NEXT GENERATION SEQUENCING OF MICROBIOMES IN POST-DRE URINE SAMPLES OF PROSTATE CANCER PATIENTS

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Introduction : Pathogenic microorganisms could be responsible for asymptomatic and symptomatic inflammatory processes in the prostate including *Escherichia coli*, *Pseudomonas spp*., *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, etc (1). Modifications of bacterial populations were observed according to different inflammatory and tumor conditions in prostate cancer (PCa) samples, which may promote the development of cancer by enhancing extracellular environmental factors within the prostate (2-4). Most of these studies have used polymerase chain reaction (PCR) techniques to identify specific microorganisms. Next-generation sequencing (NGS) has much higher sensitivity than PCR to identify microorganisms in biological samples. We investigated microorganisms in post-digital rectal exam (DRE) urine samples obtained from PCa patients and a screening population with a PSA < 1.5 ng/mL using NGS and PCR techniques.

Methods: This study was conducted with institutional approved protocol #00-812. Patients received an extensive DRE with three finger strokes for each left and right lobes of the prostate. Initial post-DRE voided urine samples of 20-30 mL were collected in specimen bottles with a buffer solution and were stored at -80^{0}C. We identified post-DRE urine samples collected from 100 patients with PCa. As the control group without PCa, 100 samples were collected from annual Prostate Cancer Awareness Week participants with PSA<1.5 ng/mL. The 5-year risk of developing high-grade PCa in men with PSA<1.5 is very small (5). Microbial PCR and NGS was performed by MicroGen DX in Lubbock, TX. The real-time qPCR panel utilizes lab developed hydrolysis probes for the detection of common UTI microbes on the Roche LightCycler 480 II instrument. The concentration of those organisms can be precisely calculated from a standard curve and the qPCR results. Microbial DNA in each specimen was sequenced using the Illumina MiSeq to establish types of bacterial and fungal species present. Universal primers for 16S and ITS barcode loci were used for PCR amplification followed by NGS to characterize bacterial and fungal communities. NGS reads went through a custom bioinformatics pipeline with steps for quality control, denoising, and chimera detection. Species identifications were made by comparison to a curated clinical database. Species relative abundances per samples were visualized by stacked bar plots. Differential abundance of species between cancer and control groups was assessed using ANCOM - Analysis of Composition of Microbiomes (6).

Results: The mean age and PSA of cancer group were 60.1±7.79 (range: 41 - 82) yrs and 8.81±2.65 (range 1.49 - 41.82) ng/mL. For the control group 64.6±10.14 (range: 27 - 85) yrs and 0.67±0.35 (range: 0.04 – 1.42) ng/mL. NGS informative rates were 40% and 56% for PCa and control groups, respectively. In the cancer group, 5, 19, 9, and 7 had Grade Group (GG) 1, GG2, GG3, and GG5 PCa, respectively. Figure shows a stacked bar plot of relative abundances of the 19 most common bacterial species found in PCa and control samples. ANCOM analysis identified *Cutibacterium acnes* ($P < 0.05$) and *Finegoldia magna* ($P < 0.05$) as significantly more abundant in cancer samples as compared to controls especially for high grade cancers. In comparison, PCR informative rates were 3% and 8% for cancer and control groups, respectively. One patient with GG2 PCa had *Klebsiella pneumoniae*, a second patient with GG3 PCa had *Gardnerella vaginalis*, *Prevotella bivia*, and *Ureaplasma parvum*, and a third patient with GG5 PCa had *Gardnerella vaginalis* and *Ureaplasma parvum*. PCR detected *Escherichia*

coli, *Prevotella bivia*, *Mycoplasma hominis*, *Gardnerella vaginalis*, and *Ureaplasma parvum* in the control samples.

Conclusions: NGS has shown higher sensitivity for identifying microbes in post-DRE urine samples than PCR technique. Human intestinal bacteria including *Finegoldia magna* are responsible for the production of equol (7). Equol is a soy metabolite associated with lower risks of breast and prostate cancer in high-soy- consuming populations. *Cutibacterium acnes* strains, on the other hand, can be divided into the major types IA, IB, II, and III. *Cutibacterium acnes* subtype IA is predominantly associated with moderate to severe acne whereas *Cutibacterium acnes* type II is reported as the most prevalent type in prostate tissue samples from patients with PCa (8). The presence of these bacteria in men with PCa may be casual or causative. Further investigation is needed to elucidate mechanisms of cancer induction. Lack of NGS microbiome signal in some samples may be the result of antibiotics. We plan to carry out additional studies using expressed prostatic secretion (EPS) samples to further validate the above findings.

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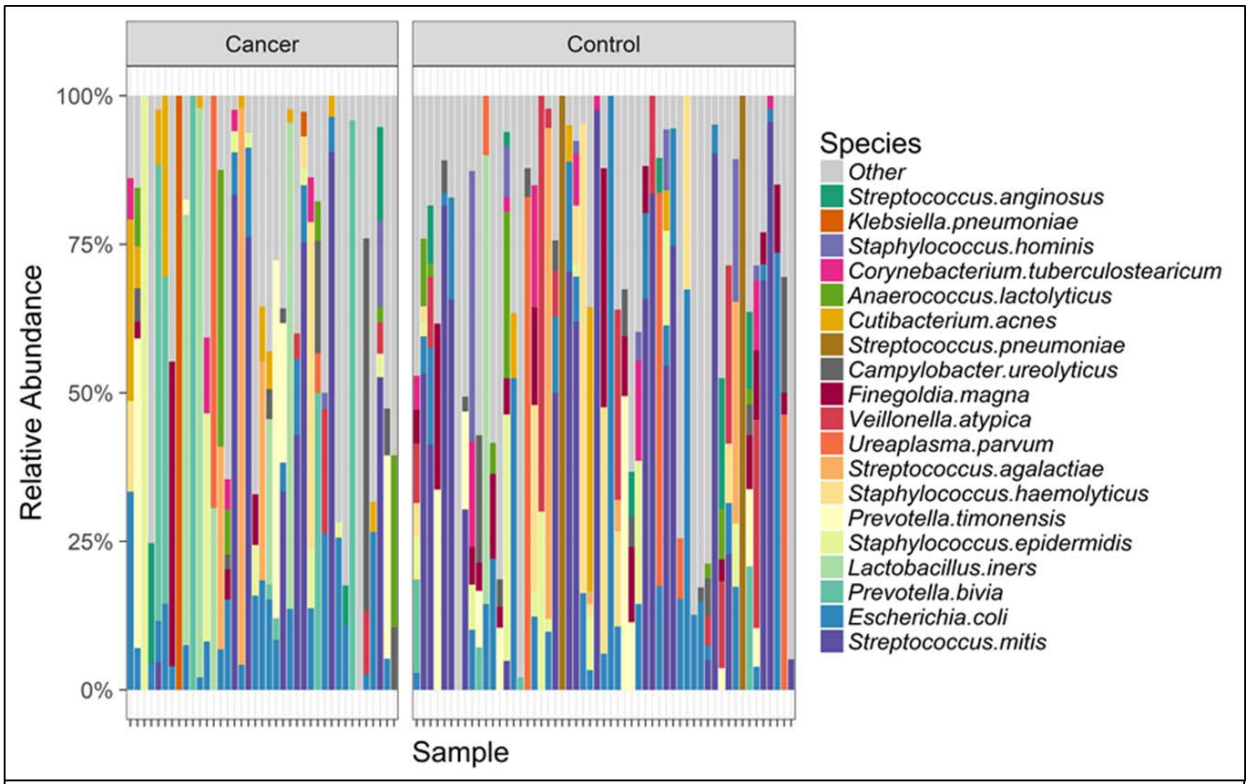

Figure: Stacked bar plot. Each sample is represented by a unique location on the x-axis. The y-axis sums to 100% and coloring is proportional to species abundance in each sample. Samples are faceted by group (i.e. Cancer/Control)