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The Clinical Urine Culture: Enhanced Techniques Improve Detection of Clinically Relevant Microorganisms

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Enhanced quantitative urine culture (EQUC) detects live microorganisms in the vast majority of urine specimens reported as "no growth" by the standard urine culture protocol. Here, we evaluated an expanded set of EQUC conditions (expanded-spectrum EQUC) to identify an optimal version that provides a more complete description of uropathogens in women experiencing urinary tract infection (UTI)-like symptoms. One hundred fifty adult urogynecology patient-participants were characterized using a self-completed validated UTI symptom assessment (UTISA) questionnaire and asked "Do you feel you have a UTI?" Women responding negatively were recruited into the no-UTI cohort, while women responding affirmatively were recruited into the UTI cohort; the latter cohort was reassessed with the UTISA questionnaire 3 to 7 days later. Baseline catheterized urine samples were plated using both standard urine culture and expanded-spectrum EQUC protocols: standard urine culture inoculated at 1 μl onto 2 agars incubated aerobically; expanded-spectrum EQUC inoculated at three different volumes of urine onto 7 combinations of agars and environments. Compared to expanded-spectrum EQUC, standard urine culture missed 67% of uropathogens overall and 50% in participants with severe urinary symptoms. Thirty-six percent of participants with missed uropathogens reported no symptom resolution after treatment by standard urine culture results. Optimal detection of uropathogens could be achieved using the following: 100 μl of urine plated onto blood (blood agar plate [BAP]), colistin-nalidixic acid (CNA), and MacConkey agars in 5% CO_2 for 48 h. This streamlined EQUC protocol achieved 84% uropathogen detection relative to 33% detection by standard urine culture. The streamlined EQUC protocol improves detection of uropathogens that are likely relevant for symptomatic women, giving clinicians the opportunity to receive additional information not currently reported using standard urine culture techniques.

The diagnostic gold standard for clinically relevant urinary tract infection (UTI) continues to be questioned for both clinical and research purposes. Since the 1950s, clinical practice has relied on detecting $\geq 10^5$ CFU/ml of a known uropathogen using the standard clinical urine culture protocol (1). The standard urine culture was initially described for detection of patients at risk for pyelonephritis (2); interpretation has been generalized to diagnose lower urinary tract infection despite studies reporting limitations of the $\geq 10^5$-CFU/ml threshold (3–6). While the clinical focus has centered on various cutoff thresholds, the basic uropathogen detection method remains unchanged.

Given emerging evidence that documents the presence of urinary microbiota in many adult women (7–15), it is clear that the mere presence of an organism should not prompt antibiotic treatment. However, clinicians are likely to benefit from a more complete report of organisms present within a symptomatic patient's urine. Recent evidence reports bacteria in ~90% of "no-growth" standard urine cultures (10, 12). We hypothesized that, in women experiencing UTI-like symptoms, an improved culture protocol would provide a more complete description of potentially treatable, clinically relevant uropathogens. This study evaluated various combinations of urine volumes, media, atmospheric environments, and incubation durations to determine conditions that optimally balance uropathogen detection with feasibility.

MATERIALS AND METHODS

Study design and patient population. Following institutional review board (IRB) approval, we enrolled 75 women who reported UTI symptoms and 75 who did not based on their yes/no response to the question "Do you feel you have a UTI?" Participants were seeking clinical care at Loyola University Medical Center's Female Pelvic Medicine and Reconstructive Surgery center between June 2014 and August 2015.

Participants gave verbal and written research consent and provided permission for abstraction of their demographic and clinical information from the electronic medical record. At baseline, urinary symptoms were characterized using a self-completed, validated UTI symptom assessment (UTISA) questionnaire, completed by both cohorts (16). Participants were dichotomized based on their yes/no response to the question "Do you feel you have a UTI?" Those who responded affirmatively (UTI cohort) completed the UTISA questionnaire again by phone 3 to 7 days postenrollment and were queried about the magnitude of symptom res-

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TABLE 1 Summary of urine cultivation protocols for catheterized urine specimens

Protocol	Vol (μl) of urine	Medium or media	Conditions	Incubation(s) (h) for microbial identification	Patient sample identifier[a]
Standard urine culture	1	BAP, MacConkey agar	Aerobic, 35°C	24	1–107
Modified urine culture	1	BAP, MacConkey agar	5% CO_2, 35°C	24, 48	108–150
Expanded-spectrum EQUC	1, 10, and 100	BAP, MacConkey agar	Aerobic, 35°C	24, 48	1–150
		BAP, chocolate agar, CNA agar	5% CO_2, 35°C	24, 48	
		CDC anaerobic BAP	Anaerobic, 35°C	48	
		CDC anaerobic BAP[b]	Microaerophilic gas mixture (5% O_2, 10% CO_2, 85% N), 35°C	48	
Streamlined EQUC	100	BAP, MacConkey agar,[c] CNA agar	5% CO_2, 35°C	48	1–150[d]

[a] Refers to the patient samples on which the corresponding protocol assessed the urinary microbiota. For diagnosis, the standard urine culture protocol was used on patient samples 1 to 107; the modified standard urine culture was used on patient samples 108 to 150. For research, all patient samples were assessed by expanded-spectrum EQUC.
[b] The CDC anaerobic BAP microaerophilic gas mixture condition was used only for samples 10 to 150.
[c] The MacConkey 5% CO_2 condition was not part of the expanded-spectrum EQUC protocol.
[d] The streamlined EQUC protocol was performed using a subgroup of agars/conditions from the expanded-spectrum EQUC protocol; therefore, it was used on all patient samples.

olution, if any. All clinical treatment was individually based on physician assessment of patient symptoms and standard urine culture results. Exclusion criteria included age of <18 years, pregnancy, catheterization (indwelling or intermittent), or insufficient English skills to complete study measures.

Sample collection and analysis. Consistent with patient care clinical protocol, urine was collected via transurethral catheter and then placed into two BD Vacutainer Plus C&S preservative tubes: one sent to the clinical microbiology laboratory for diagnostic purposes and one sent to the researchers for testing.

Table 1 displays all culture protocols used by the clinical microbiology laboratory staff and the researchers. The standard urine culture protocol used 1 μl of urine, spread quantitatively (i.e., pinwheel streak) onto 5% sheep blood (blood agar plate [BAP]) and MacConkey agars (BD BBL Prepared Plated Media; Cockeysville, MD) and incubated aerobically at 35°C for 24 h. The modified standard urine culture used the same agars and temperature but changed the incubation condition to 5% CO_2; if pinpoint growth was seen at 24 h, the agars were held for another 24 h under the same conditions. Unrelated to this study, the clinical microbiology laboratory adopted the modified standard urine culture for diagnosis during patient recruitment for this study. Thus, diagnostic testing for patients 1 to 107 was the standard urine culture, while the modified standard urine culture was used for patients 108 to 150. However, this change did not impact data presented in this study, as standard urine culture data for patients 108 to 150 were obtained by analyzing the corresponding subset of expanded enhanced quantitative urine culture (EQUC) conditions (i.e., 1 μl BAP and MacConkey agars; aerobic, 35°C; 24 h).

The conditions of the original enhanced quantitative urine culture (EQUC) protocol were described previously (10). In this study, we expanded those conditions (i.e., expanded-spectrum EQUC protocol), using three urine volumes (1 μl, 10 μl, and 100 μl) and additional plating conditions (Table 1). Each urine sample was spread quantitatively onto BAP, chocolate, and colistin-nalidixic acid (CNA) agars (BD BBL Prepared Plated Media) and incubated in 5% CO_2 at 35°C for 48 h; BAP and MacConkey agars were incubated aerobically at 35°C for 48 h; two CDC anaerobic 5% sheep blood (anaerobic BAP) agars (BD BBL Prepared Plated Media) were incubated either in microaerophilic Campy gas mixture (5% O_2, 10% CO_2, 85% N) or anaerobically at 35°C for 48 h. Three sets of these conditions were used for each urine sample, each using one of the urine volumes, for a total of 21 agars per sample. All agars were documented for growth (i.e., for morphologies and CFU per milliliter) at 24 and 48 h, except the two anaerobic BAP conditions. Each distinct colony morphology was subcultured at 48 h to obtain pure culture for microbial identification.

Microbial identification was determined using a matrix-assisted laser desorption ionization–time of flight mass spectrometer (MALDI-TOF MS; Bruker Daltonics, Billerica, MA) as described previously (10). Only clinically relevant microbes (i.e., known and emerging uropathogens) were used to calculate uropathogen detection. These uropathogens were selected based on previously published case reports of UTI.

UTISA questionnaire. This questionnaire asks the participant to rate the degree of severity and bother for seven common UTI symptoms: frequency of urination, urgency of urination, incomplete bladder emptying (urinary retention), pain or burning during urination (dysuria), lower abdominal discomfort or pelvic pain/pressure, lower back pain, and blood in the urine (hematuria). Scores for each symptom range from 0 to 3; a 0 corresponds to no symptom present, whereas a 3 indicates highest severity or bother. The seven symptoms can be clustered into four symptom domains: urination regularity (frequency and urgency), problems with urination (incomplete bladder emptying and pain or burning), pain associated with the UTI (abdominal or pelvic pain and lower back pain), and blood in the urine (16).

Statistical analyses. Continuous variables were reported as means and standard deviations (SDs) or medians and interquartile ranges (IQRs); categorical variables were reported as frequencies and percentages. Pearson chi-square tests (or Fisher's exact tests, when necessary) and 2-sample *t* tests (or Wilcoxon rank sum tests, when appropriate) were used to compare demographics and culture results (e.g., abundance and diversity) between cohorts. Measures of alpha diversity (diversity within a population) were represented as Shannon diversity indices and/or graphically by species accumulation curves (which plot accumulation of unique species per group for each additional sample included). All statistical analyses were conducted using SAS software v9.4 (SAS Institute, Cary, NC) or Systat software version 13.1 (Systat Software Inc., Chicago, IL).

RESULTS

Participant demographics and symptoms. Table 2 describes demographics of the two cohorts (75 no-UTI and 75 UTI patients). Most participants were white (81%) and overweight (mean body mass index [BMI] = 29.3 kg/m^2). Most participants (92%, 138/150) reported at least one urinary symptom; as expected, women in the UTI cohort had higher UTISA questionnaire scores.

Expanded-spectrum EQUC: urinary microbiota characteristics. Nearly all (93% [139/150]) catheterized urine samples grew bacterial colonies with at least one combination of the expanded-spectrum EQUC protocol's conditions (Table 1). The no-UTI and UTI cohorts had similar proportions of cultivatable urine

TABLE 2 Demographic characteristics and symptoms

Clinical variable	Entire cohort ($n = 150$)	No-UTI cohort ($n = 75$)	UTI cohort ($n = 75$)	P value[d]
Age (yr), mean (SD)	62.3 (14.9)	60.6 (12.3)	64.0 (17.1)	0.16[a]
BMI (kg/m^2), mean (SD)	29.3 (6.3)	28.8 (5.9)	29.9 (6.6)	0.27[a]
Race/ethnicity, no. (%)				
White	121 (81)	59 (79)	62 (83)	
Hispanic	15 (10)	9 (12)	6 (8)	
Black	9 (6)	5 (7)	4 (5)	0.90[c]
Asian	4 (3)	2 (3)	2 (3)	
Other	1 (1)	0 (0)	1 (1)	
No. of vaginal deliveries, median (IQR)	2 (0–11)	2 (0–6)	2 (0–11)	0.80[b]
Sexually active, no. (%)	58 (39)	37 (49)	21 (28)	**0.01**
Previous antibiotic treatment, no. (%)	45 (30)	20 (27)	25 (33)	0.37
Current anticholinergic treatment, no. (%)	26 (17)	9 (12)	17 (23)	0.08
Type of anticholinergic used, no. (%)				
Oxybutynin	9 (6)	3 (4)	6 (8)	
Solifenacin	8 (5)	3 (4)	5 (7)	
Fesoterodine	4 (3)	2 (3)	2 (3)	
Tolterodine	2 (1)	1 (1)	1 (1)	0.75[c]
Oxybutynin patch	1 (1)	0 (0)	1 (1)	
Trospium chloride	1 (1)	0 (0)	1 (1)	
Mirabegron (Myrbetriq)	1 (1)	0 (0)	1 (1)	
Previous vaginal estrogen use, no. (%)	32 (21)	11 (15)	21 (28)	**0.05**
Current vaginal estrogen use, no. (%)	29 (19)	10 (13)	19 (25)	0.06
Prior urogynecologic surgery, no. (%)	40 (27)	13 (17)	27 (36)	**0.01**
Symptoms of incontinence, no. (%)				
Stress urinary incontinence	17 (11)	10 (13)	7 (9)	0.44
Urgency urinary incontinence	26 (17)	11 (15)	15 (20)	0.39
Mixed urinary incontinence	42 (28)	27 (36)	15 (20)	**0.03**
Urgency-frequency syndrome, no. (%)	18 (12)	9 (12)	9 (12)	0.99
Myofascial pain, no. (%)	55 (37)	20 (27)	35 (47)	**0.01**
Painful bladder syndrome, pelvic pain, and dyspareunia, no. (%)	4 (3)	0 (0)	4 (5)	0.06[c]
UTISA score, mean (SD)[e]				
Urination regularity	6.6 (4.3)	5.4 (4.3)	7.9 (3.9)	**<0.001**[a]
Problems with urination	3.4 (3.6)	2.0 (2.5)	5.4 (3.7)	**<0.001**[a]
Pain associated with UTI	3.2 (3.6)	2.1 (3.1)	4.3 (3.8)	**<0.001**[a]
Blood in the urine	0.2 (0.8)	0.1 (0.4)	0.4 (1.1)	0.08[a]

[a] Independent *t* test.
[b] Wilcoxon rank sum test.
[c] Fisher's exact test.
[d] Chi-square test used unless otherwise indicated. Boldface indicates *P* values that are significant at ≤0.05.
[e] UTISA scores for urinary regularity, problems with urination, and pain associated with UTI range from 0 to 12. UTISA scores for blood in the urine range from 0 to 6.

samples (89% [67/75] versus 96% [72/75]; $P = 0.12$), similar numbers of total unique species detected per cohort (75 versus 66), and similar median numbers of species detected per urine sample (3 [IQR = 1 to 5] versus 2 [IQR = 1 to 4]; $P = 0.12$) (see Table S1 in the supplemental material).

However, the cohorts differed in organism diversity, genus-level composition, and species-level composition. The no-UTI cohort had more diversity with greater species richness as depicted by species accumulation curves (see Fig. S1 in the supplemental material) and greater alpha diversity as measured by the Shannon diversity index (no-UTI = 3.89 versus UTI = 3.49). The genera *Streptococcus* ($P = 0.003$) and *Gardnerella* ($P = 0.04$) were more prevalent in the no-UTI cohort, while the genus *Escherichia* ($P < 0.001$) was detected more often in the UTI cohort (see Fig. S2). Five species, namely, *Gardnerella vaginalis* ($P = 0.04$), *Streptococcus mitis/oralis/pneumoniae* ($P = 0.01$), *Streptococcus parasanguinis* ($P = 0.02$), *Streptococcus salivarius* ($P = 0.05$), and *Streptococcus sanguinis* ($P = 0.01$), were detected more often in the no-UTI cohort; in contrast, the species *Escherichia coli* ($P < 0.001$) was more prevalent in the UTI cohort (see Fig. S3).

Uropathogen detection. We next modeled our evaluation to uropathogen detection by the expanded-spectrum EQUC protocol with regard to the following parameters: detection compared to standard urine culture, detection with different urine volumes,

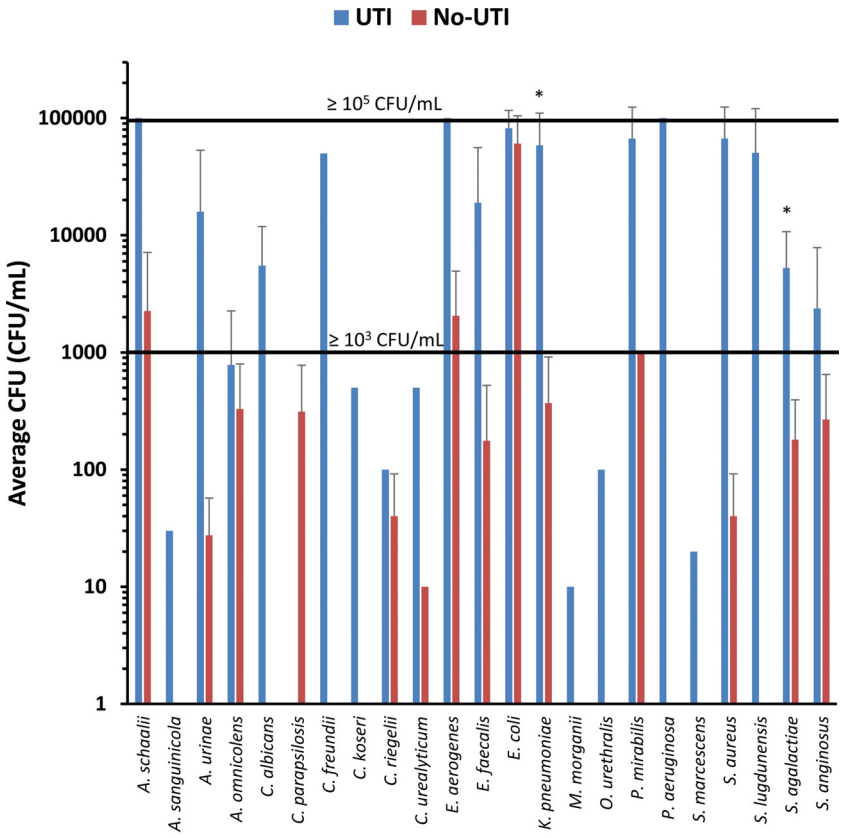

FIG 1 Average CFU per milliliter of uropathogens between the UTI and no-UTI cohorts. Depicted are the average CFU per milliliter with which the various uropathogens were cultured for both cohorts: UTI (blue bars) and no-UTI (red bars). Average CFU of *Klebsiella pneumoniae* ($P = 0.04$) and *Streptococcus agalactiae* ($P = 0.02$) are significantly higher in the UTI cohort (*). Several uropathogens had substantially lower average CFU-per-milliliter values in the no-UTI cohort than in the UTI cohort: *Aerococcus urinae* ($P = 0.12$), *Enterococcus faecalis* ($P = 0.09$), *Escherichia coli* ($P = 0.08$), *Staphylococcus aureus* ($P = 0.06$), and *Streptococcus anginosus* ($P = 0.06$). Independent *t* test (*, $P < 0.05$). Black bars depict common UTI thresholds ($\geq 10^5$ CFU/ml and $\geq 10^3$ CFU/ml).

and detection using different plating conditions. With these findings, we then identified an optimal subset of expanded-spectrum EQUC conditions for improved detection of uropathogens, which we call the streamlined EQUC protocol.

Expanded-spectrum EQUC versus standard urine culture. The expanded-spectrum EQUC protocol detected a total of 182 uropathogens in all the catheterized urine samples, 110 uropathogens in the UTI cohort urine samples, and 72 uropathogens in in the non-UTI cohort urine samples. Whereas the expanded-spectrum EQUC did not miss any uropathogen detected by standard urine culture, the standard urine culture protocol detected 33% (60/182) of all detected uropathogens, 50% (55/110) of those detected in the UTI cohort, and only 7% (5/72) of those detected in the non-UTI cohort.

The expanded-spectrum EQUC protocol detected all uropathogens at a higher average CFU per milliliter in the UTI cohort than in the no-UTI cohort (Fig. 1). This protocol detected *E. coli* in a total of 50 samples obtained from both cohorts; of these, standard urine culture detected *E. coli* in 88% (44/50). From the UTI cohort alone, expanded-spectrum EQUC detected *E. coli* in 43 samples; of these, standard urine culture detected *E. coli* in 91% (39/43). In contrast, standard urine culture detected a strikingly low fraction (12% [16/132]) of the non-*E. coli* uropathogens detected in the two cohorts by the expanded-spectrum EQUC protocol. This percentage was only slightly better in the UTI cohort alone, where standard urine culture detected 24% (16/67) of the non-*E. coli* uropathogens detected by the expanded-spectrum EQUC protocol. Therefore, standard urine culture's capacity to detect *E. coli* dramatically skewed its overall uropathogen detection value.

Expanded-spectrum EQUC: urine volumes. Uropathogen detection differed greatly based on expanded-spectrum EQUC urine volumes: for 100 μl, 96% detection (174/182); for 10 μl, 65% detection (118/182); and for 1 μl, 52% detection (95/182) versus standard urine culture (33% [60/182]). Some uropathogens were detected equally by all volumes (e.g., *E. coli* and *Pseudomonas aeruginosa*); others most often required 100 μl for detection (e.g., *Aerococcus urinae*, *Alloscardovia omnicolens*, *Enterococcus faecalis*, and *Streptococcus anginosus*) (see Fig. S4 in the supplemental material).

Expanded-spectrum EQUC: plating conditions. Table 3 displays uropathogens and the number of times that each was cultured under the various expanded-spectrum EQUC plating conditions. After 48 h incubation, CDC anaerobic BAP incubated anaerobically detected the most uropathogens (63% [115/182]), followed by BAP in 5% CO_2 (62% [112/182]), CDC anaerobic BAP incubated microaerophilically (54% [98/182]), chocolate agar in 5% CO_2 (53% [96/182]), BAP incubated aerobically (52% [94/182]), CNA agar in 5% CO_2 (43% [79/182]), and MacConkey agar incubated aerobically (34% [62/182]). Although the CNA agar condition detected fewer uropathogens, it ideally detected

TABLE 3 Optimal detection of specific uropathogens by the expanded-spectrum EQUC protocol[a]

Uropathogen (no. of isolates)	No. of times cultured under each condition: BAP, CO_2	Chocolate agar, CO_2	CNA agar, CO_2	CDC anaerobic, BAP anaerobic	BAP, O_2	MacConkey agar, O_2	CDC anaerobic BAP, microaerophilic
Actinobaculum schaalii (6)	2	0	5	3	0	0	4
Aerococcus sanguinicola (1)	1	0	0	0	1	0	0
Aerococcus urinae (15)	7	7	10	6	6	0	2
Alloscardovia omnicolens (8)	4	1	5	4	0	0	1
Candida albicans (2)	1	1	2	1	1	0	1
Candida parapsilosis (4)	1	1	2	2	0	0	1
Citrobacter freundii (1)	1	1	0	1	1	1	1
Citrobacter koseri (1)	1	1	0	1	1	1	0
Corynebacterium riegelii (4)	1	1	0	2	2	0	1
Corynebacterium urealyticum (2)	1	0	1	0	1	0	0
Enterobacter aerogenes (3)	2	2	0	1	1	2	2
Enterococcus faecalis (16)	5	4	11	5	7	0	6
Escherichia coli (50)	47	46	4	49	47	47	42
Klebsiella pneumoniae (10)	7	6	0	9	8	7	7
Morganella morganii (1)	0	0	1	0	0	0	0
Oligella urethralis (1)	0	0	0	0	1	0	0
Proteus mirabilis (4)	3	3	2	4	3	3	3
Pseudomonas aeruginosa (1)	1	1	0	1	1	1	1
Serratia marcescens (1)	0	1	0	1	0	0	0
Staphylococcus aureus (7)	4	4	4	3	4	0	4
Staphylococcus lugdunensis (2)	2	2	2	2	2	0	2
Streptococcus agalactiae (10)	8	6	6	6	5	0	5
Streptococcus anginosus (32)	13	8	24	14	2	0	15
Total (182)	112	96	79	115	94	62	98

[a] Listed are the uropathogens and the number of times that each was cultured under each expanded-spectrum EQUC plating condition. The condition(s) that best detected each uropathogen is shaded.

Gram-positive uropathogens when Gram-negative bacteria overwhelmed other agars. For example, of the 47 samples where a Gram-negative uropathogen was present at ≥50,000 CFU/ml, the CNA agar condition detected 27 underlying Gram-positive uropathogens, all of which were missed by standard urine culture (see Table S2A in the supplemental material). Conversely, of the seven samples where a Gram-positive uropathogen was present at ≥50,000 CFU/ml, the MacConkey condition detected two underlying Gram-negative uropathogens (see Table S2B).

Streamlined EQUC protocol. One hundred microliters of urine plated on a combination of BAP and CNA agars in 5% CO_2 and MacConkey agar under aerobic conditions would have detected 84% (152/182) of all uropathogens detected by the expanded-spectrum EQUC protocol. This is vastly superior to the 33% (60/182) uropathogen detection by standard urine culture. In the UTI cohort alone, the streamlined EQUC protocol (Table 1) would have detected 91% (100/110) of uropathogens, compared to only 52% (57/110) by standard urine culture.

Symptom resolution. Seventy-nine percent (59/75) of the participants in the UTI cohort completed the follow-up UTISA questionnaire. Following clinically selected treatment based on standard urine culture (or modified standard urine culture), 59% (35/59) of participants reported symptom improvement, while 41% (24/59) reported no improvement (same or worse) (Table 4). Half (12/24) of the 24 participants who did not improve had at least one uropathogen detected only by the expanded-spectrum EQUC protocol, and 13 (54%) had microorganisms of unknown pathogenicity, which were detected only by the expanded-spectrum EQUC protocol (see Table S3 in the supplemental material). Importantly, all of these missed uropathogens would have also been detected using the streamlined EQUC protocol. Ten of the 24 patients who did not improve had been clinically treated with antibiotics based on the finding of a standard urine culture-detected uropathogen. However, in 3 (30%) of these 10 patients, the expanded-spectrum EQUC (as well as streamlined EQUC) detected an additional Gram-positive uropathogen (*Aerococcus urinae*, *Corynebacterium riegelii*, or *Streptococcus anginosus*).

DISCUSSION

Accurate diagnosis for women with UTI symptoms is critical, both to target appropriate therapy and to limit inappropriate antibiotic use. Our study demonstrates deficiencies in the standard urine culture protocol that limit potentially important information that should be provided to clinicians. Our findings suggest that improved detection of clinically relevant urinary microbes can be achieved in all diagnostic clinical laboratories using the following conditions: a 100-μl urine sample obtained by transurethral catheter plated onto BAP, CNA, and MacConkey agars, with incubation of all agars in 5% CO_2 for 48 h. While incubation of MacConkey agar in 5% CO_2 may not improve Gram-negative bacillus recovery, we recommend that all agars be incubated in 5% CO_2 for the convenience of using a single incubator. All detected uropathogens will grow under the conditions described in the streamlined EQUC protocol.

Our findings support the use of the streamlined EQUC protocol to more fully describe uropathogens. We also recommend that 1 μl of the catheterized urine be plated onto BAP and MacConkey

TABLE 4 Detection of uropathogens in UTI cohort without symptom improvement[a]

Postenrollment questionnaire response (sample identifier)	Antibiotic prescribed	Uropathogen(s) detected by protocol(s):	
		Standard urine culture and expanded-spectrum EQUC	Expanded-spectrum EQUC only[d]
Same (145)	SMZ-TMP[b]	*Escherichia coli*	*Streptococcus anginosus*
Same (048)	Nitrofurantoin	*Escherichia coli*	*Streptococcus anginosus*
Same (134)	SMZ-TMP	*Escherichia coli*	*Aerococcus urinae*, *Corynebacterium riegelii*
Same (033)	Ciprofloxacin	*Klebsiella pneumoniae*	
Same (060)	Nitrofurantoin	*Escherichia coli*	
Same (109)	Nitrofurantoin	*Escherichia coli*	
Same (122)	Ciprofloxacin	*Escherichia coli*	
Same (135)	Nitrofurantoin	*Escherichia coli*	
Same (136)	Nitrofurantoin	*Escherichia coli*	
Worse (082)	Nitrofurantoin	*Escherichia coli*	
Same (140)		*Staphylococcus lugdunensis*	
Same (116)		*Escherichia coli*	
Worse (128)		*Escherichia coli*	
Same (126)		*Lactobacillus* species[c]	*Staphylococcus lugdunensis*, *Streptococcus anginosus*
Same (121)			*Proteus mirabilis*
Same (029)			*Aerococcus urinae*, *Klebsiella pneumoniae*
Same (139)			*Alloscardovia omnicolens*, *Oligella urethralis*, *Morganella morganii*
Same (052)			*Streptococcus anginosus*
Same (067)			*Streptococcus anginosus*
Worse (025)			*Candida albicans*
Worse (112)			*Streptococcus agalactiae*, *Streptococcus anginosus*
Worse (142)			*Escherichia coli*
Same (084)			
Same (108)			

[a] Uropathogens detected and missed by the standard urine culture in urine samples obtained by catheter from the UTI cohort patients who reported feeling the same or worse for the postenrollment questionnaire. Antibiotics were prescribed based on the reporting of the standard urine culture results.
[b] SMZ-TMP, sulfamethoxazole-trimethoprim.
[c] *Lactobacillus* species is not considered a uropathogen, but it was detected at >100,000 CFU/ml by standard urine culture.
[d] All of the uropathogens detected by the expanded-spectrum EQUC protocol would have been detected using the streamlined EQUC protocol.

agars and incubated in 5% CO_2 for 24 h with an option to incubate for 48 h (modified standard urine culture). The streamlined EQUC protocol provides the most thorough detection of uropathogens, while the modified standard urine culture ensures accurate colony count assessment and is beneficial for species detection of underlying uropathogens when bacterial colony counts of a predominant uropathogen exceed 10^5 CFU/ml. The need for modified standard urine culture inclusion is apparent from the observation that the expanded-spectrum EQUC (and the streamlined EQUC) protocols were not 100% sensitive. In the expanded-spectrum EQUC protocol, the use of 100 μl urine detected the most microbes and the most uropathogens. However, a small number of uropathogens were detected only with the use of a smaller urine volume (10 μl). This apparent paradox likely results from microbial overcrowding in samples containing high numbers of CFU; in these circumstances, 100 μl was not ideal for distinguishing morphologies. While addition of selective media (i.e., CNA and MacConkey agars) helped detect underlying uropathogens, some samples contained both Gram-positive and Gram-negative bacteria at high CFU numbers, likely making the 100-μl expanded-spectrum EQUC volume less efficient.

Streamlined EQUC would provide more information to clinicians who are considering the clinical need for uropathogen(s) treatment; many of these are currently missed by the standard urine culture protocol. Until better information is available concerning the relationship between clinically important UTI and CFU per milliliter, we recommend that these testing conditions (i.e., streamlined EQUC) be used for patients with recurrent UTIs or patients with clear UTI-like symptoms despite a negative standard urine culture result. Nonetheless, it is clear that treatment based on standard urine culture results limits diagnostic information that may be useful for symptom resolution. This study did not assess symptom relief in patients whose uropathogens were detected only with the streamlined EQUC protocol. Such studies are clearly needed.

Compared to the expanded-spectrum EQUC, the standard urine culture missed 67% (122/182) of all detected uropathogens and 88% (116/132) of non-*E. coli* uropathogens. Detection of uropathogens by the standard urine culture was slightly better for the UTI cohort alone (50% total missed [55/110]; 76% non-*E. coli* missed [51/67]). This improvement may result from the higher average uropathogen CFU per milliliter in the UTI cohort (Fig. 1), making detection by standard urine culture more likely. The data in Table 1 also reveal that the use of one threshold for UTI diagnosis is likely incorrect. Use of the $\geq 10^5$-CFU/ml threshold would result in untreated uropathogens in the UTI cohort (Fig. 1). Lowering the threshold to $\geq 10^3$ CFU/ml, however, creates other concerns. While use of the $\geq 10^3$-CFU/ml threshold would leave fewer uropathogens in the UTI cohort untreated, it would detect some uropathogens in the no-UTI cohort. Since individuals in the no-UTI cohort presumably do not have an infection (i.e., no/low severity of urinary symptoms), it is unlikely that they would benefit from antibiotic use. This creates a problem in diagnosis and

treatment of UTIs and is likely the reason for the current ambiguity surrounding urine cultures. From these data, we suggest the possibility that, for UTI diagnosis, each uropathogen may have its own unique threshold (e.g., $\geq 10^2$ CFU/ml for *Aerococcus urinae*, $\geq 10^3$ CFU/ml for *Streptococcus agalactiae*, and $\geq 10^4$ CFU/ml for *Klebsiella pneumoniae*).

Our findings in a selected, health care-seeking population of women should be generalized with caution to community-dwelling women who may or may not have similar microbial profiles in health or during UTI. Microbial characterization of women longitudinally may provide additional context for interpretation of standard and streamlined culture results.

It appears that simple changes to the commonly performed standard urine culture protocol have the capacity to provide potentially useful clinical information. Importantly, the urine must be collected by catheter, as we have previously shown that vulvovaginal contamination renders clean-catch voided specimens obsolete (7). At this time, we suggest that the recommended culture conditions (i.e., streamlined EQUC) be considered both as a supplemental test when individuals with UTI-like symptoms have "no growth" via standard urine culture and for use with individuals with persistent UTI-like symptoms (i.e., recurrent UTI).

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REFERENCES

1. **Garcia LS, Isenberg HD.** 2010. Clinical microbiology procedures handbook, 3rd ed. ASM Press, Washington, DC.
2. **Kass E.** 1956. Asymptomatic infections of the urinary tract. Trans Assoc Am Phys **69:**56–64.
3. **Stamm W, Counts G, Running K, Fihn S, Turck M, Holmes K.** 1982. Diagnosis of coliform infection in acutely dysuric women. N Engl J Med **307:**463–468. http://dx.doi.org/10.1056/NEJM198208193070802.
4. **Stark RP, Maki DG.** 1984. Bacteriuria in the catheterized patient. N Engl J Med **311:**560–564. http://dx.doi.org/10.1056/NEJM198408303110903.
5. **Lipsky BA, Ireton RC, Fihn SD, Hackett R, Berger RE.** 1987. Diagnosis of bacteriuria in men: specimen collection and culture interpretation. J Infect Dis **155:**847–854. http://dx.doi.org/10.1093/infdis/155.5.847.
6. **Hooton TM, Roberts PL, Cox ME, Stapleton AE.** 2013. Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med **369:**1883–1891. http://dx.doi.org/10.1056/NEJMoa1302186.
7. **Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, Fitzgerald M, Mueller ER, Schreckenberger P, Dong Q, Nelson DE, Brubaker L.** 2012. Evidence of uncultivated bacteria in the adult female bladder. J Clin Microbiol **50:**1376–1383. http://dx.doi.org/10.1128/JCM.05852-11.
8. **Fouts DE, Pieper R, Szpakowski S, Pohl H, Knoblach S, Suh MJ, Huang ST, Ljungberg I, Sprague BM, Lucas SK, Torralba M, Nelson KE, Groah SL.** 2012. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. J Transl Med **10:**174. http://dx.doi.org/10.1186/1479-5876-10-174.
9. **Khasriya R, Sathiananthamoorthy S, Ismail S, Kelsey M, Wilson M, Rohn JL, Malone-Lee J.** 2013. Spectrum of bacterial colonization associated with urothelial cells from patients with chronic lower urinary tract symptoms. J Clin Microbiol **51:**2054–2062. http://dx.doi.org/10.1128/JCM.03314-12.
10. **Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, Brubaker L, Gai X, Wolfe AJ, Schreckenberger PC.** 2014. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J Clin Microbiol **52:**871–876. http://dx.doi.org/10.1128/JCM.02876-13.
11. **Brubaker L, Nager C, Richter H, Visco A, Nygaard I, Barber M, Schaffer J, Meikle S, Wallace D, Shibata N, Wolfe A.** 2014. Urinary bacteria in adult women with urgency urinary incontinence. Int Urogynecol J **25:**1179–1184. http://dx.doi.org/10.1007/s00192-013-2325-2.
12. **Pearce MM, Hilt EE, Rosenfeld AB, Zilliox MJ, Thomas-White K, Fok C, Kliethermes S, Schreckenberger PC, Brubaker L, Gai X, Wolfe AJ.** 2014. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. mBio **5**(4):e01283-14. http://dx.doi.org/10.1128/mBio.01283-14.
13. **Nienhouse V, Gao X, Dong Q, Nelson DE, Toh E, McKinley K, Schreckenberger P, Shibata N, Fok CS, Mueller ER, Brubaker L, Wolfe AJ, Radek KA.** 2014. Interplay between bladder microbiota and urinary antimicrobial peptides: mechanisms for human urinary tract infection risk and symptom severity. PLoS One **9:**e114185. http://dx.doi.org/10.1371/journal.pone.0114185.
14. **Pearce MM, Zilliox MJ, Rosenfeld AB, Thomas-White KJ, Richter HE, Nager CW, Visco AG, Nygaard IE, Barber MD, Schaffer J, Moalli P, Sung VW, Smith AL, Rogers R, Nolen TL, Wallace D, Meikle SF, Gai X, Wolfe AJ, Brubaker L.** 2015. The female urinary microbiome in urgency urinary incontinence. Am J Obstet Gynecol **213:**347.e1-11. http://dx.doi.org/10.1016/j.ajog.2015.07.009.
15. **Thomas-White KJ, Hilt EE, Fok C, Pearce MM, Mueller ER, Kliethermes S, Jacobs K, Zilliox MJ, Brincat C, Price TK, Kuffel G, Schreckenberger P, Gai X, Brubaker L, Wolfe AJ.** 30 September 2015. Incontinence medication response relates to the female urinary microbiota. Int Urogynecol J http://dx.doi.org/10.1007/s00192-015-2847-x.
16. **Clayson D, Wild D, Doll H, Keating K, Gondek K.** 2005. 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 **96:**350–359. http://dx.doi.org/10.1111/j.1464-410X.2005.05630.x.