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The Female Urinary Microbiota

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Abstract

Purpose of review—The newly discovered female urinary microbiota has the potential to deepen our understanding of urinary tract health and disease, including common lower urinary tract conditions such as urinary incontinence and urinary tract infection. The spectrum of painful bladder disorders and other less common conditions also may benefit from additional research that includes consideration of the resident bacterial community of the female bladder. This review provides a clinical context for the rapidly emerging research regarding the female urinary microbiota and its relationships with urinary tract conditions of interest.

Recent findings—Studies using culture-independent techniques confirm prior reports of bacteria that reside in the female urinary bladder. These resident communities, the female urinary microbiota, possess characteristics that differ between women affected by urgency urinary incontinence and matched, unaffected controls. Enhanced urine culture techniques permit cultivation of organisms, including uropathogens, missed by standard urine culture, but detected by culture-independent sequencing techniques.

Summary—Clinical laboratories can modify traditional standard urine culture methods to enhance detection of uropathogens. However, given the existence of the female urinary microbiota, the simple presence of bacteria in the lower urinary tract should not be taken as evidence of infection.

Keywords

Urinary Microbiota; Urinary Incontinence; Urinary Tract Infection; Lower Urinary Tract Disorders

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Conflicts of Interest

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Introduction

The vast majority of urinary health research has been conducted without knowledge or consideration of the female urinary microbiota (FUM), communities of microbes present in the lower urinary tract of most adult women. The FUM was initially described in 2012 [1] and subsequently confirmed by others [2–6]. Prior to the discovery of the FUM, clinicians relied on the assumption of bladder sterility and depended on the standard urine culture to be the “gold-standard” for detection of clinically relevant urinary organisms [7]. They can no longer depend on either. What has changed? This review will provide context for emerging research that should begin to inform the clinical care of adult women with lower urinary tract disorders in the context of the ‘normal’ microbiota of the urinary tract in health.

The standard urine culture, a very common clinical test, has been used to determine whether living uropathogens are present in a tested urine sample. Standard urine culture was designed in the 1950s to detect specific uropathogens, especially uropathogenic strains of *Escherichia coli* that cause pyelonephritis [8,9]. As medicine costs have come under increased scrutiny, standard urine cultures have been conducted on a more selective basis (reflex cultures), based on screening tests that suggest the likelihood of uropathogen detection by standard urine culture techniques. Unfortunately, the standard urine culture does not detect most members of the FUM [3,6], including many uropathogens [10].

Bacterial Detection

An important technical advance has been the availability of culture-independent techniques, such as sequencing, that detect the DNA of microbes within a tested sample. These highly sensitive, high-throughput techniques have been used to describe the microbial communities of multiple microbial niches of the human body, most notably as part of the Human Microbiome Project [11]. Multiple studies have now used DNA sequencing to delineate the microbiota in urine collected from the bladders of women with and without lower urinary tract symptoms [1–4,6,12,13].

Sequencing and culture techniques can be complementary. Sequencing is highly sensitive but cannot quantify the detected organisms; it also cannot determine whether the DNA came from a live microbe. However, sequencing can inform culture-based approaches, detecting the presence of microbes that require “non-standard” culture conditions. This information has been used to enhance urine culture protocols; most clinical microbiology laboratories can perform this refined approach, called enhanced quantitative urine culture (EQUC). The improved protocol includes larger urine volume, additional growth media, and longer incubation in the presence of CO_2 [3,5,6,10] (Table 1). A streamlined version is recommended for use in clinical microbiology laboratories [10].

Unlike some other human microbial niches, the bladder microbiota are low biomass [6,10,14]; EQUC generally detects 10^2–10^5 colony-forming units per milliliter of urine obtained from the bladder as compared to the gut, which can contain as much as 10^{14} colony-forming units per gram of feces. This low microbial abundance is a major reason why sensitive sequencing techniques can help advance our understanding of the microbes

that reside in the bladder [6], permitting us to detect rare FUM members that may not be detected by EQUC and other enhanced culture methods. Unfortunately, the combination of this low biomass, the ubiquity of bacterial DNA, and the high sensitivity of modern DNA sequencing methods can lead to the erroneous reporting of bacterial contamination as members of the FUM. Thus, we advise extreme caution and extensive use of negative controls [6]. As we step away from the old "sterile urine" paradigm, other challenges remain; for example, investigators may find that many urine samples contain microbial communities that are below our current detection thresholds. Based on current evidence, however, it is unlikely that these samples are actually "sterile," but rather extremely low abundance [14,15]. Indeed, a recent study used large amounts of urine and detected bacteria in almost of all the tested samples [4].

Female Urinary Microbiota Characteristics

Now that we know that the FUM exist, the composition of these communities requires study. For urine samples with detectable microbial communities, we can now begin to describe them. An important descriptor is microbial diversity, which can be described by two features: richness and evenness. Richness is a measure of the total number of unique taxa within a given individual, but does not take into account the distribution of those taxa. In contrast, evenness is a measure of distribution, or equality of representation, of taxa within an ecological niche, such as the female bladder. A urine sample with 5 unique microbes would be considered richer than a sample with only 2. A sample with 5 equally abundant microbes would be considered more even than another 5-microbe sample that was predominated by one of those microbes.

Based on the available data, it appears that the FUM are similar to other human microbial niches in there is no one "normal" state, but rather variable between individuals. However, there are distinct trends. Most urine samples studied to date are not rich and contain one or two microbes that are substantially more abundant than others. These samples can be categorized on the identity of that or predominant microbe. Each category has been termed a "urotype" similar to the "enterotype" used by many gut microbiome researchers. At the genus level, the most common urotype is *Lactobacillus.* The next most common urotypes are *Gardnerella, Corynebacterium, Streptococcus* and *Staphylococcus*; other less common urotypes exist. Notably, these are all Gram-positive bacteria, quite unrelated to the Gram-negative bacteria, such as *E. coli*, responsible for the vast majority of acute uncomplicated urinary tract infection (UTI). Some samples are not predominated by a single organism or even two; they are placed in a urotype called "diverse." The biological significance of predominance by any specific organism or the lack of a predominant microbe is not yet known. However, FUM diversity appears to have associations with the host's hormonal status, body mass index and certain clinical conditions [4,6,12,13,15,16].

Clinical Associations

Despite hopes of a finding a single "causative" organism (similar to *H. pylori* for stomach ulcers), community characteristics may be more important that the presence or absence of a particular microbe. This would be expected if the FUM play a protective role. For example,

FUM diversity appears to relate to the presence of urgency urinary incontinence (UUI). A recent report suggests that treatment response may be related to the number of unique organisms (richness) present prior to solifenacin treatment for UUI [14]. Following replication of this work, it may be possible to refine clinical estimates of treatment efficacy, based on a pre-treatment assessment of that individual patient's urinary microbial community characteristics. Another report identified an association between UUI symptoms and several bacterial species, including a number of emerging Gram-positive pathogens; this report also found that *Lactobacillus crispatus* associates with the lack of symptoms [6], suggesting the possibility that *L. crispatus* may be beneficial to maintaining bladder health.

A refined estimate of risk may also be possible in women who will undergo urinary tract instrumentation (catheterization, cystoscopy, surgical procedures). Currently, the population is treated as having a "pooled" risk of UTI; often, this risk is approached with a single protocol for peri-procedure antibiotic. Despite these common clinical protocols, post-instrumentation UTI remains a common event, and is typically associated with a course of treatment antibiotic. There is DNA evidence that the FUM plays a role in within this population [13,16]. Further study is required, but indications are that sequencing (and EQUC) can assist in refining the estimate of this risk. Such information may allow modifications to peri-procedure antibiotic protocols, reducing overall antibiotic use and individualizing specific risk-reduction techniques, based on pre-instrumentation urinary microbial assessment.

The strongest evidence to date supports the hypothesis that the FUM differ in women with UUI, compared to unaffected women [4,6]. At this early stage of investigation, however, it is not known whether this intriguing association is a cause or an effect of the condition. There are many biological possibilities; for example, it is possible that the urinary frequency typically associated with UUI alters the microbial community. Yet, it is prudent to consider this association with a wider lens that takes into account other understudied aspects of lower urinary tract function. For example, significant new information has highlighted the non-barrier role of the urothelium, especially its sensory functions [17]. Evidence exists of communication between the gut microbiota and the central nervous system [18]. Given clear evidence of the communication between bladder and brain [19], it is certainly biologically plausible that a similar mechanism could be present in the urinary system, and that the urinary microbiota play some role in this communication, perhaps involving the urothelial sensory signaling.

Immune Functions of the Bladder

Another intriguing development in our understanding is the emerging evidence regarding the immune functions of the bladder, an understudied aspect of lower urinary tract health. Little is known about the immune functions of the lower urinary tract. Elegant work describes establishment of intracellular communities by uropathogenic strains of *E. coli* [20]; however, little is known about the role of most other members of the FUM in regulating immune function within the bladder. The potential for certain ("good") microbes to have an inhibitory effect on other ("bad") microbes is evident in multiple human microbial niches, where the presence of an organism clearly has a role in maintaining homeostasis [21].

Disruption of that homeostatis can cause a dysbiosis that allows an imbalance of organisms or the overt dominance of a pathogen associated with clinical infection. Unlike the conventional view of UTI that assumes invasion of a sterile field by a single uropathogen, it is more likely that there is a spectrum of urinary dysbioses. This spectrum may more appropriately explain the clinical situations that have been termed “asymptomatic bacteriuria.” This new paradigm of a spectrum of microbial community health may allow clinicians to understand the risk of meaningful clinical conditions of interest, such as UTI, UUI and, perhaps, some forms of bladder pain syndromes.

Anti-microbial peptides (AMPs) exist throughout the body and have been studied extensively in the skin and gut; AMPs have been documented in the urine [22]. Understanding the roles these peptides play in the lower urinary tract requires significantly more study; however, they are likely to play a key role in microbial community regulation, resilience following dysbiotic/infection episodes and response to treatment for lower urinary tract disorders. Similarly, the recent detection of IL-22 receptors in the urothelium allows consideration of interactions between the microbial community, known urothelial cholinergic receptors, the non-barrier role of the urothelium, and AMPs [23]. Interpretation of these interactions may provide new insights into important immune functions of the bladder.

Clinical Potential

Might there be a role for new forms of treatment for common lower urinary tract disorders? Fecal transplants have been rapidly translated from the laboratory setting to the clinic; treatment of refractory *Clostridrium difficile* infection has saved lives [24]. Some work exists to support this notion for treatment of recurrent UTI. Investigators have instilled non-pathogenic strains of *E. coli* into the bladders of men with spinal cord injury to effectively reduce subsequent clinical UTI [25]. More recently, researchers reported that intravaginal administration of a probiotic strain of *Lactobacillus crispatus* reduced episodes of recurrent UTI [26]. In the laboratory setting, Rudick et al. have demonstrated efficacy using a mouse model [27]. The possibility of this clinical treatment is enticing; it requires further rigorous testing to optimize the treatment protocol prior to widespread clinical implementation.

There are many exciting developments in lower urinary tract research that are related to the FUM. Although most studies have concentrated on bacteria, there is also preliminary evidence of viral [28] and fungal community members. As this preliminary work is replicated and published, our understanding will be expanded further.

Based on the clinical insights that the FUM can provide, the involved research community will need to ensure that foundational studies optimize specimen collection, storage and analysis.

Conclusion

The limitations of standard urine culture may affect clinical care, especially for certain subgroups of affected patients. The FUM can be assessed by enhanced urine culture techniques (EQUC) and culture-independent methods (DNA sequencing). Especially in

patients with refractory symptoms, clinicians and their patients may benefit from clinical studies that more fully describe the FUM.

Many questions remain. What roles do detected bacteria play: which ones are beneficial? Which ones detrimental? How do they interact with each other and the host? What about non-bacterial microbes? How stable/resilient is the FUM? When does it become established? Does it change with life events?

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References

1. Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, Fitzgerald M, Mueller ER, Schreckenberger P, Dong Q, Nelson DE, et al. Evidence of uncultivated bacteria in the adult female bladder. J Clin Microbiol. 2012; 50:1376–1383. [PubMed: 22278835]

2. Fouts DE, Pieper R, Szpakowski S, Pohl H, Knoblach S, Suh MJ, Huang ST, Ljungberg I, Sprague BM, Lucas SK, 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. [PubMed: 22929533]

3**. Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, Brubaker L, Gai X, Wolfe AJ, Schreckenberger PC. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J Clin Microbiol. 2014; 52:871–876. This manuscript is the earliest description of the enhanced quantitative urine culture technique (EQUC), in which the technique was used to show that bacteria in the bladder are alive. This paper sheds light on the considerable limitations of the standard urine culture in common clinical use. [PubMed: 24371246]

4*. Karstens L, Asquith M, Davin S, Stauffer P, Fair D, Gregory WT, Rosenbaum JT, McWeeney SK, Nardos R. Does the Urinary Microbiome Play a Role in Urgency Urinary Incontinence and Its Severity? Front Cell Infect Microbiol. 2016; 6:78. This is a confirmatory study documenting the presence of the female urinary microbiota; also confirming differences in women with vs. without urgency urinary incontinence. These investigators used a small group of carefully controlled women for the comparison group. [PubMed: 27512653]

5. Khasriya R, Sathiananthamoorthy S, Ismail S, Kelsey M, Wilson M, Rohn JL, Malone-Lee J. Spectrum of bacterial colonization associated with urothelial cells from patients with chronic lower urinary tract symptoms. J Clin Microbiol. 2013; 51:2054–2062. [PubMed: 23596238]

6**. Pearce MM, Hilt EE, Rosenfeld AB, Zilliox MJ, Thomas-White K, Fok C, Kliethermes S, Schreckenberger PC, Brubaker L, Gai X, et al. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. MBio. 2014; 5:e01283–01214. This manuscript describes the first meaningful use of EQUC and 16S sequencing to document the differences in the microbiome/microbiota in women with and without UUI. [PubMed: 25006228]

7. McCarter, YS., Burd, EM., Hall, GS., Zervos, M. Cumitech 2C, Laboratory diagnosis of urinary tract infections. Washington, DC: ASM Press; 2009.

8. Kass, EH. The role of asymptomatic bacteriuria in the pathogenesis of pyelonephritis. In: Quinn, EL., Kass, EH., editors. Biology of pyelonephritis. Little Brown; 1960. p. 399-412.

9. Kass EH. Pyelonephritis and bacteriuria. A major problem in preventive medicine. Ann Intern Med. 1962; 56:46–53. [PubMed: 14454174]

10**. Price TK, Dune T, Hilt EE, Thomas-White KJ, Kliethermes S, Brincat C, Brubaker L, Wolfe AJ, Mueller ER, Schreckenberger P. The Clinical Urine Culture: Enhanced Techniques Improve Detection of Clinically Relevant Microorganisms. J Clin Microbiol. 2016; 54:1216–22. This demonstrates the significant limitations of the current standard urine culture technique, which fails to detect many organisms, including well-known uropathogens; the work provides a foundation for a recommendation for a streamlined version of EQUC that can be performed in any modern clinical laboratory. [PubMed: 26962083]

11. Cho I, Blaser MJ. APPLICATIONS OF NEXT-GENERATION SEQUENCING The human microbiome: at the interface of health and disease. Nature Reviews Genetics. 2012; 13:260–270.

12. Nienhouse V, Gao X, Dong Q, Nelson DE, Toh E, McKinley K, Schreckenberger P, Shibata N, Fok CS, Mueller ER, et al. Interplay between Bladder Microbiota and Urinary Antimicrobial Peptides: Mechanisms for Human Urinary Tract Infection Risk and Symptom Severity. PLoS One. 2014; 9:e114185. [PubMed: 25486068]

13. Pearce MM, Zilliox MJ, Rosenfeld AB, Thomas-White KJ, Richter HE, Nager CW, Visco AG, Nygaard IE, Barber MD, Schaffer J, et al. The female urinary microbiome in urgency urinary incontinence. Am J Obstet Gynecol. 2015; 213:347e1–11. [PubMed: 26210757]

14**. Thomas-White KJ, Hilt EE, Fok C, Pearce MM, Mueller ER, Kliethermes S, Jacobs K, Zilliox MJ, Brincat C, Price TK, et al. Incontinence medication response relates to the female urinary microbiota. Int Urogynecol J. 2016; 27:723–33. This manuscript provides the first evidence that the female urinary microbiome/microbiota are associated with response to therapy for urgency urinary incontinence. [PubMed: 26423260]

15. Thomas-White KJ, Kliethermes S, Rickey L, Lukacz ES, Richter HE, Moalli P, Zimmern P, Norton P, Kusek JW, Wolfe AJ, et al. Evaluation of the urinary microbiota of women with uncomplicated stress urinary incontinence. Am J Obstet Gynecol. 2016 Aug 4. pii: S0002-9378(16)30522-1. Epub ahead of print. doi: 10.1016/j.ajog.2016.07.049

16. Brubaker L, Nager CW, Richter HE, Visco A, Nygaard I, Barber MD, Schaffer J, Meikle S, Wallace D, Shibata N, et al. Urinary bacteria in adult women with urgency urinary incontinence. Int Urogynecol J. 2014; 25:1179–1184. [PubMed: 24515544]

17. Keay S, Birder L, Chai T. Evidence for bladder urothelial pathophysiology in functional bladder disorders. Biomed Res Int. 2014; 2014:865463. [PubMed: 24900993]

18. Schroeder B, Bäckhed F. Signals from the gut microbiota to distant organs in physiology and disease. Nat Med. 2016; 22:1079–1089. [PubMed: 27711063]

19. Tadic SD, Griffiths D, Schaefer W, Cheng CI, Resnick NM. Brain activity measured by functional magnetic resonance imaging is related to patient reported urgency urinary incontinence severity. J Urol. 2010; 183:221–228. [PubMed: 19913803]

20. Anderson G, Palermo J, Schilling J, Roth R, Heuser J, Hultgren S. Intracellular bacterial biofilm-like pods in urinary tract infections. Science. 2003; 301:105–107. [PubMed: 12843396]

21. Ayres J. Cooperative Microbial Tolerance Behaviors in Host-Microbiota Mutualism. Cell. 2016; 165:1323–1331. [PubMed: 27259146]

22*. Becknell B, Schwaderer A, Hains DS, Spencer JD. Amplifying renal immunity: the role of antimicrobial peptides in pyelonephritis. Nat Rev Nephrol. 2015; 11:642–655. Very well-done review of anti-microbial peptides (AMP)s in the urinary tract. [PubMed: 26149835]

23**. Le PT, Pearce MM, Zhang S, Campbell EM, Fok CS, Mueller ER, Brincat CA, Wolfe AJ, Brubaker L. IL22 regulates human urothelial cell sensory and innate functions through modulation of the acetylcholine response, immunoregulatory cytokines and antimicrobial peptides: assessment of an in vitro model. PLoS One. 2014; 9:e111375. This is the first report that human urothelial (HURO) cells express receptors for the immune regulatory cytokine interleukin 22 (IL22), which regulates the sensory response through the muscarinic receptor pathway. Since muscarinic receptors are key to overactive bladder symptoms, IL22 and its receptors may represent a novel target for new therapies. [PubMed: 25354343]

24. Chapman B, Moore H, Overbey D, Morton A, Harnke B, Gerich M, Vogel J. Fecal microbiota transplant in patients with Clostridium difficile infection: A systematic review. J Trauma Acute Care Surg. 2016; 81:756–764. [PubMed: 27648772]

25. Hull R, Rudy D, Donovan W, Svanborg C, Wieser I, Stewart C, Darouiche R. Urinary tract infection prophylaxis using Escherichia coli 83972 in spinal cord injured patients. J Urol. 2000; 163:872–877. [PubMed: 10687996]

26. Stapleton AE, Au-Yeung M, Hooton TM, Fredricks DN, Roberts PL, Czaja CA, Yarova-Yarovaya Y, Fiedler T, Cox M, Stamm WE. Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. Clin Infect Dis. 2011; 52:1212–1217. [PubMed: 21498386]

27. Rudick CN, Taylor AK, Yaggie RE, Schaeffer AJ, Klumpp DJ. Asymptomic bacteriuria *Escherichia coli* are live biotherapeutics for UTI. PLoS One. 2014; 9:e109321. [PubMed: 25405579]

28. Santiago-Rodriguez T, Ly M, Bonilla N, Pride D. The human urine virome in association with urinary tract infections. Front Microbiol. 2015; 6:14. [PubMed: 25667584]

Key points

1. Standard urine culture does not detect most members of the existing female urinary microbiota (FUM), including many uropathogens.
2. Microbial detection using enhanced urine culture techniques correlates with DNA sequencing, a culture-independent method.
3. Similar to other human microbial niches, there is no one “normal” state, but rather variable between individuals.
4. Characteristics of the FUM, such as microbial diversity and predominance, vary based on hormonal status, body mass index and certain clinical conditions, especially urinary urgency incontinence.
5. Differences exist in the FUM of women with urinary urgency incontinence compared to unaffected women.

Table 1

Summary of Urine Cultivation Protocols

Protocol	Urine Volume (μl)	Media	Incubation Conditions	Microbial Identification
Standard Urine Culture	1	BAP[1], MacConkey	Aerobic 35°C	24 h
EQUC	100	BAP, MacConkey	Aerobic 35°C	24 h 48 h
		BAP, Chocolate, CNA[2]	5% CO_2 35°C	24 h 48 h
		CDC Anaerobic BAP	Anaerobic 35°C	48 h
		BAP, MacConkey, CNA	Microaerophilic gas mixture (5% O_2, 10% CO_2, 85% N) 35°C	48 h
Streamlined EQUC	100	BAP, MacConkey, CNA	5% CO_2 35°C	48 h

[1] BAP = Blood agar plate

[2] CNA = Colistin Naladixic Acid agar