Review

# Intracellular Bacterial Communities: A Potential Etiology for Chronic Lower Urinary Tract Symptoms

**Victoria C. S. Scott, David A. Haake, Bernard M. Churchill, Sheryl S. Justice, and Ja-Hong Kim**

Patients with persistent lower urinary tract symptoms and negative urine cultures are often difficult to treat. Infection may go undetected in these patients because the concentrations of bacteria in their urine are beneath the threshold of standard urine culture techniques. Empiric treatment may result in temporary relief, followed by recurrent symptoms. Occult and recurrent urinary tract infection may be due to both invasion of the bladder wall by uropathogenic *Escherichia coli* and the formation of biofilm-like intracellular bacterial communities. This review examines emerging evidence for a role of intracellular bacterial communities in human infection. UROLOGY ■: ■—■, 2015. © 2015 Elsevier Inc.

Lower urinary tract symptoms (LUTS), such as frequency, urgency, and dysuria, are extremely prevalent among adults worldwide. In an international study of 30,000 participants aged 40 to 99 years, 72.3% of men and 76.3% of women reported at least one LUTS "sometimes," and 47.9% of men and 52.5% of women reported at least one LUTS "often".[1] Patients with isolated or repeated episodes of LUTS associated with positive urine cultures are often effectively treated with short courses of antibiotics. Yet, no etiology for LUTS is found in many patients with negative results using standard urine culture techniques, who also lack a functional or anatomic abnormality of the urinary tract. Patients with urgency as their primary complaint are typically thought to have overactive bladder (OAB), and patients with pain, pressure, or discomfort are diagnosed with interstitial cystitis/bladder pain syndrome (IC/BPS). OAB and IC/BPS are diagnoses of exclusion for which there is frequently no clear etiology.[2] Treatment of such patients is targeted at symptom management, with little hope of a definitive cure.

There is great interest in the potential role of urothelial cell infection in patients with LUTS who have $\leq 10^5$ colony-forming units (CFU) of bacteria per milliliter of urine ("low-count" bacteriuria) or even negative urine cultures. Urinary tract infection in some patients may not be limited to a simple luminal infection that is reliably eradicated with a short course of antibiotics. A growing body of evidence indicates that the pathogenesis of infections caused by uropathogenic *Escherichia coli* (UPEC) may be far more complex and include invasion of urothelial cells lining the urinary bladder with formation of intracellular bacterial communities (IBCs).[3-6] In this biofilm state, IBCs may go undetected by standard urine cultures, evade host defense mechanisms, and persist despite antibiotic therapy. In this report, we explore the potential role of occult infection in OAB and IC/BPS, review the evidence for IBC formation in human bladder infections, and discuss the possible role of IBCs in recurrent UTIs.

## "CULTURE-NEGATIVE" LUTS

Patients with symptoms of urinary urgency, frequency, dysuria, or bladder pain who have normal functional and anatomic studies and negative urine cultures are frequently given the diagnosis of OAB or IC/BPS. OAB and IC/BPS are both symptom complexes, not diseases, which rely on negative urine cultures for diagnosis. A negative urine culture using the standard definition of $\leq 10^5$ CFU/mL result does not exclude "occult" UTI involving low-count bacteruria or "latent" infection involving quiescent reservoirs in the urothelium.

The International Continence Society defines OAB as urgency, with or without urgency incontinence, usually with frequency and nocturia, in the absence of infection

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*From the Department of Urology, David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, CA; the Division of Infectious Diseases, VA Greater Los Angeles Healthcare System, Los Angeles, CA; the Department of Medicine, David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, CA; the Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, CA; the Center for Microbial Pathogenesis, The Research Institute at Nationwide Children's, Hospital, Columbus, OH; and The Ohio State University School of Medicine, Columbus, OH*

*Address correspondence to: Victoria C. S. Scott, M.D., Department of Urology, David Geffen School of Medicine at UCLA, 10833 Le Conte Ave, Box 951738, Los Angeles, CA 90095. E-mail: vscott@mednet.ucla.edu*

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or other pathology.[2] Urgency, along with at least 1 other symptom, must be present for a diagnosis of OAB. There are 2 primary hypotheses for the etiology of OAB. The *neurogenic hypothesis* states that detrusor overactivity arises from generalized, nerve-mediated excitation of the detrusor muscle, whereas the *myogenic hypothesis* suggests that overactive detrusor contractions result from a combination of increased likelihood of spontaneous excitation within bladder smooth muscle and propagation of this activity to an excessive proportion of the bladder wall.[7,8]

IC/BPS is less common but remains a debilitating condition for many women and some men.[9] Although IC/BPS and OAB share the symptoms of urgency, frequency, and nocturia, pain is the hallmark symptom for IC/BPS. It can also be associated with chronic dysuria. IC/BPS encompasses a major portion of the "painful bladder" disease complex, which includes any or all of bladder, urethral, or pelvic pain, LUTS, and negative urine cultures. Multiple theories to explain the pathogenesis of IC/BPS have been proposed, including a "leaky epithelium," neurogenic inflammation, hypersensitivity response with mast cell activation, or some combination of these, leading to chronic bladder pain and voiding dysfunction.[10]

A number of studies have attempted to demonstrate an infectious etiology for OAB and IC/BPS.[11-14] Infection may be the initial insult to the urothelium that ultimately results in development of OAB or IC/BPS. UTI is the most common initial diagnosis in patients who are eventually diagnosed with IC/BPS, ranging from 42%-60% of patients. Patients with IC/BPS are twice as likely to report a history of UTI before the onset of IC/BPS symptoms compared with patients who do not have IC/BPS and 10- to 12-times as likely to have a history of childhood urinary tract disorders.[14] OAB and IC/BPS may be caused by chronic infection or may be a result of an initial infection causing persistent inflammation of the bladder after the infection has been eradicated.

There has been interest in examining the response of OAB or IC/BPS patients to empiric antibiotics. In the most successful study of antibiotic treatment of LUTS, Durier[15] reported cure of 27 of 27 patients with IC with use of up to 5 sequential antibiotics. Warren et al[16] performed a prospective, double-blind, placebo-controlled randomized trial of 50 women diagnosed with IC who were given placebo or an intensive antibiotic regimen for 18 weeks. Although the difference did not reach statistical significance, 88% of patients in the antibiotic group reported overall improvement or improvement in pain and urgency compared with 44% in the placebo group. These results suggest that the LUTS in a subset of IC/BPS patients has an infectious etiology despite negative urine cultures. An alternative interpretation is that relief of symptoms results from the placebo effect or the anti-inflammatory activity of some antibiotics, such as tetracyclines.

Infectious causes of urethritis may also play a role in some IC/BPS patients with negative urine cultures. Burkhard et al[17] treated 103 women, aged 21 to 84, with a median duration of LUTS for 60 months, with doxycycline, 100 mg twice daily for 2 weeks, followed by 100 mg once daily for 2 weeks. Patients were also given vaginal tablets (hexetidine or ciclopiroxolamine) once daily for 10 days. Patients' sexual partners were treated with the same doxycycline regimen. After 4 weeks, 70% of women reported subjective cure or improvement in symptoms. In another study of 48 women referred for chronic voiding symptoms and possible IC with negative standard urine cultures, 48% had cultures positive for *Ureaplasma urealyticum* or *Mycoplasma hominis*.[18] After treatment, 91% of the patients with positive cultures had statistically significant improvement in their symptoms.

## MICROBIOLOGIC DIAGNOSIS OF UTI

Acute UTI often presents with bacteria in the urine at $\geq 10^5$ CFU/mL, which is the standard threshold for a positive urine culture. This density cutoff was determined in studies of women with acute pyelonephritis vs asymptomatic bacteriuria in the 1950s. Lower densities of bacteria were more likely to be associated with contamination from nonurinary sources.[19] The threshold of $\geq 10^5$ CFU/mL shows high specificity for a UTI but a sensitivity of only ~50%. Lowering the threshold to $\geq 10^3$ in women with symptoms of cystitis increases sensitivity considerably, with minimal reductions in specificity.[20,21] In fact, the guidelines of the European Association of Urology and the American Academy of Family Physicians recommend $10^3$ or $10^2$ CFU/mL, respectively, as the standard for diagnosing lower UTI in symptomatic patients.[22,23]

Multiple studies have shown that a substantial number of women with symptoms of acute cystitis and pyuria have $<10^5$ CFU/mL on urine culture.[24,25] One study of 202 premenopausal, nonpregnant women who presented with at least 2 symptoms of acute cystitis compared colony formation between a clean-catch urine specimen and a catheterized urine specimen.[25] *E coli* growth was found in the catheterized specimen of 60% of patients, with 40% of these women found to have $<10^5$ CFU/mL in the voided specimen. The presence of *E coli* in midstream urine with $10^2$ CFU/mL had a 93% positive predictive value for bladder bacteriuria. An earlier study performed in a similar fashion reported the best diagnostic criteria was $\geq 10^2$ CFU/mL, which allowed for a sensitivity of 95% and a specificity of 85% for the diagnosis of bacteriuria in symptomatic women.[20]

Interestingly, all other diagnostic tests of urine constituent levels normalized with creatinine to account for dilution of the urine. However, laboratory diagnostics for bacterial burden only report the CFU/mL of urine. Taking account of urinary creatinine levels is particularly relevant considering that most women increase water