THE JOURNAL of UROLOGY®
www.auajournals.org/journal/juro

Infection/Inflammation

JU Insight

A Randomized Clinical Trial of Standard versus Expanded Cultures to Diagnose Urinary Tract Infections in Women

Hayley C. Barnes, B. Wolff, O. Abdul-Rahim et al.

Correspondence: Elizabeth R. Mueller (email: emuelle@lumc.edu) and Alan J. Wolfe (email: awolfe@luc.edu).

Full-length article available at https://doi.org/10.1097/JU.0000000000001949.

Study Need and Importance: Standard urine cultures (SUC) are commonly used to diagnose and guide treatment of urinary tract infections (UTI). However, SUC may miss certain uropathogens that can contribute to UTI symptoms. The expanded quantitative urine culture (EQUC) was developed to detect additional microbes that may not be cultivatable under SUC conditions. This study examines the clinical outcomes of women symptomatic for UTI who were treated based on results from SUC or EQUC, as randomized.

What We Found: For the overall cohort, UTI symptom resolution did not significantly differ between those treated with the 2 different culture methods. Whereas two-thirds improved regardless of culture method, approximately a third remained symptomatic for UTI despite culture-driven treatment. Research lab EQUC results revealed 3 major urotypes: *Escherichia coli*-uropathogen predominant, non-*E. coli*-uropathogen predominant and non-uropathogen predominant/culture negative. When we compared these urotypes for symptom profile and resolution, we found that the subset of women with the non-*E.coli*-uropathogen urotype trended towards better symptom resolution when treated based on EQUC results.

Limitations: The use of catheterized urine in this study may limit its generalizability to clinical care provided from voided urine. EQUC results require an additional 24 hours of reporting time.

Interpretation for Patient Care: For women with self-reported UTI symptoms, treatment based on SUC or EQUC results appears to have similar clinical outcomes. The preponderance of *E. coli*-predominant UTIs appears to mask a subset of women who would likely benefit from the use of EQUC for diagnosis. Thus, further study is warranted into the utility of EQUC in diagnosing this subset of women who have non-*E.coli* uropathogen predominant UTIs.

0022-5347/21/2065-1212/0
THE JOURNAL OF UROLOGY®

https://doi.org/10.1097/JU.0000000000001949
Vol. 206, 1212-1221, November 2021
Printed in U.S.A.

THE JOURNAL of UROLOGY®
www.auajournals.org/journal/juro

A Randomized Clinical Trial of Standard versus Expanded Cultures to Diagnose Urinary Tract Infections in Women

Hayley C. Barnes,[1] B. Wolff,[1,*] O. Abdul-Rahim,[2] A. Harrington,[3] E. E. Hilt,[2,†] T. K. Price,[2,†] T. Halverson,[2] B. R. Hochstedler,[2] T. Pham,[1] M. Acevedo-Alvarez,[1] C. Joyce,[4] C. M. Fitzgerald,[1] P. C. Schreckenberger,[3,‡] L. Brubaker,[5] Alan J. Wolfe[2] and Elizabeth R. Mueller[1,§]

[1]*Division of Female Pelvic Medicine and Reconstructive Surgery, Departments of Urology & Obstetrics/Gynecology, Loyola University Medical Center, Maywood, Illinois*
[2]*Department of Microbiology and Immunology, Loyola University Chicago, Maywood, Illinois*
[3]*Department of Pathology, Loyola University Chicago, Maywood, Illinois*
[4]*Department of Public Health Sciences, Loyola University Chicago, Maywood, Illinois*
[5]*Department of Obstetrics, Gynecology and Reproductive Sciences, University of California San Diego, La Jolla, California*

Purpose: We compared urinary tract infection (UTI) symptom resolution rates at 7–10 days in symptomatic women randomized to treatment based on standard urine culture (SUC) versus expanded quantitative urine culture (EQUC) results.

Materials and Methods: Women ≥18 years old who responded "yes" to "do you feel you have a UTI?" agreed to urethral catheterization and followup. Symptoms were assessed using the validated UTI Symptom Assessment (UTISA) questionnaire. Culture method was randomized 2:1 (SUC:EQUC); antibiotics were prescribed to women with positive cultures. The primary outcome, UTI symptom resolution, was determined 7–10 days following enrollment on all participants regardless of treatment.

Results: Demographic data were similar between groups. Of the SUC and EQUC groups 63% and 74% had positive cultures (p=0.10), respectively. Of participants with positive cultures 97% received antibiotics. Primary outcome data were provided by 215 of 225 participants (SUC 143 [95%], EQUC 72 [97%]). At the primary outcome assessment, 64% and 69% in the SUC and EQUC groups, respectively, reported UTI symptom resolution (p=0.46); UTISA scores improved from baseline in the EQUC arm compared to the SUC arm (p=0.04). In the subset of women predominated by non-*Escherichia coli* (76), there was a trend toward more symptom resolution in the EQUC arm (21%, p=0.08).

Abbreviations and Acronyms

CFU = colony forming units
EQUC = expanded quantitative urine culture
PGI-I = Patient Global Impression of Improvement
SUC = standard urine culture
UTI = urinary tract infection
UTISA = Urinary Tract Infection Symptom Assessment

Accepted for publication June 2, 2021.
Trial Registration: NCT03190421
Funding: Portions of this work were supported by an NIH award to Drs. Wolfe and Brubaker (R01 DK104718). For this study, no funding, supplies, or services were received from any commercial organization. Sponsors were not involved in the study design, collection, analysis, interpretation of the data, in writing the manuscript, or the decision to submit the manuscript for publication.
Disclosures: Mueller: UpToDate honoraria, Ethicon/Butler-Snow legal defense, research funding from the NIH. Wolfe: Membership on the Urobiome Therapeutics advisory board, membership on the Pathnostics Scientific Advisory Board, research funding from the NIH, the DOD, and Kimberly Clark Corporation. Brubaker: Editorial stipends from Female Pelvic Medicine and Reconstructive Surgery, UpToDate and JAMA, research funding from the NIH. Joyce: Research funding from the NIH, American Cancer Society, and the Hines VA Hospital. Fitzgerald: UptoDate Honoraria, Research funding from the National Institutes of Health. The remaining authors (Barnes, Abdul-Rahim, Harrington, Wolff, Hilt, Halverson, Pham, Acevedo Alvarez, Hochstedler) report no disclosures.

* Current address: Swedish Hospital—North Shore University, Chicago, Illinois.
† Current address: Department of Pathology and Laboratory Medicine, University of California Los Angeles, Los Angeles, California.
‡ Deceased.
§ Correspondence: Female Pelvic Medicine and Reconstructive Surgery, Departments of Urology & Obstetrics/Gynecology, Loyola University Medical Center, Loyola University Chicago, 2160 S. First Avenue, Maywood, Illinois 60153 (telephone: 708-216-0342; FAX: 708-216-2171; email: emuelle@lumc.edu. Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, 2160 S. First Avenue, Maywood, Illinois 60153 telephone: 708-216-2180; (email: awolfe@luc.edu).

Editor's Note: This article is the fourth of 5 published in this issue for which category 1 CME credits can be earned. Instructions for obtaining credits are given with the questions on pages 1341 and 1342.

0022-5347/21/2065-1213/0
THE JOURNAL OF UROLOGY®

https://doi.org/10.1097/JU.0000000000001949
Vol. 206, 1212-1221, November 2021
Printed in U.S.A.

Conclusions: Symptom resolution was similar for the overall population (*E. coli* and non-*E. coli*) of women treated for UTI symptoms based on SUC or EQUC. Although the sample size limits conclusions regarding the utility of EQUC in women with non-*E. coli* uropathogens, the detected trend indicates that this understudied clinical subset warrants further study.

Key Words: urinary tract infections, enhanced urine cultures, anti-bacterial agents, microbiota

THE simplistic definition of urinary tract infection (UTI) is evolving to incorporate improved microbial detection methods, increase diagnostic precision, and improved antibiotic stewardship.[1] Clinicians are aware of the limitations of empiric antibiotic prescription, especially when "UTI symptoms" overlap with chronic conditions such as overactive bladder or chronic pain in the pelvis or bladder. When UTI testing includes urine culture, the standard urine culture (SUC) method is used almost exclusively. Developed in the 1950s to non-invasively diagnose kidney infections (pyelonephritis),[2] SUC was specifically refined to detect *Escherichia coli* and other fast growing uropathogens with limited nutritional and atmospheric requirements. This initial focused use of SUC was expanded for diagnosis of bladder infections (cystitis) without strong scientific evidence of specificity and sensitivity. Recently, research teams have used next generation sequencing and enhanced urine culture methods to provide compelling evidence that urine (obtained by suprapubic aspiration, transurethral catheterization or voiding) often contains microbes (including uropathogens) that SUC either detects sporadically or not at all.[3]

One enhanced urine culture method called expanded quantitative urine culture (EQUC), designed to grow urinary microbes detected by sequencing, detects microbes in ~90% of urines deemed "no growth" by SUC.[4] EQUC detects microbes that SUC routinely misses, typically non-*E. coli* uropathogens and "normal" flora,[4,5] and reproducibly detects additional microbes that may contribute to UTI symptoms. Other enhanced methods obtain similar results.[6,7] EQUC is more labor and time-intensive than SUC because EQUC requires plating onto multiple media, and specimens are incubated for twice as long under several atmospheric conditions.[4] Although EQUC can be easily incorporated into any clinical microbiology laboratory, the clinical role of EQUC has yet to be determined.

This registered randomized controlled trial (NCT03190421) compares clinical outcomes of symptomatic women (those self-reporting a UTI) whose treatment was based on results from EQUC versus SUC.

MATERIALS AND METHODS

Study Design and Patient Population

Following institutional review board approval (IRB No. 209545), adult women ≥18 years old who presented to our tertiary care urogynecology specialty clinic (June 17, 2017–March 20, 2020) and reported symptoms of a UTI were screened for eligibility and were asked "do you feel you have a UTI?" Women who responded "yes" and agreed to phone or email contact 7–10 days after enrollment were invited to participate. We excluded women who were on antibiotics, unable to communicate or read in English, under age 18, pregnant, had an indwelling urinary catheter, were treated empirically on enrollment day, did not have sufficient collected urine volume for analysis, were performing intermittent self-catheterization, or declined to be catheterized. Following a full study explanation, potential participants provided verbal and written research consent, including permission to abstract clinical information from their electronic medical record.

Enrollment Visit

Following research consent, participants were characterized using demographic data collected by self-reported questionnaires. Pelvic floor symptoms were assessed by the Pelvic Floor Distress Inventory; UTI symptoms were characterized using the validated UTI Symptom Assessment (UTISA) questionnaire.[8,9] The UTISA questionnaire assesses severity and level of bother of 7 well-established UTI symptoms. Scores for each symptom range from 0 to 3; a 0 indicates symptom absence whereas a 3 corresponds to highest severity or bother. Using standard aseptic technique, a transurethral catheter-collected urine specimen was obtained.

Randomization

Allocation, using previously generated block randomization, was concealed in sequentially numbered, sealed, opaque envelopes. There was no stratification based on *E. coli* vs non-*E. coli* uropathogens). Following collection of the research urine specimen, participants were allocated (2:1) to SUC or EQUC, respectively; participants were masked to allocation. Urine samples were divided into 2 labeled containers. The clinical sample (1 mL) was sent to the clinical microbiology laboratory for either SUC or EQUC, as randomized; clinicians used these culture reports for treatment planning. A separate portion of urine was sent to the microbiology research laboratory, which did not provide report or results for clinicians.

Results Call

Culture results were placed into the participant's medical record by the clinical laboratory team and reviewed by the Female Pelvic Medicine and Reconstructive Surgery clinical team. SUC results were available within 48 hours, whereas EQUC results were reported within 72 hours. Participants were notified of their study urine culture results by phone without disclosing randomization assignment. Participants with positive cultures were treated based on antibiotic sensitivities when available.

Positive SUC cultures were treated with our current clinical algorithm (supplementary fig. 1, https://www.jurology.com). Prior to study commencement, clinical microbiologists reported uncertainty as to whether antibiotic sensitivities could be obtained on certain EQUC uropathogens. An EQUC treatment algorithm for these less common uropathogens (supplementary fig. 2, https://www.jurology.com) was developed by 2 authors using historical literature on microbe sensitivities and applied only to uropathogens identified in previous EQUC studies, including *Streptococcus, Corynebacterium, Aerococcus* and *Alloscardovia* species.[5] This algorithm was developed to guide treatment if antibiotic sensitivity was not available.

Primary Outcome Assessment

Participants were queried about symptoms 7−10 days after the enrollment visit via an online questionnaire, regardless of whether they had received antibiotic treatment based on a positive culture, had a negative culture (no antibiotic treatment), or grew commensal flora (no antibiotic treatment). Participants were emailed a link to complete the followup symptoms assessment; they were asked "do you continue to have UTI symptoms" and were prompted to complete the UTISA questionnaire for symptom assessment, the validated Patient Global Impression of Improvement scale (PGI-I),[10] and report how many days of antibiotics (if any) they utilized. Participants who answered "no" to "do you continue to have UTI symptoms" were categorized as treatment success while those who responded "yes" were categorized as treatment failure. If participants did not complete the online survey within 72 hours or did not have access to email, an unblinded investigator administered the symptoms assessment by phone using a standardized script that included the questionnaire linked in the email. No additional medical advice was provided by phone and study randomization was not disclosed to participants. After 3 unsuccessful attempts to reach participants by phone, nonrespondents were categorized as lost to followup and excluded from further analysis.

Clinical Microbiology Methods

All urine samples were processed on collection day in the clinical microbiology lab, according to randomization assignment (SUC or EQUC). SUC involved inoculation of 0.001 mL of urine onto a 5% sheep blood agar plate and a MacConkey agar plate, then incubated aerobically at 37C for 24 hours. The detection threshold for SUC is 10^3 CFU/mL or 1 colony of growth on either plate; this was a positive result. EQUC involved inoculation of 100× (0.1 mL) more urine onto 3 types of media (blood agar plate and MacConkey agar plate, also colistin and nalidixic acid agar plates) with incubation in 5% CO_2 at 37C for 48 hours. The detection threshold for EQUC is 10 CFU/mL or 1 colony on any plate; this was a positive result.[5]

Identification of Bacterial Isolates

After plating and initial incubation, each morphologically distinct colony type from either procedure was counted and identified via MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of Flight) mass spectroscopy (Bruker MALDI Biotyper).[5] Entry into the clinical report was based on a pre-determined list of uropathogens.[5]

Study Outcomes

The primary outcome, symptom resolution at 7−10 days after culture collection, was dichotomous based on participant's yes/no response to the question "do you continue to have UTI symptoms."

Secondary outcomes included an assessment of change in UTISA score from baseline between both groups and the PGI-I at 7−10 days. We also assessed antibiotic prescribing patterns between groups and EQUC-identified microbe sensitivities to evaluate our EQUC treatment algorithm.

Exploratory aims included 1) uropathogen detection rates between clinical microbiology and research microbiology labs, and 2) analysis of symptom resolution and severity at primary outcome based on urotype, as assessed by SUC or EQUC. For exploratory aim number 2, the entire study cohort (225) was divided into 3 major urotypes: *E. coli*-predominant (≥50% of total CFU/mL cultured from a sample were *E. coli*), non-*E. coli* uropathogen-predominant (≥50% of total CFU/mL were well-established urinary pathogens other than *E. coli* including *Klebsiella, Proteus, Streptococcus* and *Enterococcus* species), and non-uropathogen-predominant (≥50% of total CFU/mL were *Lactobacillus* or *Gardnerella* species). Non-uropathogen-predominant and culture negative were grouped into a single urotype because neither was treated with antibiotics.

Sample Size and Power

Sample size was determined for the proportion of women who would experience UTI symptom resolution following SUC and EQUC results and treatment with antibiotics after positive culture based on previous studies.[5] Assuming 66% of those with SUC and 86% of those with EQUC would report they do not feel they still have a UTI at first assessment and accounting for 10% attrition, the final sample size was set at 150 to SUC and 75 to EQUC for 84% power and alpha = 0.025. An alpha-level of 0.025 was chosen to account for 2 analyses (1 interim analysis and 1 final analysis) so that the overall alpha-level would be <0.05. The interim analysis was performed on the primary endpoint when 50% of the total number was recruited. At the interim, there was no difference in the rates of symptom resolution (64.5% [49/76] SUC vs 61.4% [27/44] EQUC; p = 0.73).

Statistical Analysis

Descriptive statistics were used to evaluate demographics and clinical characteristics at enrollment. The proportions with culture positivity, antibiotic treatment, and resolved symptoms were compared using Pearson's chi-square tests; expected cell sizes were monitored and Fisher's exact tests were reported when appropriate. UTISA scores and change in UTISA scores were compared using Wilcoxon rank-sum tests. Symptom improvement was compared using Cochran-Armitage tests for trend. Statistical analysis was performed using SAS® version 9.4.

RESULTS

Figure 1 displays the flow of 225 participants through the study, including 215 who provided primary outcome data (SUC=143 [95%], EQUC=72 [97%]). Of the participants 18% (38/215) and 77% (165/215) responded by email and phone, respectively. Ten participants (5%, 10/215) completed their followup questions in person during a clinic visit and 2 participants (1%, 2/215) mailed printed questionnaires. At baseline, both groups were not significantly different with regards to age, body mass index, race, use of bladder antispasmodics, and presenting mean Pelvic Floor Distress Inventory and UTISA scores (table 1). Vaginal estrogen use was similar (SUC=31%; EQUC=40%).

Primary Outcome

The proportion of positive cultures was not statistically different between groups (SUC=63% [95/150]; EQUC=74% [55/74], p=0.10; table 2). Similar proportions of participants with uropathogen-positive cultures were prescribed antibiotics (SUC=96% [91/95]; EQUC=95% [52/55], p=0.71); antibiotics were similar between groups, with nitrofurantoin most commonly prescribed (SUC=51%, EQUC =54%). At primary outcome assessment, 64% (92/143) and 69% (50/72) of participants in the SUC and EQUC groups, respectively, no longer reported UTI symptoms. For participants who received antibiotics and completed assessment number 1, treatment success was 65% (57/88) and 71% (36/51) for SUC and EQUC, respectively. The majority of participants (66%) who did not receive antibiotics due to non-uropathogen-predominance or negative culture also experienced symptom resolution.

Secondary Outcomes

There was no significant trend in PGI-I score improvement for those in the EQUC arm compared to SUC (p=0.28; table 2); however, those treated based on EQUC experienced a greater decrease in median UTISA score (EQUC=−8 [IQR −11, −4] compared to SUC=−6 [IQR −9, −3], p= 0.04). EQUC participants received more days of antibiotics (EQUC=4.5 [IQR 3,5]; SUC=3 [IQR 0,5], p=0.04).

Four microbes identified in previous studies were not detected in any study participants (*Corynebacterium riegelli, Corynebacterium urelytium, Oligelia urethralis* and *Alloscardovia omnicolens*). Two samples contained *Actinotignum schaalii*, which was pan-resistant despite reports documenting Amoxicillin and Nitrofurantoin as treatments of choice. Thus, the EQUC treatment protocol (supplementary fig. 3, https://www.jurology.com) was revised at the conclusion of the study.

Exploratory Aims

Uropathogen detection rates between clinical and research laboratories. The uropathogen detection rates using EQUC by the clinical and research

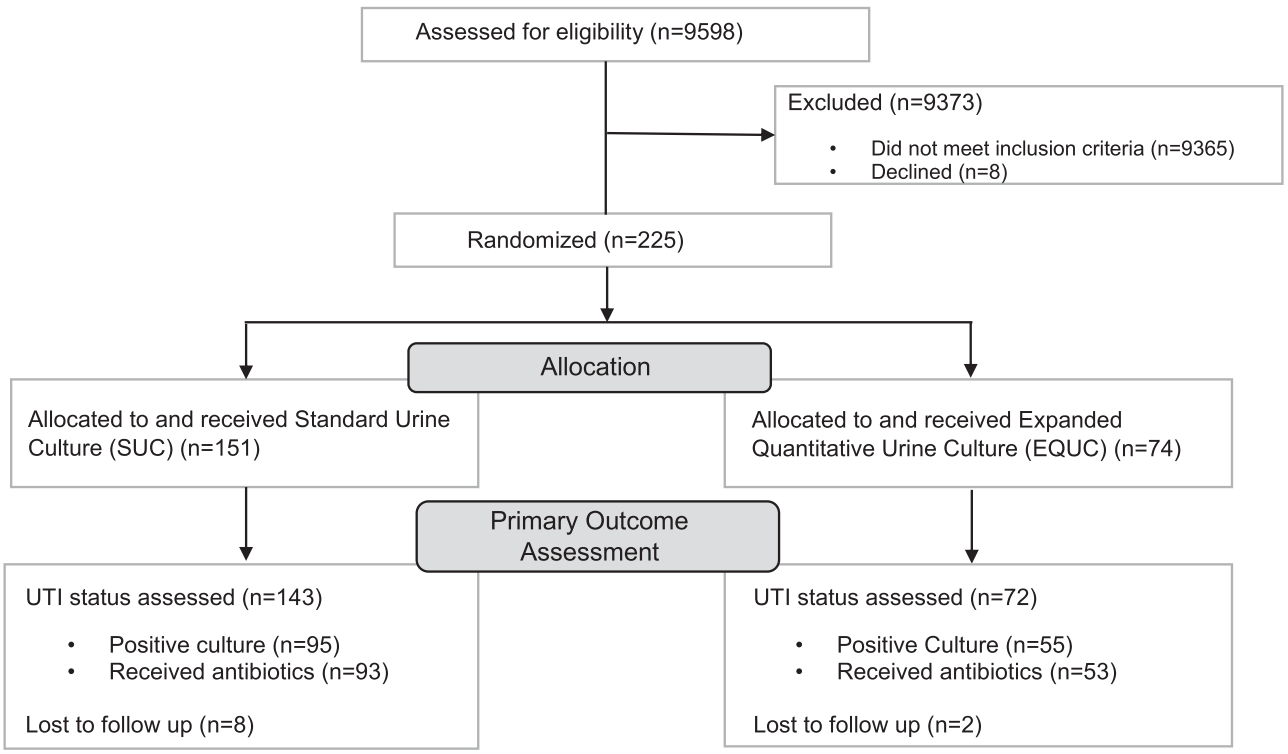

Figure 1. CONSORT diagram.

Table 1. *Baseline characteristics*

	SUC	EQUC
No. pts	151	74
Mean yrs age (SD)	65.9 (16.3)	67.3 (16.1)
Median kg/m^2 body mass index (IQR)*	29 (25–33)	28 (23–33)
No. race/ethnicity (%):		
Black or African American	22 (14.6)	8 (10.8)
White	106 (70.2)	53 (71.6)
Hispanic	21 (13.9)	11 (14.9)
Asian	1 (0.7)	1 (1.4)
Other	1 (0.7)	1 (1.4)
No. using bladder antispasmodics (%)*	16 (10.7)	6 (8.1)
No. using estrogen (%)†	47 (31.3)	29 (39.7)
Median vaginal deliveries (IQR)‡	2 (1–4)	2 (1–3)
Median Pelvic Floor Distress Inventory total score (IQR)*	75 (48–123)	63 (38–104)
Median UTISA total symptom score (IQR)	10 (7–13)	10 (7–13)

* In 224 patients.
† In 223 patients.
‡ In 216 patients.

laboratories were similar (74% [55/74]; 78% [58/74], respectively); they detected 13 and 21 unique uropathogenic species, respectively (fig. 2). The clinical laboratory was much less likely to report *Streptococcus* and *Staphylococcus* species. The clinical laboratory uropathogen detection positivity rates for SUC and EQUC differed by 11% (SUC = 63% [95/151]; EQUC = 74% [55/74]). In the research laboratory, culture results of 54/225 (24%) were non-*E. coli* uropathogen-predominant by SUC, compared to 77/225 (34%) of samples being non-*E. coli* uropathogen-predominant by EQUC.

Correlates of symptom resolution and urotypes. Regardless of culture method, most women with an *E. coli*-predominant urotype experienced symptom resolution (SUC = 71%; EQUC = 67%, p = 0.63; table 3). The majority of women with a non-*E. coli* uropathogen-predominant urotype (76) also experienced symptom resolution (SUC = 56%; EQUC = 77%, p = 0.08).

DISCUSSION

This is the first randomized clinical trial using EQUC, a culture method that has demonstrated the ability to detect additional microbes that may be the source of urinary tract symptoms in women. We found that in women with self-reported UTI treatment based on catheterized urine samples cultured using SUC and EQUC have similar self-reported symptom resolution. Antibiotic usage and classes were similar; while we had originally developed algorithms for antibiotic usage based on limited literature, the clinical laboratory was able to obtain sensitivities on most cultured uropathogens. The

Table 2. *SUC and EQUC results and primary outcome assessment*

	SUC	EQUC	p Value
No. results call	151	74	
No. culture pos at enrollment (%) (224 pts*):			
Yes	95 (63.3)	55 (74.3)	0.10†
No	55 (36.7)	19 (25.7)	
No. given antibiotics following enrollment culture (%):			
Yes	93 (61.6)	53 (71.6)	0.14†
No	58 (38.4)	21 (28.4)	
No. antibiotics given for pos culture (%) (144/146 pts):			
1st generation cephalosporin	14 (15.2)	8 (15.4)	0.99‡
3rd generation cephalosporin	1 (1.1)	0 (0.0)	
Fluoroquinolone	7 (7.6)	3 (5.8)	
Fosfomycin	2 (2.2)	1 (1.9)	
Nitrofurantoin	47 (51.1)	28 (53.8)	
Trimethoprim	1 (1.1)	0 (0.0)	
Trimethoprim/sulfamethoxazole	20 (21.7)	12 (23.1)	
No. assessment (primary outcome)	143	72	
No. still feel have UTI (%):			
Yes	51 (35.7)	22 (30.6)	0.46†
No	92 (64.3)	50 (69.4)	
Median days antibiotics (IQR)	3 (0, 5)	4.5 (3, 5)	0.04§
No. symptom improvement PGI-I (%) (214/215 pts):			
Very much better	43 (30.1)	19 (26.8)	0.28‖
Much better	46 (32.2)	31 (43.7)	
A little better	28 (19.6)	16 (22.5)	
No change	21 (14.7)	3 (4.2)	
A little worse	1 (0.7)	1 (1.4)	
A lot worse	4 (2.8)	1 (1.4)	
Median UTISA score (IQR)	3 (0, 6)	2 (1, 4)	0.26§
Median change in UTISA score from baseline (IQR)	−6 (−9, −3)	−8 (−11, −4)	0.04§

* Missing culture data.
† Pearson's chi-square test.
‡ Fisher's exact test.
§ Wilcoxon rank-sum test.
‖ Cochran-Armitage test for trend.

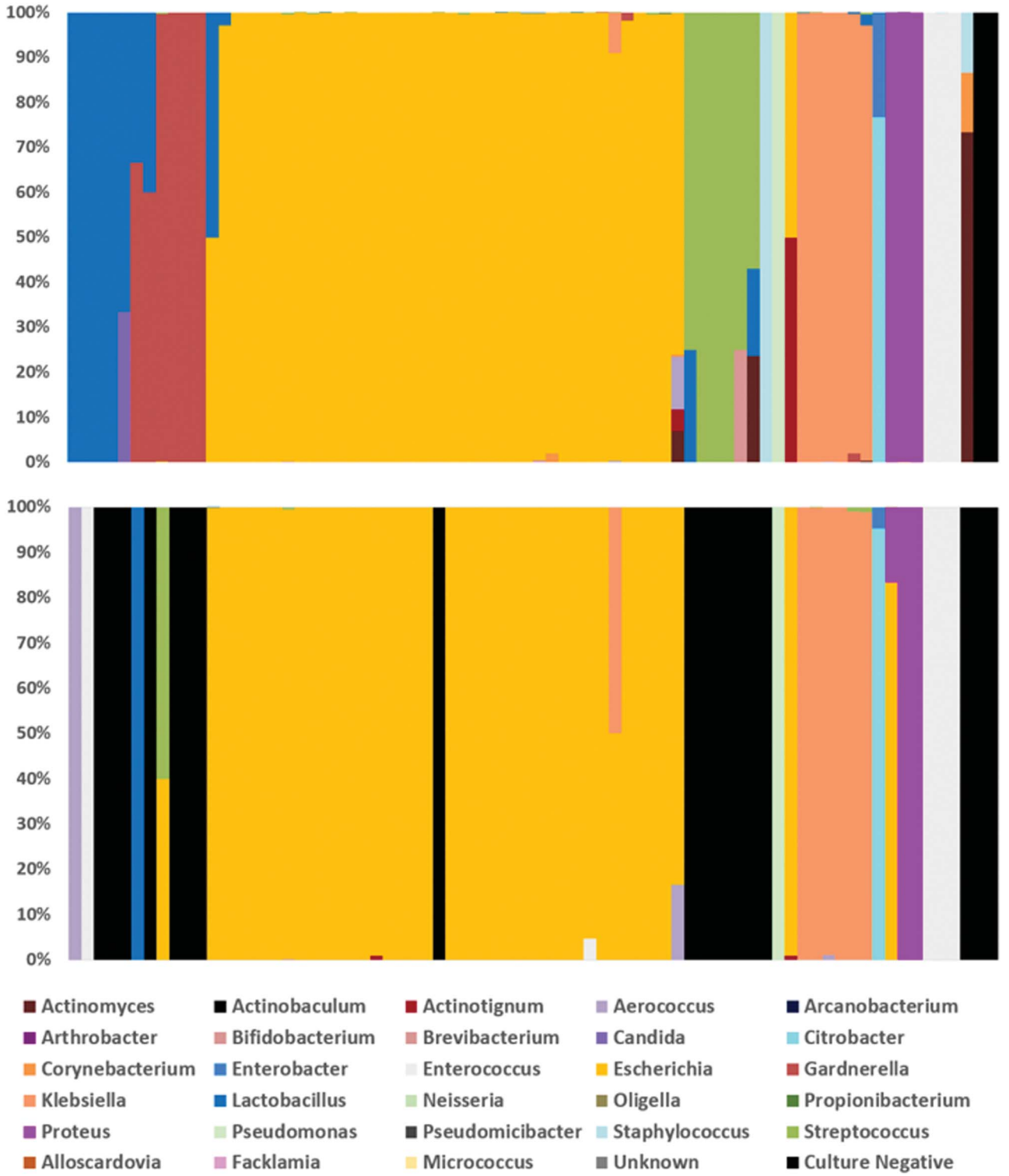

Figure 2. Relative abundance EQUC samples research vs clinical microbiology lab. EQUC results from both research microbiology (top) and clinical microbiology (bottom) labs are presented here for 74 patients in EQUC cohort. Culture procedures were identical across both labs. Each column represents 1 patient, and color proportions denote relative proportions of CFU/mL of each microbe (at genus level) cultured from that patient sample.

treating clinicians chose antibiotics based on sensitivities and patient characteristics such as age, allergies and renal function.

Regardless of culture method, ~33% of participants had persistent UTI symptoms after appropriate antibiotic treatment. Symptoms women

Table 3. *Symptom resolution comparison between and within urotypes*

	E. coli Predominant					Non-*E.coli* Uropathogen Predominant					Non-Uropathogens and Culture Negative				
	SUC		EQUC		p Value	SUC		EQUC		p Value	SUC		EQUC		p Value
No. pts	56		36			54		22			33		14		
Median days antibiotics (IQR)	5	(3–5)	5	(4, 6)	0.25*	3	(0, 5)	3	(3, 5)	0.40*	0	(0, 0)	0	(0, 3)	0.27*
No. still feel have UTI (%):															
Yes	16	(28.6)	12	(33.3)	0.63†	24	(44.4)	5	(22.7)	0.08†	11	(33.3)	5	(35.7)	0.99‡
No	40	(71.4)	24	(66.7)		30	(55.6)	17	(77.3)		22	(66.7)	9	(64.3)	
No. symptom improvement (%):															
Very much better	21	(37.5)	10	(28.6)	0.53§	14	(25.9)	6	(27.3)	0.30§	8	(24.2)	3	(21.4)	0.82§
Much better	20	(35.7)	19	(54.3)		17	(31.5)	9	(40.9)		9	(27.3)	3	(21.4)	
A little better	7	(12.5)	5	(14.3)		14	(25.9)	6	(27.3)		7	(21.2)	5	(35.7)	
No change	7	(12.5)	1	(2.9)		7	(13.0)	1	(4.5)		7	(21.2)	1	(7.1)	
A little worse	0	(0.0)	0	(0.0)		1	(1.9)	0	(0.0)		0	(0.0)	1	(7.1)	
A lot worse	1	(1.8)	0	(0.0)		1	(1.9)	0	(0.0)		2	(6.1)	1	(7.1)	
Median UTISA score (IQR)	3	(0, 5)	2	(0.5, 3)	0.47*	3.5	(0, 7)	2	(1, 4)	0.25*	3	(0, 6)	3	(0, 8)	0.79*
Median change in UTISA score from baseline (IQR)	−7 (−9.5, −4)		−8.5 (−10.5, −5)		0.18§	−5 (−8, −2)		−7.5 (−11, −5)		0.06§	−6 (−9, −4)		−5.5 (−11, −3)		0.89§

* Wilcoxon rank-sum test.
† Pearson's chi-square test.
‡ Fisher's exact test.
§ Cochran-Armitage exact test for trend.

attribute to a UTI may be due to other causes. Myofascial pain of the pelvic floor, urgency-frequency syndrome, and overactive bladder share symptoms similar to UTI that would not be alleviated by antibiotic treatment, as previously reported.[11] Obrien et al described 113 women who self-reported UTI symptoms to their general practitioner. While 61% of participants received empiric antibiotics, only 40% had a positive urine culture.[12] In our study, participants' self-assessment of UTI was 63%–74% accurate based on SUC or EQUC results. This success rate might increase if an additional marker were used for study inclusion; however, we reasoned that many women receive care based on their self-reported symptoms, and on-line companies are now advertising next generation sequencing to women with UTI symptoms but negative urine cultures.

Since SUC and EQUC perform similarly in detecting *E. coli* but SUC often fails to detect uropathogens detected by EQUC,[5] we hypothesized that outcomes for participants without an *E. coli* urotype might increase when treated on EQUC. Thus, we sorted participants into urotypes (*E. coli*-predominant, non-*E. coli* uropathogen-predominant and non-uropathogen-predominant/negative culture). Treatment based on SUC and EQUC had similar success for women with the *E. coli* urotype and our results suggest that future studies on efficacy of EQUC or other enhanced urine culture methods should focus on non-*E. coli* uropathogens.

The major limitation of this study is our assumption that 66% of participants who received SUC and 86% of participants who received EQUC would have symptom resolution based on previous studies.[5] The proportion of participants with symptom resolution did not differ as much as we estimated. Our findings are strengthened by the randomized study design with participant blinding. We accurately calculated the dropout rate and met our enrollment objectives. Inclusion of women with self-reported UTI symptoms increases generalizability to the practice of primary care providers who often rely solely on a patient's description of symptoms. While catheterized samples decrease contamination from other genitourinary sources,[13] these results may not be applicable for clinical care provided from voided urine. A validated questionnaire was used to assess UTI symptoms. Finally, by comparing culture results from 2 laboratories, clinical and research, we assessed quality control. We noted similarity between the labs in uropathogen detection rate but a difference in the number of uropathogenic species identified. We believe this is due to the low CFU/mL of certain species which can lead to natural variation in culture results.

This work is clinically significant because of the increasing beliefs of patients and advocacy groups

that negative cultures miss significant bacteria. A simple search on the Internet using the phrase "I have a UTI but my cultures are negative" reveals the information being shared by women about recent findings on the urobiome. Many advocate advanced testing methods such as MicroGenDX that in the U.S. require physician authorization. This study clearly demonstrates that SUC detects *E. coli* uropathogens and advanced testing is not indicated, which is reassuring. Our work also shows that for a bare majority of patients predominated by non-*E. coli* uropathogens, SUC was sufficient; however, a larger proportion of patients diagnosed with EQUC had an improved outcome (SUC=56% versus EQUC=77%, p=0.08). Thus, we strongly encourage future research efforts to focus on the clinical utility of EQUC or other sensitive detection methods to diagnose individuals predominated with non-*E. coli* uropathogens.

CONCLUSIONS

This randomized trial using 2 different culture techniques on catheterized urine from women with self-reported UTI that include a pooled population of infections caused by *E. coli* and non-*E. coli* uropathogens suggests that treatment based on EQUC and SUC have similar symptom resolution rates that range between 64%–69%. The observed trend suggesting a potential difference in patient outcomes based on microbial etiology (*E. coli* vs non-*E. coli* uropathogens) likely warrants focused studies of culture techniques in women with UTI symptoms and associated non-*E. coli* uropathogens.

REFERENCES

1. Trautner BW: Urinary tract infection as a continuum-implications for diagnostic and antibiotic stewardship. Clin Infect Dis 2021; **72:** 1339.
2. Kass EH: Asymptomatic infections of the urinary tract. J Urol 1956; **167:** 1016.
3. Price TK, Hilt EE, Dune TJ et al: Urine trouble: should we think differently about UTI? Int Urogynecol J 2018; **29:** 205.
4. Hilt EE, McKinley K, Pearce MM 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.
5. Price TK, Dune T, Hilt EE et al: The clinical urine culture: enhanced techniques improve detection of clinically relevant microorganisms. J Clin Microbiol 2016; **54:** 1216.
6. Khasriya R, Sathiananthamoorthy S, Ismail S et al: Spectrum of bacterial colonization associated with urothelial cells from patients with chronic lower urinary tract symptoms. J Clin Microbiol 2013; **51:** 2054.
7. Coorevits L, Heytens S, Boelens J et al: The resident microflora of voided midstream urine of healthy controls: standard versus expanded urine culture protocols. Eur J Clin Microbiol Infect Dis 2017; **36:** 635.
8. Barber MD, Walters MD and Bump RC: Short forms of two condition-specific quality-of-life questionnaires for women with pelvic floor disorders (PFDI-20 and PFIQ-7). Am J Obstet Gynecol 2005; **193:** 103.
9. Clayson D, Wild D, Doll H et al: Validation of a patient-administered questionnaire to measure the severity and bothersomeness of lower urinary tract symptoms in uncomplicated urinary tract infection (UTI): the UTI Symptom Assessment questionnaire. BJU Int 2005; **96:** 350.
10. Yalcin I and Bump RC: Validation of two global impression questionnaires for incontinence. Am J Obstet Gynecol 2003; **189:** 98.
11. Wolff BJ, Joyce CJ, Brincat CA et al: Pelvic floor myofascial pain in patients with symptoms of urinary tract infection. Int J Gynaecol Obstet 2019; **145:** 205.
12. O'Brien K, Hillier S, Simpson S et al: An observational study of empirical antibiotics for adult women with uncomplicated UTI in general practice. J Antimicrob Chemother 2007; **59:** 1200.
13. Chen YB, Hochstedler B, Pham TT et al: The urethral microbiota: a missing link in the female urinary microbiota. J Urol 2020; **204:** 303.

EDITORIAL COMMENTS

This well-designed and well-implemented negative study confirms that our traditional diagnostic algorithm to direct antibiotic therapy in women with typical UTI symptoms can remain simple and uncomplicated; a standard of practice urine culture of a carefully collected midstream urine specimen. The added value of subjecting women with simple uncomplicated UTI to a bladder catheterization has been suggested but not confirmed. The question as to the clinical value of sophisticated bacterial detection strategies such as expanded quantitative urine culture (and next-generation sequencing technologies) using catheterized urine specimens to direct focused antibiotic therapy in patients with recurrent or chronic cystitis symptoms with negative, non-*E. coli* or nebulous standard urine culture results, remains to be answered.

J. Curtis Nickel[1]
[1]*Department of Urology*
Queen's University
Kingston, Ontario, Canada

This was a well-conceived randomized controlled trial (RCT) of standard vs expanded quantitative urine culture to aid in diagnosis and management of adult women who felt they had UTI. The topic is timely and useful to consider within our current paradigm of UTI diagnosis and with the known limitations of the standard urine culture.

While a well-designed and properly executed RCT may provide evidence to guide health care decisions, trials with intentional or unintentional bias could potentially give exaggerated results.[1] Here, the investigators describe appropriate methods of randomization and allocation concealment that minimize bias. Another crucial aspect of trial design is to pre-specify a primary outcome and assess this in an unbiased manner. Blinding is an important safeguard against bias, especially when a subjective outcome is used.[2] In this study the investigators pre-specified their primary outcome as a subjective question asked 7−10 days after enrollment; specifically, "do you continue to have UTI symptoms." However, only 40/215 (19%) participants responded with a blinded technique via email or mailed questionnaire. The remaining 175/215 (81%) participants responded in-person during a clinic visit (10) or to an unblinded investigator by phone (165). The investigators state that these personnel followed a standardized script. Yet, there is always the potential for unintentional bias to creep in when unblinded individuals are administering a subjective question. This reader is left wondering if any of the results might have been different if the primary objective was assessed in a truly blinded manner.

Despite this limitation, this trial adds important data to the literature, especially in an era when molecular-based tests (eg those that utilize sequencing to identify microbes) are being expanded and marketed to patients and providers. The authors appropriately conclude that focused studies of culture techniques in women with UTI symptoms are still warranted.

Nazema Y. Siddiqui[1]
[1]*Duke University*
Durham, North Carolina

REFERENCES

1. Moher D, Hopewell S, Schulz KF et al: CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomized trials. BMJ 2010; **340:** c869.

2. Wood L, Egger M, Gluud LL et al: Empirical evidence of bias in treatment effect estimates in controlled trials with different interventions and outcomes: meta-epidemiological study. BMJ 2008; **336:** 601.