

Assessment of the Lower Urinary Tract Microbiota during Symptom Flare in Women with Urologic Chronic Pelvic Pain Syndrome: A MAPP Network Study

J. Curtis Nickel,* Alisa Stephens, J. Richard Landis, Chris Mullins, Adrie van Bokhoven, M. Scott Lucia and Garth D. Ehrlich, the MAPP Research Network†

From the Department of Urology, Queen's University, Kingston, Ontario, Canada (JCN), Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania (AS, JRL), and Departments of Microbiology & Immunology and Otolaryngology-Head and Neck Surgery, Drexel University College of Medicine (GDE), Philadelphia, Pennsylvania, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland (CM), and Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, Colorado (AvB, MSL)

Abbreviations and Acronyms

EP = Epidemiology and Phenotyping
GUPI = Genitourinary Pain Index
IC/BPS = interstitial cystitis/bladder pain syndrome
ICSI = Interstitial Cystitis Symptom Index
MAPP = Multidisciplinary Approach to the study of Pelvic Pain
SYMQ-1 = pain, pressure, discomfort scale
UCPPS = urological chronic pelvic pain syndrome
UTI = urinary tract infection
VB1 = voided bladder 1 or initial stream urine (urethral) specimen
VB2 = voided bladder 2 or midstream urine (bladder) specimen

Purpose: We compared culture independent assessment of microbiota of the lower urinary tract in standard culture negative female patients with urological chronic pelvic pain syndrome who reported symptom flare vs those who did not report a flare.

Materials and Methods: Initial stream (VB1) and midstream (VB2) urine specimens (233 patients with urological chronic pelvic pain syndrome) were analyzed with Ibis T-5000 Universal Biosensor system technology for comprehensive identification of microorganism species. Differences between flare and nonflare groups for presence or number of different species within a higher level group (richness) were examined by permutational multivariate analysis of variance and logistic regression.

Results: Overall 81 species (35 genera) were detected in VB1 and 73 (33) in VB2. Mean (SD) VB1 and VB2 species count per person was 2.6 (1.5) and 2.4 (1.5) for 86 flare cases and 2.8 (1.3) and 2.5 (1.5) for 127 nonflare cases, respectively. Overall the species composition did not significantly differ between flare and nonflare cases at any level ($p=0.14$ species, $p=0.95$ genus in VB1 and VB2, respectively) in multivariate analysis for richness. Univariate analysis, unadjusted as well as adjusted, confirmed a significantly greater prevalence of fungi (*Candida* and *Saccharomyces*) in the flare group (15.7%) compared to the nonflare group in VB2 (3.9%) ($p=0.01$). When adjusted for antibiotic use and menstrual phase, women who reported a flare remained more likely to have fungi present in VB2 specimens (OR 8.3, CI 1.7–39.4).

Accepted for publication September 19, 2015.

Supported by National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health MAPP Network Awards U01DK82370, U01DK82342, U01DK82315, U01DK82344, U01DK82325, U01DK82345, U01DK82333 and U01DK82316.

No direct or indirect commercial incentive associated with publishing this article.

The corresponding author certifies that, when applicable, a statement(s) has been included in the manuscript documenting institutional review board, ethics committee or ethical review board study approval; principles of Helsinki Declaration were followed in lieu of formal ethics committee approval; institutional animal care and use committee approval; all human subjects provided written informed consent with guarantees of confidentiality; IRB approved protocol number; animal approved project number.

* Correspondence: Department of Urology, Queen's University, Kingston, Ontario Canada K7L 2V7 (telephone: 613-548-2497; e-mail: jcn@queensu.ca).

† Described in supplementary Appendix 1 (<http://jurology.com/>).

For another article on a related topic see page 499.

Conclusions: Among women with urological chronic pelvic pain syndrome the prevalence of fungi (*Candida* and *Saccharomyces* sp.) was significantly greater in those who reported a flare compared to those who did not.

Key Words: microbiota; infection; cystitis, interstitial; symptom assessment

INTERSTITIAL cystitis/bladder pain syndrome is characterized by waxing and waning symptoms of bladder pain and storage urinary symptoms.¹ Symptom exacerbations among patients with IC/BPS, often called flares, well recognized by physicians and patients, have not been well characterized. Many patients and physicians attribute flares to an infection, and antibiotics are often prescribed.^{1,2} Following the culture status of women with IC/BPS for almost 2 years failed to provide evidence to support the bacterial etiology flare hypothesis.³ However, standard culture techniques have many limitations, including the fact that 99% of known bacteria cannot be cultivated using standard culture media techniques.⁴ Enhanced culture techniques are reportedly better (larger volumes, multiple media, multiple atmospheric conditions, longer incubation times) at capturing more bacterial and fungal species,^{5,6} while new culture independent methods (such as Ibis T-5000 Universal Biosensor technology⁷) allow for the detection of up to 1% to 3% of the total microbiome without needing an a priori hypothesis of which species are present.

In this study we use a novel, state-of-the-art, culture independent method to compare the microbiota of the lower urinary tract in standard culture negative (for bacteria) female patient with UCPPS (ie IC/BPS) enrolled in the MAPP-EP study^{8,9} who reported a current flare at study entry compared to those women who did not.

METHODS

The Trans-MAPP EP Study

The MAPP-EP study recruited UCPPS participants for baseline phenotyping and 12-month longitudinal followup of the treated history of UCPPS symptoms, with standardized data acquisition and analysis and biological sample collection across network sites.⁸ Inclusion and exclusion criteria have been described.⁹ This study evaluated female participants with a diagnosis of IC/BPS, with urological symptoms present a majority of the time during any 3 of the last 6 months. Further details of the study design are available, including descriptions of the study population enrollment criteria, and disease specific questionnaires are available^{8–10} or can be accessed online at <http://www.biomedcentral.com/content/pdf/1471-2490-14-58.pdf>.

Participants and Specimens

At the time of enrollment (baseline) each participant was asked the question, “Are you currently experiencing a

flare of your urologic or pelvic pain symptoms? By this we mean are you currently experiencing symptoms that are much worse than usual?” (possible responses yes or no). Participants who responded with yes were included in the flare group. Patients with a flare at baseline were compared to women who did not report a flare. Each participant was asked to provide an initial stream urine specimen (VB1, approximately 20 ml) and classic midstream clean catch specimen (VB2, approximately 20 ml) at the baseline clinic visit. Participants were asked to clean the genital area using saline wipes before urine collection. A second VB2 urine taken at the same in-clinic visit was cultured (standard culture technique) on-site to identify active infection. Participants with a positive urine culture (traditional uropathogen colony forming units 10⁵/ml or greater) were excluded from the EP study.

Specimen Handling

Urine specimens were collected using standardized collection kits and frozen as soon as possible (85% were frozen within 15 minutes, more than 95% within 30 minutes). After collection at MAPP Network Discovery sites,^{8,9} specimens were transferred from urine cups to 50 ml conical tubes and frozen at –80C until shipping to the central MAPP Network TATC (Tissue Analysis and Technology Core). Upon receipt at the TATC the specimens were thawed, thoroughly mixed and aliquoted into 1 and 3 ml aliquots. Specimens were frozen at –80C until used.

DNA Extraction and Ibis Eubacterial and Fungal Domain Ibis T-5000 Assays

We subjected VB1 and VB2 specimens to next generation molecular diagnostic Ibis T-5000 Universal Biosensor technology.⁷ Total DNA was extracted using 3 ml from VB1 and VB2 urine samples, and the bacterial and fungal DNA was amplified by polymerase chain reaction using the 16 primer pair BAC (Bacterial:Antibiotic resistance genes:*Candida*)/Fungal detection systems developed by Ibis.⁷ The individual amplicons were weighed using the Ibis instrumentation for electrospray ionization time-of-flight mass spectrometry, which reports molecular mass. The species identities of the amplicons were then revealed using a database containing base composition data on virtually all bacterial/fungal species sequenced to date. Details of the methodology, including limitations, are available (supplementary Appendix 2, <http://jurology.com/>).⁷

Statistical Analysis

Demographic characteristics and relevant clinical factors were compared between flare and nonflare cases by chi-square tests. Differences in the overall microbial composition for flare vs nonflare IC/BPS cases were assessed by permutational multivariate analysis (PERMANOVA).¹¹ This procedure is a nonparametric analogue of multivariate ANOVA that uses resampling for

Download English Version:

<https://daneshyari.com/en/article/3858322>

Download Persian Version:

<https://daneshyari.com/article/3858322>

[Daneshyari.com](https://daneshyari.com)