The Urinary Microbiome

Improving Diagnostics and Management of Urinary Tract Infections in Adult Females

Toby C. Chai, MD[a,*], Alan J. Wolfe, PhD[b], Linda Brubaker, MD[c]

KEYWORDS

• Microbiome • Urinary microbiome • UTI • Diagnostics • UTI management

KEY POINTS

- Urine specimens collected from normal adult female bladders are populated by different genera of bacteria termed the urinary microbiome or urinary microbiota.
- The female urinary tract outlet is located within the genital tract; therefore, there exist interactions between the urinary and genital microbiomes.
- The significance of the urinary microbiome in the pathobiology of urinary tract infection (acute bacterial cystitis) is unknown currently.
- The standard urine culture test has been designed to identify only the most common uropathogenic bacteria. However, other genera of bacteria which can cause UTI do not grow with standard urine culture methodology.
- While bacterial molecular genetic testing has a distinct advantage of shortened time, compared to bacteriologic culture technique, to identify bacteria present in urine, the optimal integration of these techniques into evaluation and management of UTI has not been defined.

INTRODUCTION

Although urinary tract infections (UTI) are common, most clinicians do not think about them much until they experience patients (or family/friends) with frequent recurrences or serious infectious complications, such as pyelonephritis or urosepsis. The current clinical view is that UTIs are common—like the common cold—and that treatment is

[a] Department of Urology, Boston University Chobanian & Avedisian School of Medicine and Boston Medical Center, 725 Albany Street, Suite 3B, Boston, MA 02118, USA; [b] Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, 2160 South First Avenue, Building 115, Room 215, Maywood, IL 60153, USA; [c] Department of Obstetrics, Gynecology, and Reproductive Sciences, Division of Urogynecology and Reconstructive Pelvic Surgery, University of California, 9300 Campus Point, Mail Code 7433, La Jolla, CA 92037-7433, USA

* Corresponding author. 725 Albany Street, Suite 3B, Boston, MA 02118, USA

E-mail address: tobychai@bu.edu

Infect Dis Clin N Am 38 (2024) 241–253

https://doi.org/10.1016/j.idc.2024.03.003

id.theclinics.com

straightforward based on a sound evidence base with a clear goal. Yet, for affected patients, emerging evidence suggests that there are opportunities to better understand UTI, improve treatment, and refine goals of therapy to reduce potential harms. This article reviews common clinical beliefs regarding UTI and provides the evidence for an updated concept that the bladder has a resident bacterial community (microbiome) and that the mere presence of a microbe in the bladder should not trigger UTI diagnosis or antibiotic therapy.

The term "UTI" is familiar within and beyond clinical medicine, with multiple forms of direct-to-consumer advertising for UTI-associated products. Within medicine, it is a broad term that can be applied to any uropathogenic microorganism (bacterial, fungal, viral) infecting any part of the urinary tract from the kidneys to the urethra in both sexes. In clinical practice, however, the term "UTI" is generally usually used to mean uncomplicated acute bacterial cystitis, typically in a non-pregnant patient who is otherwise healthy. UTIs in individuals with urinary tract abnormalities/obstruction, renal calculi (stones), urinary catheters, or who are pregnant, male, or immunocompromised are considered "complicated UTIs."

Women are disproportionately affected by UTI and UTI-mimics (ie, conditions that cause symptoms attributed to UTI without evidence of uropathogenic microbes). In women, the reproductive tract and urinary tract are separate until the end of the urinary tract (ie, urethral meatus), which has a separate external opening above the vagina. Although the length of the female urethra has been implicated (without evidence) as a UTI risk factor, it is more logical to consider the biological events in a woman's life course, such as menstrual cycles, pregnancy/delivery, coital activity, and estrogen levels as important factors that impact UTI pathogenesis and UTI risk.

In men, the lower urinary tract, downstream of the bladder, is anatomically connected to the reproductive tract (prostate, vasa, epididymis, testes); thus, a UTI in men also could involve simultaneous prostatitis, epididymitis, and/or epididymoorchitis. This is because uropathogens that access the bladder retrogradely through the urethra also can access the reproductive tract. These unique and differing characteristics between men and women require that any discussion of UTI be separate for the 2 sexes. This article discusses the urinary microbiome in relation to uncomplicated acute bacterial cystitis (UTI) in women unless otherwise specified.

Lay and scientific awareness of the human microbiome has increased significantly over the past decade. Most individuals assume "microbiome" refers to the huge number of microbes present in the gut, and the multiple studies that relate gut microbes to human health states. Yet, over the past decade there have been an increasing number of investigations into clinical questions related to the discovery of the female urinary microbiome or microbiota. The concept that bladder sterility was the state of a normal bladder and non-sterility represented a UTI-diseased state was accepted by the medical community. Two publications clearly showed that this concept was not correct: these publications reported the use of 16S rRNA gene sequencing[1] and an enhanced urine culture technique[2] to demonstrate the presence of microbes in the female urinary bladder, now known as the female urinary microbiota. In retrospect, there were several earlier clinical observations that should have prompted clinicians to challenge "the bladder is sterile" concept. In 1956, Edward Kass from Boston City Hospital (now Boston Medical Center)/Harvard Medical School published that 6% to 23% of urine specimens obtained by urethral catheterization from women with different medical conditions, but all without symptoms of UTI, had greater than 10^5 bacteria/mL by culture.[3] The bacteria were identified as bacillus-form and gram-negative, which we might presume to be *Escherichia coli*. As early as 1956, there was concern for high prevalence (thought to be ~20%) of "occult" renal infections (pyelonephritis, renal

abscess). Furthermore, pyelonephritis was thought to be the leading cause of end-stage renal failure.[4] Dr Kass believed that these "asymptomatic" individuals had UTI and would go on to develop pyelonephritis and therefore used the term "asymptomatic *infections.*" Today, however, we would use the term "asymptomatic bacteriuria" to describe these individuals.

The introduction by Jack Lapides, MD, at the University of Michigan of clean intermittent self-catheterizations (CISC) for bladder emptying dysfunction also challenged the notion that a healthy bladder required sterility.[5] CISC was initially rejected by the medical community for fear that clean, and not sterile, technique of urethral catheter reuse would convert the sterile bladder into an infected, non-sterile bladder, resulting in UTI and then pyelonephritis. Dr Lapides stated his hypothesis in the 1972 publication that "*organisms supposedly ascending through the urethra are of doubtful importance in the genesis of urinary infection,*" which is prescient of the development in the literature over the past 10 to 15 years. A 10-year follow-up study of individuals using CISC (60 total, of which 39 were females) found that none had long-term sequelae such as deterioration of renal function from pyelonephritis.[6]

In separate work, an observant physician, Rosalind Maskell, noted slow-growing microbes in urine obtained from patients with UTI-like symptoms, but negative standard urine cultures.[7,8] These slow-growing microbes require growth conditions that differ from those of the standard urine culture. Although Maskell correctly concluded that standard urine culture was insufficient for diagnosis of many urinary disorders, unfortunately, her conclusion was repudiated and ignored, an all-too-common event in the history of women in medicine[9]

In our current era, the risk of pyelonephritis within 30 days following diagnosis of UTI (specifically stated as uncomplicated cystitis) was investigated in a large nationwide sample of ~750,000 women in Sweden.[10] The odds ratio for developing pyelonephritis after UTI was ~1%. Most of these women (78%) did not fill their antibiotic prescriptions after being diagnosed with UTI. For women who filled their antibiotic prescriptions, the risk reduction in developing pyelonephritis, compared to those not taking antibiotics, was too low to be clinically significant. The authors concluded that non-antibiotic treatments for UTIs could be considered, though future studies should be done to define those at highest risk for pyelonephritis to allow better selection of those who should be treated with antibiotics. This work highlights the need for clinicians to be specific about treatment goals: whether it is symptom reduction (occurs quickly—supporting very short course antibiotics), prevention of pyelonephritis (difficult to justify based on rarity of event), microbial eradication (requires follow-up testing), or other goals. Clinically, it is clear that currently the main reason for UTI treatment is symptom reduction. Given this, clinicians should prescribe adjuvants to facilitate symptom relief, such as anti-inflammatory and analgesic agents, and discontinue antibiotics once symptoms resolve (often within 24–48 hours). However, most clinical studies comparing single dose to 3 or more days of therapy have found lower efficacy with the shorter regimen and thus current guidance for treatment of uncomplicated UTI ranges between 3 and 5 days depending on the agent used. It is generally accepted that symptoms are caused by a host response, although the specific mechanisms of symptom generation and resolution are poorly understood.

It is within this context that we need to understand the role of the urinary microbiota in UTI pathogenesis. Identifying the microbes present in health versus those present during UTI is only the beginning to understand the complexity of UTI pathogenesis, including differentiating normal variations in the urinary microbiota from pathologic states that require clinical intervention. Since the bladder was considered to be sterile, mechanisms underlying the host response to microbes are understudied. It makes

biologic sense that the microbiota of different niches (bladder, vagina, and gut) interact with each other in health, as well as during a UTI event; however, these relationships remain poorly understood. Future research should close knowledge gaps regarding the interactions between the urinary microbiota and the host, amongst the microbiota of adjacent niches, and between the microbes within the same microbiota. The new knowledge should result in improved UTI treatment in the age of antibiotic stewardship.

PRESENCE OF URINARY MICROBIOTA IN ASYMPTOMATIC WOMEN

In a seminal study, 16S rRNA gene sequencing was used to detect many different types of bacteria in urine samples obtained from asymptomatic women using urethral catheterization and suprapubic aspiration techniques.[1] Soon after, similar results were observed by another research team.[11] To determine whether these bacteria were alive, the first team of investigators developed an enhanced culture method called "expanded quantitative urine culture" (EQUC). Relative to the standard urine culture (SUC) method typically used by clinical microbiology laboratories, EQUC used greater volumes of urine, more types of culture media, varied atmospheric conditions, and prolonged incubation times. In this early study, EQUC detected 35 different bacterial genera and 85 different bacterial species,[2] most of which SUC did not detect. The most common bacterial genera detected with EQUC in the urinary microbiota were *Lactobacillus* (15% of study subjects), *Corynebacterium* (14.2%), *Streptococcus* (11.9%), *Actinomyces* (6.9%), and *Staphylococcus* (6.9%). The authors concluded that EQUC was more sensitive than SUC in detecting both uropathogenic and non-uropathogenic bacteria in the urine and confirmed the presence of living resident female urinary microbiota. In another study with a larger population of asymptomatic women (n = 224), using both EQUC and 16S rRNA gene sequencing, investigators found that the main constituents of the urinary microbiota were members of the genera *Lactobacillus*, *Streptococcus*, *Gardnerella,* and *Escherichia*.[12] A recent study compared results obtained by EQUC with 16S rRNA sequencing technique applied to urine specimens obtained from urethral catheterization of 59 asymptomatic women,[13] finding that 16S rRNA gene sequencing was more sensitive than EQUC in detecting bacteria. The most common families of bacteria identified in this study with 16S rRNA sequencing included *Streptococcaceae, Staphylococcaceae, Pseudomonadaceae, Lactobacillaceae,* and *Enterobacteriaceae*. However, EQUC also identified bacteria not detected by 16S rRNA gene sequencing, including *Enterococcaceae, Peptoniphilaceae*, *Morganellaceae*, *Corynebacteriaceae,* and *Leuconostocaceae*. There was only 15% concordance in identities of bacteria detected by 16S rRNA sequencing compared to EQUC. While EQUC and bacterial DNA sequencing each have advantages and disadvantages, these tests complement each other. DNA sequencing detects more taxa but does not quantify the microbes; EQUC detects fewer taxa but is quantitative. Each test detects microbes that the other tests do not. These studies showed that many types of bacteria, both uropathogens and non-uropathogens, exist in the bladder of healthy asymptomatic women and that sequencing and enhanced culture capture overlapping but distinct parts of the urinary microbiota.

EXPANDED QUANTITATIVE URINE CULTURE IN URINARY TRACT INFECTION MANAGEMENT

While clinicians currently rely on SUC results to guide management of UTI, it is important to point out that obtaining a careful patient history, performing physical examination including pelvic examination when appropriate, interpreting the laboratory urinalysis,

and applying clinical acumen and judgment also are important in determining diagnosis and planning treatment. Although a UTI diagnosis may seem simple, a UTI diagnosis has complexities that are not always recognized. Starting with symptoms, it is generally understood that clinicians expect to obtain a history that indicates a change in urinary symptoms. In young, healthy women, this is commonly an abrupt symptom change with acute onset of dysuria, frequency, and urgency. However, later in life when the prevalence of lower urinary tract symptoms is much higher, it can be difficult to determine which urinary tract symptoms indicate UTI. Generally, the most important symptom that supports a UTI diagnosis, based on EQUC and SUC results, is dysuria.[14] The current definition of a "positive" SUC result is growth of a single predominant uropathogen at the quantity of greater than 10^5 colony-forming units (CFU)/mL from a "clean catch" midstream voided urine specimen and a lower cutoff of greater than 10^3 CFU/mL from a catheterized urine specimen. The culture conditions and media used in SUC are designed to primarily detect growth of the *Enterobacteriaceae* family of gram-negative bacteria, which includes *E coli*, *Klebsiella* species, and *Enterobacter* species, among others. EQUC is more sensitive than SUC because EQUC uses 100 × more urine and additional culture conditions for growth of bacteria that grow poorly or do not grow at all under SUC conditions.[15] However, as with other diagnostic tests, increasing sensitivity often comes with a price of decreasing specificity. Identification of more species by EQUC does not necessarily mean that these species are causative for UTI. A way to increase the specificity of EQUC is by defining what constitutes a positive EQUC result based on analysis of differences in EQUC results in women with and without symptoms of UTI.[16] These data showed that, with EQUC, while there were higher uropathogen loads (higher CFU/mL) in women with UTI symptoms, the differences between uropathogen loads between the 2 cohorts differed depending on species of the uropathogen. Thus, a potential way to increase specificity of EQUC is to define different cutoff positive values (eg, set different cut-points of CFU/mL) based on which uropathogenic species grows out from EQUC.

These studies lead to a larger question—that is, what is the "gold standard" for UTI diagnosis? An even bigger question is—which symptoms are related, or caused, by which microbe (alone or through actions of the entire microbial community)? For many years, once testing identified the presence of *E coli*, further thought and investigation was set aside, with the belief that the causative agent had been identified. To see whether associations existed between specific microbes and symptoms, investigators analyzed EQUC results obtained from culturing catheterized urine from 43 post-menopausal women with a history of recurrent UTIs.[17] Most of the women (63%) did not feel like they had a current UTI at time of urine collection, though more participants who did not feel like they had current UTI had dysuria (74%) compared to those who felt like they had UTI (50%). These participants stratified

Table 1
Five different clinical phenotypes

Phenotype	Characteristics
A	Odor, Cloudiness, Vaginal Estrogen Use
B	Frequency, Back Pain, Incomplete Emptying, Vaginal Estrogen Use
C	Pain/Burning, Odor, Cloudiness, Urgency
D	Frequency, Urgency, Pain/Burning, Vaginal Estrogen Use
E	Frequency, Urgency, Pain/Burning, Odor, overactive bladder (OAB), Sexual Activity

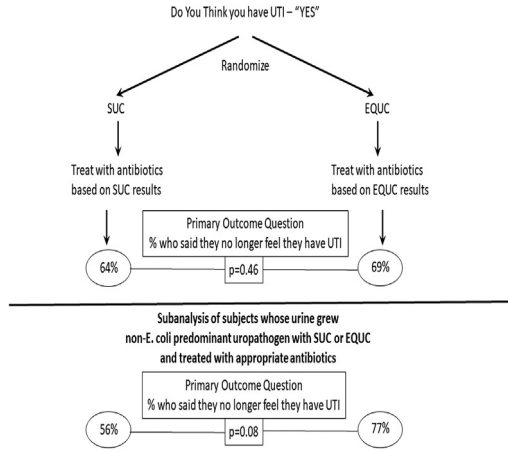

Fig. 1. Summarization of expanded quantitative urine culture (EQUC) studies.

into 5 different clinical phenotypes (**Table 1**): Phenotype A: cloudy and malodorous urine using vaginal estrogen; Phenotype B: urinary frequency, low back pain, incomplete emptying; Phenotype C: dysuria, urgency, urine cloudiness/malodorous; Phenotype D: dysuria, urgency, urine cloudiness/malodorous with vaginal estrogen use; Phenotype E: frequency, urgency, dysuria, malodorous, overactive bladder, and sexually active. Whereas the investigators detected *E coli* in each of the phenotypic groups, they found different bacterial species associated with each of these groups. These results suggest either that the *E coli* detected in each phenotypic group differed somehow or that the different symptoms resulted from the functions of the entire microbial community.

In a randomized trial, EQUC was compared to SUC to determine which diagnostic test was better in guiding clinicians to treat or not to treat (with antibiotics) women presenting with symptoms suspected to be a UTI, with the primary outcome being symptom relief based on antibiotic treatment guided by results from EQUC or SUC.[18] The schematic of this trial is shown in **Fig. 1**. Women who thought they had UTI were randomized to either EQUC or SUC testing on catheterized urine specimens and, based on these culture test results, clinicians followed a protocol to treat or not to treat with antibiotics. The definition of a "positive" SUC result was based on the presence of any uropathogen (no cutoff CFU/mL); the definition of a "positive" EQUC result was similar to SUC with additional growth of any of these following 8 species using no cutoff CFU/mL: *Actinotignum schaalii, Aerococcus sanguinicola, Aerococcus urinae, Alloscardovia omnicolens, Corynebacterium riegelii, Corynebacterium urealyticum, Oligella urethralis,* and *Streptococcus anginosus*. If there was growth of more than 1 uropathogen on EQUC or SUC, a common antibiotic that covered each uropathogen was used. Multiple antibiotics were prescribed to cover every cultured uropathogen, if needed. The primary outcome was the symptom response of the participants. A responder or non-responder was defined by the participant's binary response (yes or no) to a single symptom question ("do you continue to have UTI symptoms?"). The outcome was similar between SUC (64% responder rate) and EQUC (69% responder rate) without statistical difference. Though this trial was not powered prehoc to detect benefit of EQUC over SUC in the specific situation of women whose cultures grew non-*E coli* uropathogens, a sub-analysis revealed that EQUC did result in a trend toward a significantly higher responder rate than SUC (77% vs 56%, $P = .08$).

This is the only randomized trial that compared clinical utility of 2 diagnostic culture testing methods in UTI management. The symptom response rate of 69% using EQUC to guide antibiotic treatment suggests that there is additional room for improvement in UTI treatment. While other trials have incorporated test-of-cure culture as part of outcome data, this trial did not perform test-of-cure culture (with either SUC or EQUC). Potentially, paired comparisons of SUC and EQUC culture changes pre-antibiotic and post-antibiotic treatment in relationship to symptom response changes might have provided additional useful information, such as possibly correlating response rates to change in uropathogen growth on EQUC pre-antibiotic and post-antibiotic treatment. This is especially important in women with frequent/recurrent UTI events, as these individuals are more likely to have non-*E coli* microbes identified during their UTI events.

Since neither EQUC nor SUC measures host responses, increasing diagnostic specificity could include combining EQUC results with another diagnostic test measuring host responses, such as urinary cytokines.[19,20] By combining diagnostic tests that measure both the host and pathogen factors/characteristics occurring during UTI, one would be able to maximize both sensitivity and specificity in UTI diagnosis.

A practical factor needs to be taken into consideration if EQUC is to be broadly used in UTI management. This factor is the need to obtain urine specimens via urethral catheterization to minimize contamination from vagina/perineum that would occur if voided specimens were used for testing. While urethral catheterization is routinely performed in an urogynecology or urology clinic, this is not necessarily true for primary care, general internal medicine, ID clinics, and emergency departments. These ambulatory care environments do not routinely catheterize women to obtain urine for diagnostic testing. While an alternative voided collection method requiring patient education and use of a collection aid device did decrease vaginal/perineal flora detected by EQUC,[21] urine from urethral catheterization is still the best in minimizing contamination. The addition of urethral catheterization for these clinics may impose a hurdle difficult to overcome. Finally, urethral catheterization may induce pain during the catheterization and post-catheterization dysuria for some women, though the vast majority tolerates this minor procedure.

In summary, EQUC is a more sensitive culture technique compared to SUC for detecting more types of uropathogens. To maximize specificity, EQUC should be used on catheterized specimens. However, one of the main hurdles to overcome in EQUC, similar to SUC, is that the growth of uropathogens in culture may not be clinically important. Finding uropathogens on EQUC does not necessarily mean that the symptoms are caused by these uropathogens. Further research into host factors that are elicited during UTI will be a useful adjunct test to EQUC. Other areas for research could include longitudinal EQUC testing accompanied by correlative symptom changes in order to potentially associate a specific bacterial species with symptom changes. Test-of-cure EQUC done at multiple post-treatment time points in antibiotic trials with correlation to symptom changes can result in finding stronger associations between symptoms and EQUC findings.

BACTERIAL GENETIC (MOLECULAR) DIAGNOSTIC TESTING IN URINARY TRACT INFECTION MANAGEMENT

Clinicians may be offered the opportunity to order non-culture tests to identify microbes in urine. There are 2 general high-throughput approaches to identifying microbes in a urine specimen: targeted and non-targeted. The targeted approach

utilizes select polymerase chain reaction (PCR) primer sequences that can uniquely detect a pre-determined set of bacteria, primarily those known to cause UTI. In one way, this multiplex PCR (mPCR) technique can be analogized to SUC as both tests target certain pre-determined bacteria, usually those bacteria most likely to cause UTIs. However, the mPCR technique is faster than the SUC, delivering results as fast as 6 hours. Also, if designed properly, mPCR can detect microbes that SUC cannot. For example, a set of investigators found that an mPCR method pre-determined to detect 31 types of bacteria considered to be uropathogens was more sensitive in detecting the presence of these bacteria than SUC.[22] The non-targeted approach takes advantage of next generation sequencing (NGS). In this approach, universal PCR primers are used to amplify all the 16S rRNA genes within a urine specimen. These amplicons are then sequenced, and those sequences are compared to a database to identify each bacterium. 16S rRNA gene sequencing can be analogized to the EQUC in that both tests "widen the net" by identifying more bacteria compared to the more targeted methods, such as PCR and SUC. One downside to 16S rRNA gene sequencing is that it can only detect bacteria and then often only to the genus level. In contrast, mPCR, EQUC, and SUC can reach the species level. While both mPCR and 16S rRNA gene sequencing identify many more types of bacteria than culture-based tests, identification of bacteria by these molecular tests does not necessarily mean that these bacteria are alive. On the other hand, molecular tests can detect bacteria that are alive but are not cultured. Another problem with 16S rRNA gene sequencing is that some reports provide "recommendations" or "considerations" for treatment. Clinicians are cautioned to avoid acceptance of such recommendations without careful evaluation of the patient, her clinical status, and the risks of antibiotic therapy. The mere presence of bacterial DNA should not be equated with a UTI. Moreover, clinicians should evaluate whether there is a lack of beneficial microbes and minimize further disruptions of a recovering urinary microbial community.

In a non-randomized study, 16S rRNA gene sequencing was compared to SUC to determine the percentage of positive results and treatment outcomes (symptom relief).[23] The study found that individuals who were treated based on positive 16S rRNA gene sequencing results had more symptomatic relief compared to those that were treated based on positive SUC results. In this small study (n = 44), every individual (100%) undergoing 16S rRNA gene sequencing had a positive result, whereas only 30% undergoing SUC had a positive result. It might not be surprising that non-blinded treatment with antibiotic in 100% of individuals who had positive sequencing test resulted in better outcomes compared to 30% of individuals who were treated with antibiotic in the SUC testing arm.

16S rRNA gene sequencing was performed on catheterized urine specimens collected from 49 women presenting with symptoms of UTI.[24] SUC was also performed on all urine specimens. The urine specimens were stratified into 2 groups based on whether women had recurrent UTI defined as $\geq$2 SUC-proven UTI in the past 6 months or $\geq$3 SUC-proven UTIs in the past 12 months (n = 31) or had no UTIs in the past 3 years (n = 11 without recurrent UTI). The theory was that a difference in the urinary microbiota could possibly explain why some women have recurrent UTI and others do not. While the demographics of the 2 strata had no statistical differences with the small sample sizes, the mean age of women with recurrent UTI were older (55 years of age) compared to those with without recurrent UTI (51 years of age). Furthermore, the women were not matched for menopausal status (68% of recurrent UTI group were menopausal, 55% of without recurrent UTI group were menopausal). Because prior antibiotic use would likely affect the urinary microbiome, it was surprising that prior antibiotic use was actually higher in the group without

recurrent UTI (64% had prior antibiotic use) compared to the group with recurrent UTI group (52% had prior antibiotic use). This was counterintuitive and there were no details of how investigators defined prior antibiotic use. Similar to other studies, the detection rate for bacteria was significantly higher with 16S rRNA gene sequencing compared to SUC in both cohorts. Clinicians should interpret these studies with caution. Just because a new telescope sees more stars does not mean there ARE more stars. Just because a new test finds more bacteria, this does not mean there ARE more bacteria. What clinicians need is helpful information to guide assessment and treatment planning. Broad knowledge about the state of the urinary microbiota should be helpful for clinicians who know their patients; clinicians should avoid reflex prescribing based on a laboratory recommendation.

A drawback espoused about sequencing compared to urine culture technique is that antimicrobial resistance cannot be measured. However, this drawback can be addressed. Using PCR to sequence antibiotic resistance (ABR) genes, antibiotic resistance patterns can be measured.[25] The concept of modulation of ABR genes by bacteria within the urinary microbiota was described in this publication. The idea is that the constituent bacteria work together to regulate ABR gene expression within the community, including within uropathogens. This community effect would not be detectable by culture techniques because uropathogens lose communication with the resident microbiota when they are isolated by culturing on a Petri dish.

Investigators have studied how uropathogens interact with other bacteria within the urinary microbiota.[26] These investigators found that uropathogens, cultured from UTI individuals, were better able to control growth of commensal bacteria isolated from the urinary microbiota of asymptomatic individuals than vice-versa. Furthermore, gram-positive uropathogens regulated growth of commensal urinary microbiome bacteria differently than gram-negative uropathogens. While this study utilized *in vitro* culture techniques, future studies should seek methods and techniques to study *in vivo* interactions within the urinary microbiota since what happens *in vitro* may not reflect what occurs *in vivo* within the urinary microbiota.

While 16S rRNA gene sequencing and mPCR, compared to culture-based techniques, result in increased sensitivity of detection of bacteria from urine specimens, the question becomes the clinical significance of the additional sensitivity. There is likely decreased specificity as the sensitivity of the test goes up. Investigating how molecular testing changes longitudinally after antibiotic treatment for UTI could result in better test performance for these molecular tests.

INFLUENCE ON OTHER MICROBIOMES (GUT AND VAGINAL) ON URINARY MICROBIOME AND URINARY TRACT INFECTION

Since the 3 tracts (urinary, reproductive, gut) are in close proximity in the pelvic outlet in the female, it is plausible that these microbiomes (urinary, vaginal, and stool) interact with each other. The urinary and vaginal microbiota are similar, while both are dissimilar to the gastrointestinal tract.[27] This is likely because of the closer proximity of the bladder/urethra to the vagina. Many bacteria seem to see the urogenital tract as 1 niche. A recent study touting similarity of urinary microbiota with gut microbiota was based on a highly heterogeneous population (subjects aged <3 months to adult, renal transplant individuals, subjects who underwent fecal transplants, individuals with UTI) and heterogenous urine collection techniques (voided and catheterized).[28] Given these issues, especially the use of voided urine, the findings from this study are difficult to interpret.

Though the GI tract microbiome is dissimilar to the urinary microbiome, several studies suggested that changing the gut microbiome altered the risk of UTI. Investigators used 16S rRNA gene and shotgun metagenomic sequencing to obtain evidence for the hypothesis that the presence of certain pathogens in the gut microbiome are associated with increased risk of UTI development post-renal transplant, a clinical group that has especially high risks of UTI and serious consequences, including potential loss of the transplanted kidney.[29,30] Furthermore, case reports and single-cohort retrospective studies have provided evidence that fecal transplant for *Clostridium difficile* colitis can reduce UTI risk.[31–34] However, no analysis of the urinary microbiota was performed in these fecal transplant studies, so how the urinary microbiota changed after fecal transplant remains unknown.

The vagina is another ecologic niche that likely contributes to the urinary microbiota and may play a role in UTI pathogenesis. The identities of bacteria that populate the vagina and urine showed that these 2 niches are similar.[27] Randomized controlled trials have clearly demonstrated that low-dose intravaginal estrogen can decrease the frequency of UTI recurrence in post-menopausal women who have recurrent UTI.[35,36] Changes in both the vaginal and urinary microbiomes have been observed with low-dose vaginal estrogen use in post-menopausal women.[37] Although probiotics that target the vaginal microbiota to reduce risk of UTI have been suggested, the evidence does not support a change in clinical recommendations at this time.[38] Some have proposed vaginal microbiota transplant to improve vaginal health and to resolve vaginal dysbiosis[39]; however, there is no current evidence that vaginal microbiome transplant is effective in reducing UTI risk. Since the vagina and bladder niches are adjacent, study of mechanisms by which the vaginal and urinary microbiota interact is likely to elucidate additional mechanisms by which UTI develops.[27,40]

THE FUTURE OF URINARY TRACT INFECTION MANAGEMENT IN THE URINARY MICROBIOME ERA

The traditional clinical concept of a UTI is based on the incorrect framework of causation by a single uropathogenic bacterial species that normally resides in the gut and migrates into a sterile bladder to incite a host immune response, giving rise to acute lower urinary tract symptoms, including dysuria, urinary frequency, and urinary urgency. Diagnosis and treatment based on this incorrect framework are likely associated with missed opportunities to refine treatment and reduce harm associated with antibiotic misuse and over-use. There are promising non-antibiotic prevention strategies, such as vaccines, that are beyond the scope of this manuscript.

Scientific honesty about the limitations of our knowledge will help prioritize clinically relevant research studies that close knowledge gaps about the detailed physiologic interactions between the host and the urinary microbiota during health and UTI and how the microbiota between adjacent niches interact with each other. While a small proportion of individuals with UTIs progress to develop serious UTI manifestations, including pyelonephritis, urosepsis, and renal abscess, we do not understand how the different microbial niches might be involved in or prevent these outcomes. Currently, the main rationale to treat UTIs with antibiotics is to relieve patients' symptoms, although treatment often continues well beyond symptoms resolution, often with the unsubstantiated goal of reducing bladder microbial load. In the era of antibiotic stewardship, treatment of UTIs presents a challenge to balance the use antibiotics to treat UTI against potential collateral damage caused by those antibiotics. To advance this field, more in depth understanding of the myriad of biologic mechanisms

underlying UTI pathogenesis in the context of the existence of the urinary microbiota will be required. While antibiotics will remain the mainstay of treatment for the time being, non-antibiotic therapies as adjuncts or solo treatments must be developed and specific strategies tested in rigorously designed randomized trials. Understanding the host response to UTI will help deliver other treatments that modulate the host response, resulting in reduction in symptoms without concomitant increase in morbidity from UTI.

Clinicians who want to help their patients with improved UTI care must learn about the evolving evidence related to the urinary microbiota and the implications for UTI diagnosis and treatment. No clinician wants to miss an important diagnosis or provide incorrect treatment. The necessary research should not be delayed, given the prevalence and cost of UTI care.

AUTHOR CONTRIBUTIONS

T.C. Chai conceived of the manuscript and wrote the first draft. All authors contributed to the final manuscript and approved the submitted version.

FUNDING

This work is funded by internal funds to A.J. Wolfe. Our funding sources had no role in the process of writing this article.

ACKNOWLEDGMENTS

The authors acknowledge the clinicians, study participants, and scientists who recruited the participants, who provided the samples, and who performed the analyses that resulted in the primary research papers upon which this article is based.

DISCLOSURE

L. Brubaker discloses editorial stipends from JAMA and UpToDate. T.C. Chai discloses his role as a consultant for Glaxo Smith Kline involved in the commercialization of their new antibiotic, gepotidacin, for uncomplicated UTI. A.J. Wolfe discloses advisory board membership for Pathnostics and Urobiome Therapeutics, funding from Pathnostics, the Craig Neilsen Foundation, National Institutes of Health, United States, and an anonymous donor.

REFERENCES

1. Wolfe AJ, Toh E, Shibata N, et al. Evidence of uncultivated bacteria in the adult female bladder. J Clin Microbiol 2012;50(4):1376–83.
2. 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(3):871–6.
3. Kass EH. Asymptomatic infections of the urinary tract. Trans Am Physiol 1956;69:56–64.
4. Anumudu S, Eknoyan G. Pyelonephritis: A Historical Reappraisal. J Am Soc Nephrol 2019 Jun;30(6):914–7.
5. Lapides J, Diokno AC, Silber SJ, et al. Clean, intermittent self-catheterization in the treatment of urinary tract disease. J Urol 1972 Mar;107(3):458–61.
6. Diokno AC, Sonda LP, Hollander JB, et al. Fate of patients started on clean intermittent self-catheterization therapy 10 years ago. J Urol 1983 Jun;129(6):1120–2.

7. Maskell R, Pead L, Sanderson RA. Fastidious bacteria and the urethral syndrome: a 2-year clinical and bacteriological study of 51 women. Lancet 1983 Dec 3; 2(8362):1277–80.
8. Maskell RM. The natural history of urinary tract infection in women. Med Hypotheses 2010;74(5):802–6.
9. Wynn R. Saints and Sinners: Women and the Practice of Medicine Throughout the Ages. JAMA 2000;283(5):668–9.
10. Jansåker F, Li X, Vik I, et al. The Risk of Pyelonephritis Following Uncomplicated Cystitis: A Nationwide Primary Healthcare Study. Antibiotics (Basel) 2022;11(12): 1695.
11. Fouts DE, Pieper R, Szpakowski S, et al. 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 2012;10:174.
12. Price TK, Hilt EE, Thomas-White K, et al. The urobiome of continent adult women: a cross-sectional study. BJOG 2020;127(2):193–201.
13. Vaughan MH, Zemtsov GE, Dahl EM, et al. Concordance of urinary microbiota detected by 16S ribosomal RNA amplicon sequencing vs expanded quantitative urine culture. Am J Obstet Gynecol 2022;227(5):773–5.
14. Dune TJ, Price TK, Hilt EE, et al. Urinary Symptoms and Their Associations With Urinary Tract Infections in Urogynecologic Patients. Obstet Gynecol 2017;130(4): 718–25.
15. Hochstedler BR, Burnett L, Price TK, et al. Urinary microbiota of women with recurrent urinary tract infection: collection and culture methods. Int Urogynecol J 2022;33(3):563–70.
16. 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(5):1216–22.
17. Burnett LA, Hochstedler BR, Weldon K, et al. Recurrent urinary tract infection: Association of clinical profiles with urobiome composition in women. Neurourol Urodyn 2021;40(6):1479–89.
18. Barnes HC, Wolff B, Abdul-Rahim O, et al. A Randomized Clinical Trial of Standard versus Expanded Cultures to Diagnose Urinary Tract Infections in Women. J Urol 2021;206(5):1212–21.
19. Bilsen MP, Treep MM, Aantjes MJ, et al. Diagnostic accuracy of urine biomarkers for urinary tract infection in older women: a case-control study. Clin Microbiol Infect 2023. S1198-743X(23)00488-3.
20. Parnell LKS, Luke N, Mathur M, et al. Elevated UTI Biomarkers in Symptomatic Patients with Urine Microbial Densities of 10,000 CFU/mL Indicate a Lower Threshold for Diagnosing UTIs. Diagnostics 2023;13(16):2688.
21. Southworth E, Hochstedler B, Price TK, et al. A Cross-sectional Pilot Cohort Study Comparing Standard Urine Collection to the Peezy Midstream Device for Research Studies Involving Women. Female Pelvic Med Reconstr Surg 2019 Mar/Apr;25(2):e28–33.
22. Wojno KJ, Baunoch D, Luke N, et al. Multiplex PCR Based Urinary Tract Infection (UTI) Analysis Compared to Traditional Urine Culture in Identifying Significant Pathogens in Symptomatic Patients. Urology 2020;136:119–26.
23. McDonald M, Kameh D, Johnson ME, et al. A Head-to-Head Comparative Phase II Study of Standard Urine Culture and Sensitivity Versus DNA Next-generation Sequencing Testing for Urinary Tract Infections. Rev Urol 2017;19(4):213–20.

24. Yoo JJ, Shin HB, Song JS, et al. On The Behalf Of Sms Soonchunhyang Microbiome Multi-Disciplinary Study Group. Urinary Microbiome Characteristics in Female Patients with Acute Uncomplicated Cystitis and Recurrent Cystitis. J Clin Med 2021;10(5):1097.
25. Vollstedt A, Baunoch D, Wolfe A, et al. Bacterial Interactions as Detected by Pooled Antibiotic Susceptibility Testing (P-AST) in Polymicrobial Urine Specimens. J Surg Urol 2020;1:101.
26. Zandbergen LE, Halverson T, Brons JK, et al. The Good and the Bad: Ecological Interaction Measurements Between the Urinary Microbiota and Uropathogens. Front Microbiol 2021;12:659450.
27. Thomas-White K, Forster SC, Kumar N, et al. Culturing of female bladder bacteria reveals an interconnected urogenital microbiota. Nat Commun 2018;9(1):1557.
28. Dubourg G, Morand A, Mekhalif F, et al. Deciphering the Urinary Microbiota Repertoire by Culturomics Reveals Mostly Anaerobic Bacteria From the Gut. Front Microbiol 2020;11:513305.
29. Magruder M, Sholi AN, Gong C, et al. Gut uropathogen abundance is a risk factor for development of bacteriuria and urinary tract infection. Nat Commun 2019;10(1):5521.
30. Magruder M, Edusei E, Zhang L, et al. Gut commensal microbiota and decreased risk for Enterobacteriaceae bacteriuria and urinary tract infection. Gut Microb 2020;12(1):1805281.
31. Tariq R, Pardi DS, Tosh PK, et al. Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection Reduces Recurrent Urinary Tract Infection Frequency. Clin Infect Dis 2017;65(10):1745–7.
32. Aira A, Rubio E, Vergara Gómez A, et al. rUTI Resolution After FMT for Clostridioides difficile Infection: A Case Report. Infect Dis Ther 2021;10(2):1065–71.
33. Bier N, Hanson B, Jiang ZD, et al. A Case of Successful Treatment of Recurrent Urinary Tract Infection by Extended-Spectrum β-Lactamase Producing Klebsiella pneumoniae Using Oral Lyophilized Fecal Microbiota Transplant. Microb Drug Resist 2023;29(1):34–8.
34. Tariq R, Tosh PK, Pardi DS, et al. Reduction in urinary tract infections in patients treated with fecal microbiota transplantation for recurrent Clostridioides difficile infection. Eur J Clin Microbiol Infect Dis 2023;42(8):1037–41.
35. Perrotta C, Aznar M, Mejia R, et al. Oestrogens for preventing recurrent urinary tract infection in postmenopausal women. Cochrane Database Syst Rev 2008;(2):CD005131.
36. Ferrante KL, Wasenda EJ, Jung CE, et al. Vaginal Estrogen for the Prevention of Recurrent Urinary Tract Infection in Postmenopausal Women: A Randomized Clinical Trial. Female Pelvic Med Reconstr Surg 2021;27(2):112–7.
37. Thomas-White K, Taege S, Limeira R, et al. Vaginal estrogen therapy is associated with increased Lactobacillus in the urine of postmenopausal women with overactive bladder symptoms. Am J Obstet Gynecol 2020;223(5):727.e1–11.
38. Reid G, Dols J, Miller W. Targeting the vaginal microbiota with probiotics as a means to counteract infections. Curr Opin Clin Nutr Metab Care 2009;12(6):583–7.
39. DeLong K, Bensouda S, Zulfiqar F, et al. Conceptual Design of a Universal Donor Screening Approach for Vaginal Microbiota Transplant. Front Cell Infect Microbiol 2019;9:306.
40. Komesu YM, Dinwiddie DL, Richter HE, et al. Eunice Kennedy Shriver National Institute of Child Health and Human Development Pelvic Floor Disorders Network. Defining the relationship between vaginal and urinary microbiomes. Am J Obstet Gynecol 2020;222(2):154.e1–10.