Improving Treatment Pathways for Patients with Persistent Lower Urinary Tract Symptoms

A resource for clinicians who have patients experiencing lower urinary tract symptoms (LUTS) despite negative standard urine culture (SUC), as well as patients who do not respond to treatment based on positive SUC.

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“The literature on urinary microbiome testing is expanding, and can be useful to review as we treat patients with either recurrent culture-positive UTIs or what I refer to as ‘culture-negative UTIs’. I’ve seen patients who have been labeled as having IC, however, microbiome analysis clearly shows pathogens in their urine. When we’ve treated these pathogens, their symptoms have resolved.

“I think some patients with the diagnosis of interstitial cystitis have an occult UTI with difficult to culture organisms. By utilizing more accurate testing methods, we are able to identify pathogens in many cases, and develop appropriate treatment. Even as a physician who has conducted microbiome research for a number of years, I was initially skeptical of urine microbiome testing as a means to diagnose UTI. However, based upon patient and clinical experience, microbiome testing appears to not only be accurate in the right setting, but also may predict imminent UTI in some patients.”

— Michael Hsieh, MD, PhD

BLADDER HEALTH ALLIANCE PARTNER

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INTRODUCTION

The purpose of this document is to provide an overview of the limitations of standard urine culture (SUC) for patients with lower urinary tract symptoms (LUTS) and persistent urinary tract infection (UTI) that responds poorly to standard treatment. Here, clinicians are provided with additional diagnostic resources for improving clinical outcomes in patients with persistent UTI and LUTS.

This document highlights the limitations of current gold standard diagnostics and presents methods available to address these limitations. The peer-reviewed articles referenced below indicate that a shift in UTI diagnostics improves treatment and quality of life (QOL) outcomes for patients experiencing persistent or difficult to diagnose urinary tract symptoms.

While SUC may be negative for some patients experiencing LUTS, when additional diagnostics are utilized, uropathogenic organisms may be identified in these patients and greater treatment success achieved. The term 'culture-negative UTI' is applied in these circumstances. Therefore, a culture-negative UTI should be considered as part of a differential diagnosis, and enhanced testing methods should be utilized in an effort to improve diagnostics and treatment decisions for patients with lower urinary tract symptoms.

FIGURE 1: Microbe Detection Rate in Symptomatic Patients[1,11-13,21]

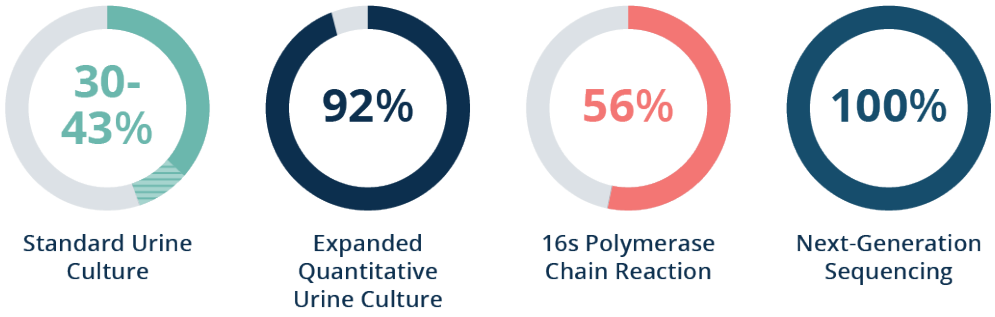
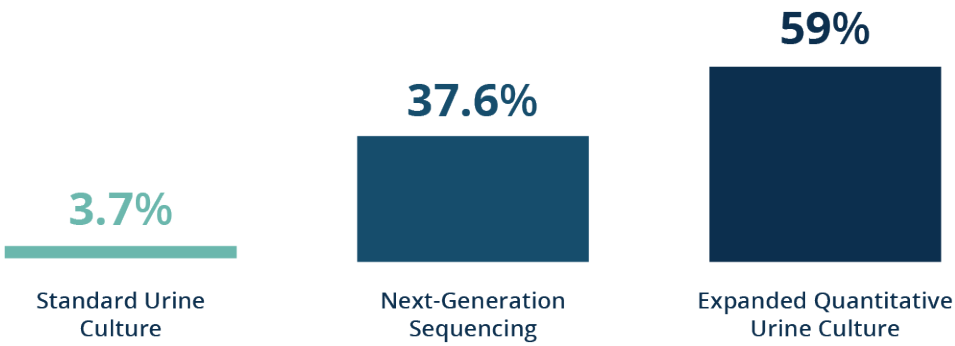


FIGURE 2: Urinary Symptom Improvement Following Treatment Based upon Diagnostic Method[11,14]



KEYWORDS AND DEFINITIONS

- **Biofilm:** Bacterial communities encased in a polysaccharide matrix capable of adhering to and inside surfaces and tissues; contribute to diagnostic and treatment difficulties
- **Culture-negative urinary tract infection**: UTI is present and contributing to lower urinary tract symptoms despite a negative standard urine culture
- **Dysbiosis:** A decrease of microbial diversity in which a reduction of beneficial bacteria or an increase of pathogenic bacteria exist in a microbiome
- **Expanded Quantitative Urine Culture (EQUC)**: A more sensitive culture-dependent diagnostic tool which adjusts for the limitations of standard urine culture
- **Horizontal Gene Transfer:** The transfer of resistance behaviors between microbes
- **Intracellular bacterial communities (IBC)**: Bacterial communities that exist within urothelial cells in a biofilm-like state
- **Next-Generation Sequencing (NGS)**: An enhanced DNA-based microbial detection method that can examine all microbes present in a sample
- **Polymerase Chain Reaction (PCR)**: An enhanced DNA-based microbial detection method that amplifies microbial DNA using 16s and 18s rRNA to identify microbes from a panel
- **Polymicrobial infection**: An infection that consists of multiple pathogens
- **Shotgun metagenomic sequencing**: An advanced NGS diagnostic method in which the entire genome of an organism is sequenced
- **Urobiome**: The microbiome of the urinary tract

THE BLADDER IS NOT STERILE

Identifying the urinary microbiome and addressing standard urine culture limitations

"Our previous study showed that bacterial genomes can be identified using 16S rRNA sequencing in urine specimens of both symptomatic and asymptomatic patients who are culture negative according to standard urine culture protocols... Our current study demonstrates that urine contains communities of living bacteria that comprise a resident female urine microbiota."

— Hilt et al. (2013), Urine Is Not Sterile: Use of Enhanced Urine Culture Techniques To Detect Resident Bacterial Flora in the Adult Female Bladder

Current research into the urinary microbiome:

- Bacterial communities have been observed in 80% of samples obtained by transurethral catheter of female participants, with up to 92% of the samples being reported as 'no growth' using SUC.[1] A dysbiosis of this healthy urinary microbiome (the urobiome) is correlated with the development of symptoms and urinary disorders.[1-3]
- Participants with urinary symptoms demonstrated a more diverse urobiome with larger quantities of bacteria than asymptomatic controls.[3,4] The frequency of bacterial detection was between 81% and 86% for symptomatic cohorts compared to only 57% in the control cohort.[1,2,4,5]
- When compared with asymptomatic controls, patients experiencing urgency incontinence had statistically significant differences in their urobiome, with lower levels of *Lactobacillus* and higher levels of *Gardnerella*.[6,7,8]

TABLE 1: Documented Limitations of Standard Urine Culture
Inability to detect slow-growing microorganisms
Inability to grow non-aerobic organisms
Poor detection of gram-positive organisms
Poor detection of organisms under 10^3 CFU/ml
Threshold developed for pyelonephritis applied to acute cystitis
Distinctions of unique organism thresholds not accounted for
Polymicrobial infection reported as contamination
Poor detection of organisms contained within biofilm
Poor detection of organisms contained within urothelial cells

Fallacies of standard urine culture (SUC) and urinary dipsticks:

- Urinary dipsticks are often utilized as the first method of UTI diagnostics, however, their detection of leukocyte-esterase has been shown to have low sensitivity (0.76) and specificity (0.46).[9] While urinary dipsticks can provide evidence of infection, they cannot accurately determine that no infection is present and are, therefore, an unreliable method for ruling out a UTI in a symptomatic patient.[10]

- The standard urine culture has been determined to be up to 90% inaccurate when tested against more sensitive testing methods, such as an Expanded Quantitative Urine Culture (EQUC) or 16s rRNA sequencing. Cultures reported as "no growth" or "insufficient growth" may be missing a significant portion of infections.[2,6,7,11-14]

- SUC has been shown to be ineffective at detecting Gram-positive microorganisms. Additionally, SUC fails to detect microorganisms in the following categories: slow-growing, anaerobic, colonies present under the threshold of 10^3 CFU/ml, and microbes encased within biofilm or within urothelial cells, also known as intracellular bacterial communities (IBCs).[6,11,12]

- Polymicrobial infections are often reported as "mixed growth" or "contamination." However, current SUC methods result in an overdiagnosis of *E. coli* infection and misdiagnosis of up to 65% of other infections that contain multiple species.[11,14,15]

- Existing SUC procedures do not account for IBCs contained within exfoliated urothelial cells. Up to 10^5 CFU of bacteria can be present in a single cell. However, without proper homogenization to release the microbes, current SUC methods may detect and report these bacteria as only a single colony.[6,15]

- The issue of culture-negative infection is not isolated to the urinary tract and SUC. Studies completed by Kuzmar et al. and Bernard et al. demonstrate that 29-68% of patients diagnosed with sepsis receive a negative blood culture, and empiric antimicrobial treatment is initiated.[16-18]

- In patients with culture-negative sepsis, advanced microbial diagnostics result in a 20% increased detection rate and a reduction in inadequate treatment.[18]

- More sensitive testing methods, reviewed in the following section, may more effectively diagnose an infection or an imbalance within the urobiome of symptomatic patients.

OVERCOMING THE LIMITATIONS OF STANDARD URINE CULTURE

Testing methods that more accurately represent the state of the urobiome

> "Enhanced [expanded] quantitative urine culture (EQUC) detects live microorganisms in the vast majority of urine specimens reported as "no growth" by the standard urine culture protocol…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."
>
> **— Price et al. (2016), The Clinical Urine Culture: Enhanced Techniques Improve Detection of Clinically Relevant Microorganisms**

****A quick-reference table summarizing testing methods can be viewed below.****

Standard urine culture (SUC) compared to alternative diagnostic methods:

The diagnostic methods reviewed in this section have been shown to more accurately detect dysbiosis of the urobiome, with links to improved patient outcomes.

- Expanded Quantitative Urine Culture (EQUC) detected known uropathogenic bacteria in 84% of urine samples compared to only 33% using SUC.[12]
- DNA and RNA based next-generation sequencing (NGS) detected bacteria in 100% of samples compared to 29% with SUC. Due to the high sensitivity rate of NGS, treatment recommendations should be carefully considered, as not all microbes present may be contributing to LUTS.[1,12,14]
- Patients treated according to either fresh urine microscopy results or more sensitive diagnostic methods reported increased symptom improvement when compared to patients treated according to SUC results only.[10,14]
  - On a 21 point scale, patients who were treated according to SUC antibiotic sensitivity testing (AST) reported an average symptom severity decrease of 3.7 points.[14]
  - Patients treated according to NGS results reported an average symptom severity decrease of 7.9 points.[14]

Enhanced detection methods:

- **Expanded Quantitative Urine Culture (EQUC):** Because SUC misses between 67% and 90% of bacteria and is unable to detect certain microorganisms, a more sensitive culture-dependent approach has been established. EQUC adjusts the following conditions to improve detection of slow-growing, anaerobic, and Gram-positive bacteria: volume of urine, media used, atmospheric conditions, and incubation period.[12]

- **Fresh Urine Microscopy:** This diagnostic method examines a urine sample under a microscope to assess the pyuria count. Due to cell integrity being compromised during centrifugation, a fresh, unspun urine sample is necessary. Epithelial cells may also be present, as the bladder lining sheds within six hours of exposure to bacterial strains, such as *E. coli,* in an effort to clear the attached bacteria.[19] Microscopy sidesteps the limitations of SUC as it does not rely on plating conditions, but rather considers the patient's immune response as an indicator of infection. When urine samples of 624 patients experiencing LUTS were examined with both microscopy and SUC, 100% of the samples revealed high pyuria count, whereas only 16% had a positive SUC.[10]

- **Polymerase Chain Reaction (PCR)**: PCR amplifies small sections of microbial DNA in order to analyze the genome. Microbes can be identified based on a pre-selected panel, which varies by the lab. An enhanced approach dependent on PCR is 16s sequencing (metataxonomics). While metataxonomic provides more detailed analysis, limitations in differentiating bacterial strains still exist. When compared to EQUC, similar urobiomes have been observed. This similarity indicates that organisms identified via PCR testing are likely living.[6,20,21]

- **Next-Generation Sequencing (NGS):** NGS is a non-targeted testing method that uses either 16s rRNA or shotgun sequencing (metagenomic) to detect microbes from a database of up to 50,000 organisms, dependent upon the lab. Millions of DNA strands are independently sequenced, minimizing the need for DNA amplification and providing a detailed look into the urobiome.[22,23] Treatment recommendations according to resistance genes are available through some NGS testing facilities.

****For a list of available diagnostic tests utilizing these methods, see the Enhanced Diagnostics Directory****
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TABLE 2: Comparison of urinary microbe testing methods

Comparison of UTI Diagnostic Methods

	SUC	EQUC	PCR (16s)	NGS*	Fresh Unspun Urine Microscopy
Polymicrobial detection	☐	☑	☑	☑	Detects indicators of infection
Detects anaerobic microbes	☐	☑	☑	☑	Detects indicators of infection
Detects microbes within biofilm	☐	☑	☑	☑	Detects indicators of infection
Threshold requirement for diagnosis	≥10^5	Unique to the microbe	N/A	N/A	N/A
Microbe detection rate in symptomatic patients	30-43%[10,11]	92%[1]	56%[20]	100%[12]	N/A
Polymicrobial detection rate in symptomatic patients	5.2-6.6%[11,20]	18-42%[10]	28.5-33%[12,20]	77%[13]	N/A
Antibiotic recommendations method	Standard Antibiotic Susceptibility Testing (AST)	Standard Antibiotic Susceptibility Testing (AST) - *where available*	Typically resistance gene detection only Pooled-Antibiotic Susceptibility Testing (P-AST) - *where available*	Resistance gene detection only	N/A
Advantages	N/A	Threshold unique to microbe Greater response to treatment	Higher sensitivity rate Rapid identification Genetic resistance detection	Database of up to 50,000 organisms Distribution of organisms reported Greater response to treatment	N/A
Improved detection of:	N/A	• Polymicrobial infection • Slow-growing organisms • Anaerobic organisms • Microbes within biofilm • Microbes attached to epithelial cells	• Polymicrobial infection • Slow-growing organisms • Anaerobic organisms • Microbes within biofilm • Microbes attached to epithelial cells	• Polymicrobial infection • Slow-growing organisms • Anaerobic organisms • Microbes within biofilm • Microbes attached to epithelial cells	• White blood cells • Epithelial cells • Host immune response
Limitations	Biased toward *E. coli* detection **Unlikely to detect:** • Slow-growing organisms • Anaerobic organisms • Organisms under 10^3 • Gram-positive organisms • Polymicrobial infection	Not easily accessible	Detection limited to specific panel of organisms; varies by lab May not detect dominant species	Interpretation by physician necessary	Interpretation by physician necessary Detection of pathogenic organisms limited
Turnaround Time	2-7 days	2-4 days	6-24 hours	3-5 days	Immediate

* Please note that current research around NGS has been completed by MicroGen Diagnostics

Download the table in full size here

CONTAMINATION VS. POLYMICROBIAL INFECTION

Recognition of polymicrobial infection helps guide treatment

"Retrospective record review of 582 consecutive elderly patients presenting with symptoms of lower urinary tract infection (UTI) was conducted. All patients had traditional urine cultures and PCR molecular testing run in parallel.

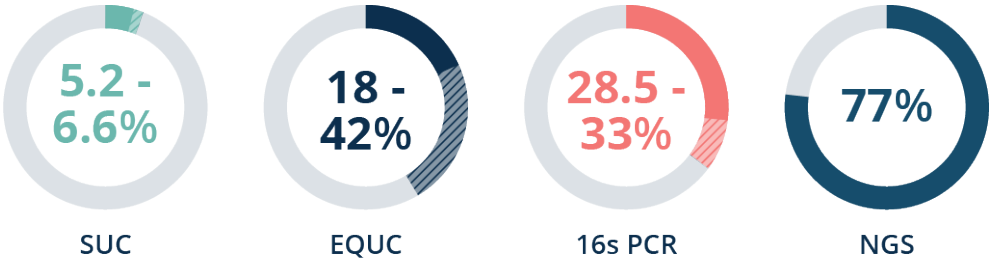
"Polymicrobial infections were reported in 175 patients (30%, 175/582), with PCR reporting 166 and culture reporting 39. Further, polymicrobial infections were identified in 67 patients (12%, 67/582) in which culture results were negative."

— Wojno et al. (2019), Multiplex PCR Based Urinary Tract Infection (UTI) Analysis Compared to Traditional Urine Culture in Identifying Significant Pathogens in Symptomatic Patients

Evidence of polymicrobial infection:

- The limited capabilities and *E. coli*-centric bias of standard urine culture (SUC) has been well established. SUC identifies only 24% of non-*E. coli* uropathogens, and evidence of polymicrobial infection has emerged. Price et al. used Expanded Quantitative Urine Culture (EQUC) to examine polymicrobial infections. 81% of the samples in which *E. coli* was detected also contained at least one additional pathogen.[11]
- Vollstedt et al. utilized polymerase chain reaction (PCR). Out of 1,352 specimens that tested positive for bacteria, 56.1% were reported as being polymicrobial. While not all organisms within a sample are necessarily pathogenic, the possibility of a polymicrobial infection should be considered in symptomatic patients.[24]
- According to Swamy et al., because "the diagnostic picture has become even more complex with the recent discovery that UTI can legitimately involve polymicrobial infection; mixed growth cultures do not necessarily reflect contamination."[10]
- When the limitations of SUC are removed, the opportunity for more informed decision making arises. The interactions between organisms present within an individual's urobiome should be considered as they impact patient-reported outcomes.[7,12,25]

FIGURE 3: Polymicrobial Detection Rate in Symptomatic Patients[11-14,21]



VAGINAL AND URINARY MICROBIOME INTERCONNECTEDNESS

How the health of the vagina influences the urobiome

"Detailed genomic and functional comparison of the bladder microbiota to the gastrointestinal and vaginal microbiotas demonstrates similar vaginal and bladder microbiota, with functional capacities that are distinct from those observed in the gastrointestinal microbiota.

Whole-genome phylogenetic analysis of bacterial strains isolated from the vagina and bladder in the same women identifies highly similar *Escherichia coli, Streptococcus anginosus, Lactobacillus iners, and Lactobacillus crispatus*, suggesting an interlinked female urogenital microbiota that is not only limited to pathogens but is also characteristic of health-associated commensals."

— Thomas-White et al. (2018), Culturing of female bladder bacteria reveals an interconnected urogenital microbiota

Understanding the vaginal and urinary microbiome connection

- While the microbiomes of the urinary tract and bladder are unique, there is significant overlap between the species and protein functions of the two environments, giving way to the theory that they could be considered one single urogenital microbiome.[5]
- *Lactobacillus crispatus* is considered a protective species in both the bladder and vagina.[5,34,35] A decrease of *L. crispatus* and an increase of *L. gasseri* in the bladders of women with LUTS has been observed.
  - *L. crispatus* levels have been shown to increase in the bladder during and after treatment with vaginal *L. crispatus* or estrogen[29], with modest improvement of LUTS, episodes of UTI recurrence, and prolonged time between UTI recurrences being observed.[36,41-43]
- For women with vaginal dysbiosis, there exists an increased risk of developing a UTI compared to women with a *Lactobacillus*-dominated vaginal microbiome.[37]
  - 75%, 46%, and 13% of women with bacterial vaginosis, candidiasis, or trichomoniasis respectively, also have a UTI.[38]
- Similar to the urobiome, the presence of vaginal pathogens alone may not result in vaginal symptoms.[39] Yet, asymptomatic vaginal dysbiosis can result in the introduction of vaginal pathogens to the urinary tract.[35,37,40]

- This introduction of vaginal pathogens (such as *Streptococcus* and *Gardnerella*) can initiate a response from bacteria lying dormant in the urinary tract.[35,37,40] While the instigating organism is often cleared from the urinary tract, the brief exposure and host response may result in the development of UTI pathogenesis.

How *Lactobacillus* and estrogen vaginal suppositories can reduce recurrent UTI (rUTI) by more than 5 episodes per year:[29,36.41,42]

- *Lactobacillus* species produce 2 types of lactic acid (L- and D-lactic acid) which are toxic to many pathogens. *L. crispatus* are particularly good at making highly potent D-lactic acid and are therefore considered the most protective of the *lactobacilli*. When *lactobacilli* levels are decreased in the vagina, as can occur with antibiotic use or low levels of estrogen, this protective mechanism is reduced.[36]

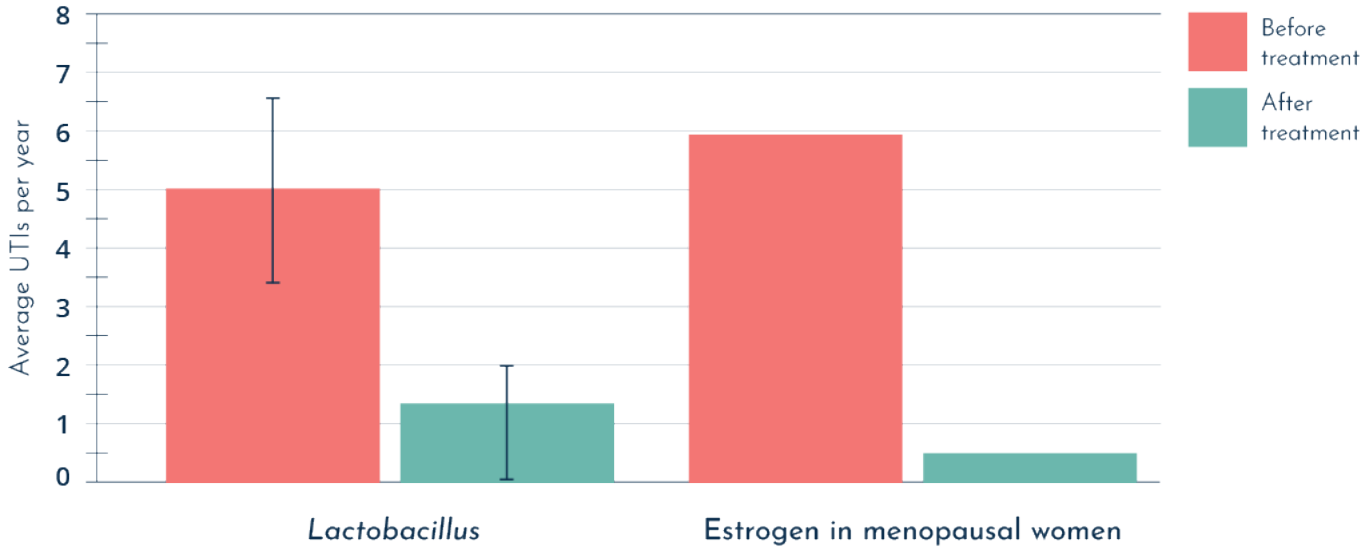
- The repopulation of the vaginal microbiome with *L. crispatus* vaginal suppositories show promise in colonizing the vagina for prevention of bacterial vaginosis, thereby limiting the risk of UTI.[41,43]

- For females with a history of rUTI, a randomized, placebo-controlled trial of *L. crispatus* vaginal suppositories demonstrated a recurrence rate of 15% in the treatment group compared to 27% in the placebo group at a 10 week follow up.[44] A separate study comparing the effect of *L. crispatus* vaginal suppositories on UTI recurrence rates found a significant reduction throughout the 12 month treatment period - 5.0 ± 1.6 episodes per year to 1.3 ± 1.2.[41]

- As estrogen encourages the production of glycogen, a food source for *Lactobacilli*[39], the replacement of estrogen through topical or suppository hormone therapy can be significant in preventing acute and persistent UTI in the postmenopausal population.[29,36]

- Increased *Lactobacilli* have been observed in the urine of participants undergoing estrogen treatment,[36] with 60% of participants in a treatment group having *Lactobacilli* compared to 0% in the placebo group.[29,45]

FIGURE 4: UTIs Per Year Using *Lactobacillus* or Estrogen[29,41,45]



A MORE ACCURATE APPROACH TO DETECTING RESISTANCE

Advanced susceptibility testing may result in better clinical outcomes

"Antimicrobial susceptibility is well characterized in monomicrobial infections, but bacterial species often coexist with other bacterial species. Antimicrobial susceptibility is often tested against single bacterial isolates; this approach ignores interactions between cohabiting bacteria that could impact susceptibility.

"Bacterial interactions in polymicrobial specimens can result in antimicrobial susceptibility patterns that are not detected when bacterial isolates are tested by themselves. Optimizing an effective treatment regimen for patients with polymicrobial infections may depend on accurate identification of the constituent species, as well as results obtained by Pooled Antibiotic Susceptibility Testing."

— Vollstedt et al. (2020), Bacterial Interactions as Detected by Pooled Antibiotic Susceptibility Testing (P-AST) in Polymicrobial Urine Specimens

Types of antimicrobial susceptibility testing:

- **Antibiotic Susceptibility Testing (AST):** When a standard urine culture (SUC) identifies bacteria, the individual pathogen is tested against an antibiotic. This isolated approach to determine susceptibility is limited, as interactions between organisms are not considered.[24] Because SUC fails to identify up to 65% of polymicrobial infection, AST and treatment recommendations may be impacted.[11,14]
- **Pooled Antibiotic Susceptibility Testing (P-AST):** P-AST considers the presence of bacterial interactions and horizontal gene transfer (HGT) when reporting antibiotic susceptibility. While SUC tests antibiotics against a single pathogen, P-AST is conducted in the context of the entire microbiome to determine overall susceptibility.[24]
  - Using P-AST, antibiotic resistance behaviors have been observed to shift within polymicrobial infection. The increased or decreased likelihood of resistance in polymicrobial infection is dependent upon the combination of microbes present, not a single pathogen, due to HGT.[24]
  - Additionally, the use of PCR and P-AST guided treatment has been shown to decrease the rate of hospital admissions for UTI patients by 13.7%.[24]

> "Based on these findings, P-AST testing might more closely approximate the polymicrobial environment in the patient and possibly provide more clinically important information regarding antibiotic susceptibility."
>
> **— Vollstedt et al. (2020), Bacterial Interactions as Detected by Pooled Antibiotic Susceptibility Testing (P-AST) in Polymicrobial Urine Specimens**

- **Resistance Genes**: Labs that utilize DNA-based diagnostic methods may provide information on resistance genes detected, which can assist in making antibiotic recommendations. Due to HGT and interactions between organisms, the presence of resistance genes does not guarantee resistance to a specific antibiotic.[24] This differs from a traditional susceptibility report included with SUC, as the organisms are not tested against antibiotics in vitro, but rather, resistance factors specific to certain classes of antibiotics are reported as either present or absent.[26]

SYMPTOMS OF A PERSISTENT UTI

Overlapping symptoms of urinary conditions present a need to consider culture-negative UTI

"Lower urinary tract symptoms (LUTS) may be associated with chronic urinary tract infection (UTI) undetected by routine diagnostic tests. Antimicrobial therapy might confer benefit for these patients. Over 10 years, we treated patients with chronic LUTS. Pyuria was adopted as the principal biomarker of infection. Urinary leucocyte counts were recorded from microscopy of fresh midstream urine (MSU) samples. Antibiotics were prescribed and the prescription adjusted to achieve a measurable clinical response and a reduction in pyuria.

"This large case series demonstrates that patients with chronic LUTS and pyuria experience symptom regression and a reduction in urinary tract inflammation associated with antimicrobial therapy. Disease regression was achieved with a low frequency of AEs. These results provide preliminary data to inform a future randomized controlled trial (RCT)."

— Swamy et al. (2018), Recalcitrant chronic bladder pain and recurrent cystitis but negative urinalysis: What should we do?

- The symptom presentation of urinary conditions such as overactive bladder (OAB), interstitial cystitis/painful bladder syndrome (IC/PBS), and persistent UTI often overlap. Given the established limitations of standard urine culture (SUC), in the presence of any of the below symptoms, a negative culture should not be considered conclusive, and a culture-negative or persistent UTI should be considered.[6,10]

- Study participants with urinary urgency incontinence (UUI) have more urobiome diversity than non-UUI controls.[4,7,34] When lower urinary tract symptoms are present, consideration of a patient's unique microbiota and microscopy examination can have a positive impact on treatment outcomes.[4,10]

- A prospective, double-blind study performed by Warren et al. demonstrated that 48% of participants diagnosed with IC who underwent antibiotic treatment for 18 weeks reported either a reduction in urgency and pain, or an overall improvement in symptoms, compared to 24% of those in the placebo group. While further studies are needed, this outcome suggests that patients with urinary symptom complexes may have an undiagnosed UTI.[6,27]

Undiagnosed Persistent UTI Should be Considered in Patients Experiencing the Following Symptoms:

TABLE 3: Symptom Overlap Between UTI, OAB, and IC/PBS

KEY: Persistent UTI ● IC / PBS ● OAB ●

Urgency	● ● ●
Frequency	● ● ●
Incontinence	● ● ●
Nocturia	● ● ●
Double voiding	● ● ●
Dysuria	● ●
Hematuria	● ●
Bladder filling & voiding pain	● ●
Pain unchanged by voiding	● ●
Loin pain	● ●
Pain radiating to genitals & legs	● ●
Urethral pain	● ●
Pelvic pain	● ●
Vaginal pain	● ●
Pain during sex	● ●
Reduced stream	● ●
Straining to void	● ●
Post-micturition dribbling	● ●
Foul smelling urine	●

THE ROLE OF BIOFILM IN PERSISTENT AND CULTURE-NEGATIVE UTI

How intracellular bacterial communities and biofilms contribute to treatment difficulty

"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."

— Scott et al. (2015), Intracellular Bacterial Communities: A Potential Etiology for Chronic Lower Urinary Tract Symptoms

"We discovered that the intracellular bacteria matured into biofilms, creating pod-like bulges on the bladder surface. Pods contained bacteria encased in a polysaccharide-rich matrix surrounded by a protective shell of uroplakin. Within the biofilm, bacterial structures interacted extensively with the surrounding matrix, and biofilm associated factors had regional variation in expression. The discovery of intracellular biofilm-like pods explains how bladder infections can persist in the face of robust host defenses."

— Anderson et al. (2003), Intracellular Bacterial Biofilm-Like Pods in Urinary Tract Infections

TABLE 4: Characteristics of Biofilm that Contribute to Antibiotic Resistance
Antibiotic-inactivating enzymes
Horizontal Gene Transfer between organisms
Barriers against: • Host immune cells • Antibodies • Antimicrobials

How biofilm contributes to approximately 80% of recurrent infections and influences antibiotic resistance[28]:

- **Rate of recurrence:** After the initial onset of an acute UTI, the risk of future recurrence increases. 19-24% of women will have a rUTI within 6 months of their first infection, and for those patients who have a history of UTIs, 70% will have a recurrence within one year.[10,29] Multiple factors previously discussed, such as standard urine culture (SUC) bias and sensitivity report limitations, contribute to increased recurrence rates. However, the presence of biofilm plays a significant role.

- **Bacterial biofilms:** Biofilms are bacterial communities encased in a polysaccharide matrix capable of adhering to and inside surfaces and tissues, expressing antibiotic resistance genes, and greatly influencing the development of chronic infections.[28] *E. coli* specifically is a high biofilm-producing bacterium, responsible for contributing to chronic and recurrent infection, with 62.5% of *E. coli* infections shown to produce biofilm.[6,30,31]

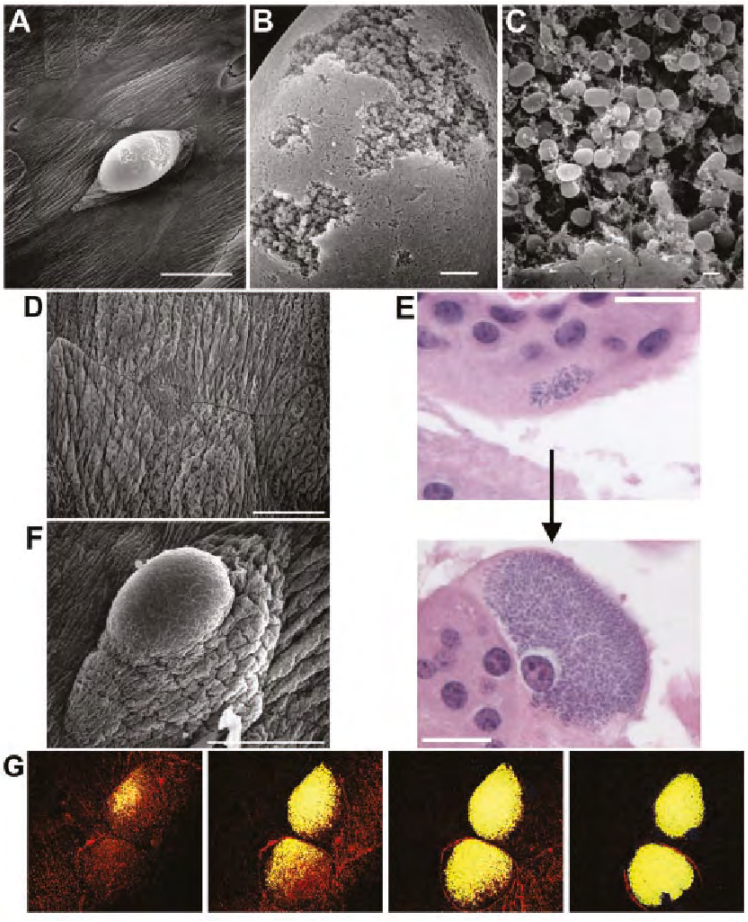
- **Intracellular bacterial communities (IBCs):** IBCs occur when bacteria invade urothelial cells and can be found at varying depths of the bladder epithelia. IBCs take on biofilm-like qualities and, like biofilms, are difficult to detect using standard urine culture (SUC) and are extremely difficult to treat.[30] As much as 10^5 CFU of bacteria are capable of existing in one single shed urothelial cell.[6] However, because SUC methods do not encourage a release of bacteria within the cell, the community is reported as only a single colony. See Figure 5-A below.

- **Adherence to urothelium:** Biofilms and IBCs in the bladder adhere to and inside the urothelium. At times dormant, bacteria within these communities are difficult to detect and effectively treat, however, they continue to colonize and modify gene expression. Biofilm and IBC pods eventually break open, releasing planktonic bacteria and reinfecting the host. Without intervention, the process continues.[6,30]

- **Prevalence:** When compared with asymptomatic controls, 75% of patients with lower urinary tract symptoms (LUTS) had evidence of IBCs compared to 17% found in controls, indicating the potential role of biofilm in urinary symptoms.[6] As explained by Scott et al., "IBCs may have a role not only in the etiology of recurrent UTI but also of chronic LUTS experienced by some women who are given the diagnosis of OAB or IC/BPS."[6]

- **Other biofilm-associated infections:** Biofilms and IBCs are recognized as being associated with other tissue infections, such as dental infections, respiratory tract infections, endocarditis, prostatitis, and more.[28]

FIGURE 5: Intracellular bacterial community



Intracellular bacterial communities attached to the bladder wall of a mouse. (A to C) The biofilm-like pod is magnified to show bacterial communities.[30]

Increasing antibiotic resistance and horizontal gene transfer:

- Compared to planktonic bacteria, microbes encased in biofilm are 10-1,000 times more resistant to antibiotics,[28] with 64% of biofilm-forming *E. coli* infections being multi-drug resistant (MDR) compared to 36% for non-biofilm forming *E. coli* infections.[32]
- Increased resistance is due to the following: Biofilms and IBCs provide microbes with a barrier against host immune cells and antibodies and antimicrobials, in addition to harboring antibiotic-inactivating enzymes. The prominent differentiation between conventional antibiotic resistance and biofilm antibiotic resistance is the altered environment that takes place within the biofilm due to their multicellular nature.[28]
- The transfer of resistance behaviors between microbes, known as horizontal gene transfer (HGT), can occur as a result of increased inflammation in the host as well as in response to antibiotics that promote bacterial lysis. Through the process of HGT, bacterial resistance increases.[24,33]
- The prevalence and defense behaviors of biofilms and IBCs make them a necessary consideration for patients with culture-negative, recurrent, persistent, and multi-drug resistant UTI, as early intervention may disrupt the multicellular structure and aid in achieving better clinical outcomes.[6,30]

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