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"Sterile Urine" and the Presence of Bacteria

A. J. Wolfe, PhD.[1] and **L. Brubaker, MD MS**[2]

[1]Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, Chicago IL USA

[2]Departments of Obstetrics & Gynecology and Urology, Stritch School of Medicine, Loyola University Chicago, Chicago IL USA

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Modern clinicians have equated the presence of bacteria in urine with infection, or, less commonly, an ill-defined phenomenon termed "asymptomatic bacteriuria." These and other existing concepts are based on the long-held "sterile urine" paradigm. Recently, however, bacterial communities (microbiota) have been discovered in the female bladder (Brubaker et al., 2014; Fouts et al., 2012; Hilt et al., 2014; Khasriya et al., 2013; Lewis et al., 2013; Nienhouse et al., 2014; Pearce et al., 2014; Siddiqui et al., 2011; Wolfe et al., 2012). Thus, the "sterile urine" paradigm is no longer valid. This new discovery of the female urinary microbiota (FUM) offers an exciting opportunity to advance our understanding of bladder health and disease. Clinicians and scientists must reassess their assumptions concerning the etiologies of lower urinary tract disorders. Reassessment will facilitate consideration of new approaches for prevention and treatment of these poorly understood disorders.

The terms microbiota and microbiome are only now becoming part of the clinical lexicon. These terms have different meanings, yet are often used interchangeably. The urinary microbiota is defined as the microorganisms that exist within the bladder and the urinary microbiome is the collection of all their genomes.

Recognizing the importance of bacterial communities in human health, the NIH initiated the Human Microbiome Project (HMP). The HMP has clearly shown that the microbiota of various anatomical sites contribute to multiple and diverse human health and disease states. However, the female urinary tract was not included in the initial HMP studies. In contrast to the rich diversity of bacterial species at other human mucosal surfaces, the urinary tract was generally considered to be "sterile", likely due to the use of culture-dependent methods of bacterial detection (Kass, 1962). However, culture-dependent techniques are severely limited because the vast majority of bacteria are not or cannot be cultured by standard clinical laboratory techniques.

Corresponding Author: Alan J. Wolfe, PhD, Professor, Department of Microbiology and Immunology, Stritch School of Medicine Loyola University Chicago, 2160 South First Avenue, Maywood, IL USA, awolfe@luc.edu, Phone: 708-216-5814, FAX: 708-216-9574.

Fortunately, high-throughput DNA sequence-based analyses can identify bacteria without culturing. Our group (Nienhouse et al., 2014; Pearce et al., 2014; Wolfe et al., 2012) and others (Fouts et al., 2012; Lewis et al., 2013; Siddiqui et al., 2011) have used such approaches to systematically characterize bacteria directly from urine samples. The workhorse of these efforts is broad-range 16S rRNA gene sequence analysis, the primary tool used by bacterial ecologists – think of the female bladder as just another ecological niche – to characterize complex bacterial phylogenetic relationships. The 16S rRNA gene sequence is highly conserved, a direct result of its critical cellular role. Within the gene, however, some stretches can evolve, becoming hypervariable regions that can measure evolutionary distance and thus phylogenetic relatedness. All nine known hypervariable regions (V1–V9) of the 16S rRNA gene contain sufficient polymorphisms, so that sequencing one V region often suffices to achieve accurate taxonomic classification. Using this approach, we have begun to phenotype women on the basis of their individual urinary microbiome, drawing urine directly from the bladder by suprapubic aspiration (Wolfe et al., 2012) and/or transurethral catheter (Nienhouse et al., 2014; Pearce et al., 2014; Wolfe et al., 2012). Transurethral catheter samples are similar to suprapubic samples that bypass vulvo-vaginal contamination, demonstrating that the bladder possesses its own microbiome.

Clinicians may be wondering about traditional bacterial detection tests that have been used for decades. For example, a common screening test is the urinary dipstick (for leukocyte esterase and/or nitrates). However, the specificity of this test is limited. Clinicians may also use formal urinalysis with reflex urine culture testing. However, standard urine culture protocol (1 microliter of urine on blood and MacConkey agar plates incubated at 35°C in air for 24 hours) is designed to quickly detect a select group of known uropathogens, most notably uropathogenic *Escherichia coli*. The standard urine culture protocol is not designed to detect bacteria that require special nutrients, grow slowly, cannot tolerant oxygen, or are present in small numbers ($<10^3$ colony forming units per milliliter); organisms that may be involved in urinary disorders. Furthermore, the assumption that urine is sterile has led clinical microbiologists to overlook colonies that resemble those known to be part of the vaginal microbiota. Because standard testing limits detection to certain organisms, clinicians have no ability to detect new or previously unappreciated uropathogens.

Fortunately, new approaches are being developed. For example, 16S rRNA sequence analysis (Wolfe et al., 2012) revealed that urine deemed ‘no growth’ by the standard protocol contained bacteria that could be cultured, but not by the standard approach. This led our research team to develop an expanded quantitative urine culture (EQUC) protocol (Hilt et al., 2014), which can isolate and identify many organisms that standard culture misses because it uses 100 times more urine and a variety of media and atmospheric conditions. A direct comparison between EQUC and the standard protocol revealed an astounding 90% false negative rate for the standard clinical approach (Hilt et al., 2014).

While the clinical feasibility and future roles for these new assessment tools (sequencing and EQUC) are yet to be determined; it is clear that the prior, widespread testing methods (e.g., standard urine culture and dipstick) are inadequate for research purposes, and may also be inadequate for clinical purposes.

In women, emerging evidence suggests that the FUM may contribute to symptoms of urinary incontinence. The bladders of both women with and without lower urinary tract symptoms contain a FUM (Brubaker et al., 2014; Fouts et al., 2012; Hilt et al., 2014; Khasriya et al., 2013; Nienhouse et al., 2014; Pearce et al., 2014; Siddiqui et al., 2011; Wolfe et al., 2012); the bacteria that comprise the FUM are clearly distinct from those that cause overt clinical urinary tract infection (Hilt et al., 2014; Nienhouse et al., 2014; Wolfe et al., 2012). Using only baseline urine samples from a study planned prior to the knowledge of the FUM existence, NIH investigators have discovered that the FUM is associated with pre-treatment urgency urinary incontinence symptoms and protection against urinary tract infection (Brubaker et al., 2014). Finally, several bacterial species are more common in women with urgency urinary incontinence than in asymptomatic controls (Pearce et al., 2014). These exciting findings open previously unappreciated opportunities for scientific inquiry concerning prevention, etiology and treatment of various lower urinary tract disorders.

The opportunity to expand our scientific vision is a welcome one. Freed from the incorrect assumption that urine is sterile, we can explore the role of resident urinary bacterial communities in health and disease. Undoubtedly, we will learn much more about common lower urinary tract disorders, including, but not limited to, urinary tract infections, overactive bladder, urinary incontinence and painful bladder syndromes.

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Take home message

Adult human urine is not sterile. The resident bacterial community may contribute to urinary health and disease in undiscovered ways. Bacterial genomic sequencing and expanded urine cultures techniques are major complementary tools for scientific exploration in urologic research.