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ORIGINAL ARTICLE

Urinary bacteria in adult women with urgency urinary incontinence

Linda Brubaker · Charles W. Nager · Holly E. Richter · Anthony Visco · Ingrid Nygaard · Matthew D. Barber · Joseph Schaffer · Susan Meikle · Dennis Wallace · Noriko Shibata · Alan J. Wolfe

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Abstract
Introduction and hypothesis This study's aims were to detect and quantify bacterial DNA in the urine of randomized trial participants about to undergo treatment for urinary urgency incontinence (UUI) without clinical evidence of urinary tract infection (UTI) and to determine if the presence of bacterial DNA in baseline urine relates to either baseline urinary symptoms or UTI risk after urinary tract instrumentation.
Methods Women without clinical evidence of baseline UTI were randomized to cystoscopic onabotulinum toxin A injection and oral placebo medication versus cystoscopic placebo injection and active oral medication. Bacterial DNA in participants' catheterized urine was measured by quantitative polymerase chain reaction (qPCR).
Results Bacterial DNA was detected in the urine of 38.7 % of participants (60 out of 155). In these 60 qPCR-positive participants, baseline daily UUI episodes were greater than in the 95 qPCR-negative participants (5.71 [±2.60] vs 4.72 [±2.86], $p=0.004$). Neither symptom severity by questionnaire nor treatment outcome was associated with qPCR status or with qPCR level in qPCR-positive subjects. In contrast, the presence of urinary bacterial DNA was associated with UTI risk: only 10 % of the qPCR-positive women developed a UTI post-treatment, while 24 % of the qPCR-negative women did

The ABC trial is registered at www.clinicaltrials.gov as NCT01166438.

L. Brubaker (✉)
Departments of Obstetrics and Gynecology and Urology, Stritch School of Medicine, Loyola University Chicago, 2160 S. First Avenue, Boulevard 120, Room 420, Maywood, IL 60153, USA
e-mail: Lbrubaker@lumc.edu

C. W. Nager
Department of Reproductive Medicine, UC San Diego Health System, San Diego, San Diego, CA, USA

H. E. Richter
Department of Obstetrics and Gynecology, University of Alabama at Birmingham, Birmingham, AL, USA

A. Visco
Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, USA

I. Nygaard
Department of Obstetrics and Gynecology, University of Utah, Salt Lake City, UT, USA

M. D. Barber
Obstetrics, Gynecology and Women's Health Institute, Cleveland Clinic, Cleveland, OH, USA

J. Schaffer
Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, TX, USA

S. Meikle
Gynecologic Health and Disease Branch, The Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA

D. Wallace
Health Sciences Division, Research Triangle Institute, Research Triangle Park, NC, USA

N. Shibata · A. J. Wolfe
Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, Maywood, IL, USA

so. The median qPCR level for qPCR-positive samples did not differ significantly by UTI status (UTI 2.58×10^5 vs no UTI 1.35×10^5 copies/mL, $p=0.6$).
Conclusions These results may indicate a urinary bacterial contribution to both baseline UUI and the risk of post-treatment UTI.

Keywords Microbiome · Urinary bacteria · Urinary urgency incontinence · Urinary tract infection

Introduction

Urinary tract infections (UTI) are common in women with pelvic floor disorders, including women with urgency urinary incontinence (UUI) or those undergoing urinary tract instrumentation during stress incontinence surgery [1, 2]. Urinary catheterization, urodynamic testing and cystoscopy are often used for diagnostic and/or therapeutic purposes; however, these instrumentations may increase UTI risk despite proper techniques and antibiotic prophylaxis. UTI following urinary tract instrumentation affects a significant minority of women. Specific risk factors are not known, and the potential contribution of sub-clinical bacteriuria to the multifactorial etiology of UUI (or other lower urinary tract disorders) remains unknown.

Recent DNA and culture-based evidence suggests that even when urine cultures are negative, detectable bacterial communities (microbiota) exist in the urine of some adult women, regardless of their urinary symptoms [3–6]. In addition to broader implications of this discovery for a variety of lower urinary tract disorders, these findings may allow us to identify baseline factors that predispose or even protect women to subsequent UTI. The goals of this study were to detect and quantify bacterial DNA in the baseline urine of participants in a randomized clinical trial for the treatment of UUI without clinical evidence of UTI. In addition, we wished to determine if the presence of urinary bacteria related to either baseline urinary tract symptoms or the risk of subsequent UTI after urinary tract instrumentation.

Materials and methods

The Anticholinergic Versus Botulinum Toxin A Comparison (ABC) Trial is a registered, randomized, double blind, active-controlled clinical trial for the treatment of bothersome UUI conducted by the NICHD Pelvic Floor Disorders Network (pfdn.org). The full methods of the ABC trial and its primary results have been published elsewhere [7, 8]. Clinical sites are supported by a data coordinating center, an NIH Project Scientist, and an external Steering Committee Chair. The ABC trial was initiated following IRB approval at the data coordinating center and each clinical site and all participants completed an informed consent process. The study participants were women with moderate to severe UUI, defined as ≥5 urgency urinary incontinence episodes (UUIE) on a 3-day bladder diary. Subjects were randomized 1:1 using a dual placebo approach: they were randomized either to onabotulinum toxin A 100 units plus an oral placebo or to a 6-month anticholinergic regimen plus a placebo (saline) bladder injection. The primary outcome measure of the primary trial was the change from baseline in the mean number of UUI episodes/day from 3-day bladder diaries, measured monthly, over the 6-month, double-blind active treatment period (i.e., at months 1, 2, 3, 4, 5, and 6). All cystoscopic injections were done using standard sterile procedures and all women received prophylactic antibiotic therapy.

The subsequent analysis was restricted to participants with no clinical evidence of UTI who provided a baseline urine sample that was negative on urinalysis and who had sufficient outcome data. Subjects without clinical evidence of UTI provided a baseline catheterized urine sample following baseline assessment and prior to randomization to cystoscopic study injection. Baseline urine samples that were negative on dipstick urinalysis were frozen to –80 °C within 1 h of collection and shipped in batches on dry ice to the test site (Loyola University Chicago, Maywood, IL, USA). No clinical information was available to the basic science investigators prior to laboratory analysis of the urine specimens.

The urine samples were assessed for urinary bacterial DNA using quantitative polymerase chain reaction (qPCR). To avoid contamination, isolation of DNA from baseline urine specimens was performed in a laminar flow hood, according to strict standard operating procedures. For the same reason, all bar-coded containers containing urine were wiped with ethanol and thawed under direct UV light. To isolate DNA for molecular analysis, urines were centrifuged in nucleic acid-free tubes, the supernatant aspirated, the pellet suspended in lysis buffer, and the DNA isolated using a stringent protocol, according to the manufacturer's instructions (Qiamp DNA Micro kit, Qiagen). DNA was eluted with molecular grade water from purification columns into bar-coded collection tubes, quantified by UV spectroscopy, and stored at −80ºC.

All specimens were processed in batches, and each batch included:

1. Subject samples with universal 16S ribosomal DNA (rDNA) primers F785 and R926 and PCR reagents (GoTaq® qPCR Master Mix, Promega Cat # 6001)
2. A negative control to ensure sterility consisting of molecular grade water, PCR reagents, the universal 16S rDNA primers with no target DNA
3. A positive control consisting of molecular grade water spiked with *E. coli*, the 16S rDNA primers, and PCR reagents

Laboratory-introduced contamination was monitored in control samples and periodically in all DNA preparation materials using PCR with universal 16S rDNA primers. We defined "positive" qPCR as samples with DNA above the detection limit, which we determined to be 5,000 rRNA gene copies per milliliter.

Consistent with the ABC trial, in this analysis, we defined UTI dichotomously, either as $>10^5$ CFU/mL or as any treatment with antibiotics for a UTI (suspected or documented) at any point between randomization and 6 months.

Differences were examined descriptively at baseline and changes in clinical outcome measures for individuals defined as qPCR-positive and qPCR-negative with differences in binary measures examined via contingency tables with *p* values generated from Chi-squared tests and differences in median values for continuous measures evaluated using Wilcoxon rank sum tests. Because all analyses were considered descriptive, no adjustments were made for multiple comparisons and *p* values should be interpreted accordingly. Statistical analyses were conducted using SAS version 9.3.

Results

The ABC study had 249 participants, 155 (62 %) of whom contributed baseline urine samples for this analysis. The participants in this analysis were similar in demographic characteristics to participants in the overall ABC study (Table 1) and those who contributed baseline urine were similar to participants who did not. Consistent with study eligibility criteria, all participants were free of clinical UTI according to the inclusion criteria, including negative urine dipstick at baseline.

Table 1 Demographics of randomized and treated subjects

Characteristic		Included in microbiome analyses		*p value	All ABC (n=247[a])
		Yes (n=155)	No (n=92)		
Age at randomization (years)	Mean (SD)	57.7 (11.3)	58.4 (11.2)	0.63	57.9 (11.3)
Hispanic or Latina: *n* (%)		33 (21)	11 (12)	0.06	44 (18)
Race: *n* (%)	White	113 (73)	81 (88)	0.01	194 (79)
	Black/African American	30 (19)	11 (12)		41 (17)
	Other	12 (8)	0		12 (5
BMI	Mean (SD)	32.9 (7.7)	31.8 (7.5)	0.29	32.5 (7.6)
Marital status: *n* (%)	Married/living as married	69 (45)	46 (50)	0.79	115 (47)
	Divorced/separated/widowed	59 (38)	33 (36)		92 (37)
	Other	1 (1)	0		1 (0
	Single, never married	20 (13)	11 (12)		31 (13)
Some college or greater: *n* (%)		112 (72)	64 (70)	0.44	176 (71)
Health insurance: *n* (%)	Private only	75 (48)	46 (50)	0.49	121 (49)
	Medicare or Medicaid only	13 (8)	13 (14)		26 (11)
	Other only	40 (26)	22 (24)		62 (25)
	Combination of types	26 (17)	11 (12)		37 (15)
	Not reported	1 (1)	0		1 (0)
Smoking: *n* (%)	Never	84 (54)	56 (61)	0.03	140 (57)
	Previous	58 (37)	21 (23)		79 (32)
	Current	13 (8)	14 (15)		27 (11)
Menopause: *n* (%)	Pre-menopausal	23 (15)	14 (15)	0.85	37 (15)
	Post-menopausal	123 (79)	71 (77)		194 (79)
	Not sure	9 (6)	7 (8)		16 (6)
No prior anticholinergic exposure: *n* (%)		68 (44)	34 (37)	0.29	102 (41)
3-day diary total IE/day	Mean (SD)	6.1 (3.3)	5.7 (3.0)	0.38	6.0 (3.2)
3-day diary urge IE/day	Mean (SD)	5.1 (2.8)	4.8 (2.5)	0.44	5.0 (2.7)
Baseline PVR (mL)	Mean (SD)	34.3 (46.7)	38.8 (44.8)	0.47	36.0 (46.0)

IE incontinent episodes, *PVR* postvoid residual urine, *ABC* Anticholinergic Versus Botulinum Toxin A Comparison Trial

*p value based on the linear regression model with the treatment group as the explanatory value for continuous variables and Chi-squared tests for categorical measures

[a] Of the 249 randomized individuals, 2 withdrew prior to treatment and did not provide baseline data

Table 2 Baseline and change in baseline measures

Clinical measure	Negative qPCR, mean (±SD)	Positive qPCR, mean (±SD)	*p* value* for mean difference
Baseline UUIE per day	4.72 (±2.86)	5.71 (±2.60)	0.0045
Baseline OABq SF symptom severity	70.86 (±18.03)	67.33 (±19.38)	0.34
Change in UUIE per day[a]	−3.79 (±2.81)	−4.35 (±2.80)	0.12
Change in OABq SF symptom severity	−45.39 (±25.12)	−44.69 (±22.43)	0.88

UUIE urgency urinary incontinence episodes, *OABq SF* overactive Bladder Questionnaire Short Form, *qPCR* quantitative polymerase chain reaction

*p value based on the Wilcoxon rank-sum test

[a] Measured as mean change from baseline over months 1 through 6

We detected qPCR evidence of urinary bacterial DNA in 38.7 % of the participants (60 out of 155). These qPCR-positive participants had a higher mean number of UUIE per day (5.71±2.60) at baseline than qPCR-negative participants (4.72±2.86; p=0.004), and there were no significant differences in baseline symptom severity as measured by (OABq SF; Table 2). After treatment, qPCR-positive participants had similar reductions in UUIE per day and severity symptoms as qPCR-negative participants. Neither subjective symptom severity nor treatment outcomes were associated with either the proportion of qPCR-positive subjects or the qPCR level in qPCR-positive subjects.

The presence of urinary bacterial DNA was negatively associated with the risk of developing a UTI after treatment (p=0.03; Table 3). Of the 60 women who were qPCR-positive, 6 (10 %) developed a UTI after treatment, compared with 23 out of 95 (24 %) of the women who were qPCR-negative. The median qPCR level for samples above the detection limit did not differ significantly in the women who developed a UTI compared with those who did not (2.58×10^5 vs 1.35×10^5, p=0.6).

Discussion

Studies based on DNA and culture have provided evidence of live bacteria in urine samples that are deemed "culture-negative" by standard clinical microbiological procedures [3, 5]. We have now detected DNA evidence of microbiota in the urine of women seeking treatment for UUI without clinical symptoms of a UTI and with a negative urine dipstick. The existence of bacteria in the female lower urinary tract appears to have potential clinical significance, as a positive qPCR result was related both to an increase in the number of UUIE prior to treatment and to a decreased risk of UTI following cystoscopic bladder injection. Although our study does not clarify the biologically "preferred" state regarding bacteria in the urine, it suggests that "protective" or "vulnerable" bacterial communities may exist in the urinary tract, similar to findings in other parts of the human body.

Table 3 Risk of UTI after treatment, by qPCR status

	Negative qPCR (n=95)	Positive qPCR (n=60)
No UTI (n=126)	72 (57.1 %)	54 (42.9 %)
UTI (n=29)	23 (79.3 %)	6 (20.7 %)
	*p=0.027	

*p value based on Chi-squared tests

In women affected by UUI, the diagnosis of UTI can be challenging, given that there is an overlap in the symptoms (urgency and frequency) of both conditions. UTIs may exacerbate current overactive bladder symptoms, including urgency, frequency, UUI, and nocturia, or cause de novo symptoms that may have an impact on both clinical care and outcomes measurements for research purposes. The gold standard diagnostic approach has been a clinical urine culture to detect uropathogens [9]. Clinical urine cultures have been considered positive when the colony count of a recognized uropathogen, such as *Escherichia coli*, *Pseudomonas*, *Klebsiella* or Group B *Streptococcus*, reaches a pre-defined threshold, typically 10^2–10^5 CFU/mL [10]. The clinical urine culture at the time of instrumentation has not been used to predict the risk of UTI following lower urinary tract instrumentation. This may be because of reliance on less costly methods, such as urinalysis or dipstick testing, or the cost and familiarity with standard culture-dependent clinical microbiological procedures. Standard clinical urine cultures are designed to favor the detection of fast growing aerobic bacteria, consistently undercount slow-growing bacteria, and can detect neither anaerobic bacteria nor those whose preferred growth conditions remain unknown [11]. This limitation is reinforced by repeated discoveries of previously "uncultivated" bacteria as emerging agents of urinary tract disease [11, 12]. Because the symptoms of overt UTIs overlap significantly with UUI, a culture-independent technique may augment the current clinical practice of using urine culture to rule out infection before diagnosing UUI.

Significant scientific advances in our understanding of the bacterial diversity of the human body have resulted from the Human Microbiome Project [13, 14]. This project uses multiple massive parallel DNA-sequencing technologies (sometimes called "next-generation" sequencing). These technologies have revolutionized biological research, largely because they permit sequencing of multiple samples in a single run (termed multiplexing) [15–19]. These culture-independent techniques hold great promise in advancing our ability to detect and study urinary bacteria. Such techniques have already shown that urine from asymptomatic women is often not "sterile" [3, 20] and that voided urine of adult males contains diverse bacteria. In both cases, many of the bacteria identified cannot be or are not routinely cultivated by clinical microbiology laboratories [20].

This initial analysis is limited to a dichotomous identification of the presence of bacterial DNA found in catheterized urine. Therefore, without sequencing the organisms contributing to the bacterial DNA detected, we are unable to comment on the specific microbes present in the urinary bacterial community. While not every urine specimen was subjected to conventional clinical culture techniques, emerging evidence suggests that these techniques may be inadequate without specialized culture techniques [6]. Given the relatively low bacterial loads in the urine, the use of PCR may underestimate the presence of bacterial DNA. Finally, our study cannot comment on the stability/variability of the microbiome over time or whether the DNA detected is connected to microbes associated with tissue or free in the urine itself.

Clearly, the next step is to incorporate sequencing of bacterial DNA to further advance our understanding of the bacterial communities that appear to exist in some adult women affected by UUI, and perhaps other lower urinary tract disorders. These findings, coupled with emerging evidence regarding the urinary bacterial community, are likely to influence the diagnosis and treatment of a variety of lower urinary tract disorders, including urinary tract infection and potentially, urgency urinary incontinence.

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