaro M, Simons MP, Marguet C, Santomauro M, Auge B, et al. Rectal cultures before transrectal ultrasound-guided prostate biopsy reduce post-prostatic biopsy infection rates. Urology. 2012;79:556–61.

15. Evaldson G, Heimdahl A, Kager L, Nord CE. The normal human anaerobic microflora. Scand J Infect Dis Suppl. 1982;35:9–15.

16. Lloyd AL, Smith SN, Eaton KA, Mobley HL. Uropathogenic *Escherichia coli* suppresses the host inflammatory response via pathogenicity island genes sisA and sisB. Infect Immun. 2009;77:5322–33.

17. Gorvitovskaia A, Holmes SP, Huse SM. Interpreting Prevotella and Bacteroides as biomarkers of diet and lifestyle. Microbiome. 2016;4:15.

18. Yu H, Meng H, Zhou F, Ni X, Shen S, Das UN. Urinary microbiota in patients with prostate cancer and benign prostatic hyperplasia. Arch Med Sci. 2015;11:385–94.