FROM THE LAB

Urinary Tract Infections & Antimicrobial Resistance

A Role of PCR-Based Diagnosis

Epidemiology of UTI

Urinary tract infections (UTIs) are the most common bacterial infections encountered by primary care physicians in the United States, accounting for approximately 7 million visits and $1.6 billion in cost annually.[19, 29] Because of the shorter length of the female urethra and its proximity to the rectum, UTIs are more common in women than in men—accounting for 84% of visits.[13, 19] Fifty to 70% of women will have a UTI sometime during their lifetime, and 20 to 30% will have a recurrent infection.[2, 14] Known risk factors for developing uncomplicated and recurrent cases of UTIs include sexual intercourse, use of spermicides, a previous UTI, a new sex partner, and a history of UTI in a first-degree female relative.[19] Changes in the normal vaginal microbiome have also been recently identified as an important risk factor for UTIs.[26]

The prevalence of UTIs increases with age and affects 20% of women over 65 years, which is twice the prevalence in the general population.[7] Pregnancy and diabetes mellitus (DM) are also associated and have an increased risk of both first-episode and recurrent UTIs, with recurrence defined as three episodes of UTI in the last 12 months or two episodes in the last 6 months. UTIs during pregnancy may also increase the risk of preeclampsia.[38]

UTI is also the most common bacterial infection in children and is often associated with the presence of anatomic abnormalities. Nonetheless, the diagnosis of UTI in children is often hampered by the diversity of symptoms that can range from irritability to fever and vomiting. In this young population, encopresis—soiling of underwear with stool—is associated with recurrent UTIs.[30]

Hospital- and healthcare facility-acquired UTI also represent a significant health and economic burden. Compared with individuals who develop community-acquired UTIs, those in healthcare settings such as nursing homes are more likely to be older, to have more co-morbidities, and to have been previously treated with antimicrobial agents.[1] Hospital-acquired UTIs, which are predominantly linked to the use of urinary catheters, are often polymicrobial and are more frequently caused by multiple-drug resistant uropathogens.[20]

Classification of UTI

UTIs are generally classified as “uncomplicated” and “complicated.” Traditionally, uncomplicated UTIs comprise episodes of acute cystitis in healthy, non-pregnant women who have no history of urinary tract abnormalities, immunocompromised disorders, or signs of systemic infection.[19] More recent classifications have expanded the list to include cases of acute uncomplicated pyelonephritis, asymptomatic bacteriuria, and recurrent uncomplicated UTIs in this category.[25]

By default, all other UTIs are classified as complicated and refer to infections that occur in men, pregnant women, immunocompromised patients, or those who have an anatomical or functional abnormality of the urogenital tract (for example, individuals with renal stones, urinary catheters, neurogenic bladder, spinal cord injury, and renal transplantation).

Clinical Considerations for Management

Common symptoms of acute cystitis include frequency, dysuria, suprapubic pain, and hematuria. Uncomplicated cystitis is extremely rare in young men, while in elderly men, even though symptoms of acute prostatitis, such as frequency and dysuria, can be misdiagnosed as UTI, urinary-tract obstruction resulting from prostatic edema can increase the risk of UTI.[13]

The prevalence of asymptomatic bacteriuria (ASB), defined as a positive urine culture with at least 105 cfu/ml collected from someone without symptoms, is reported to be between 1-5% in healthy women, 12-26% in women with DM (also increased in men with DM), and over 20% in the elderly.[13] However, treatment of ASB is generally reserved for specific populations at risk for serious complications, such as renal transplant patients and pregnant women.[13, 16, 23, 27] During pregnancy, ASB is associated with an increased risk of preterm delivery and a 20 to 30-fold increased risk of pyelonephritis; furthermore, antibiotic treatment of ASB has been shown to significantly reduce these risks.[35]

The presence of Group B streptococcus (GBS) in the urine of pregnant women indicates vaginal colonization and, therefore, also warrants treatment. GBS is the leading cause of morbidity and mortality among infants in the United States and results from vertical transmission from mother to newborn *in utero* or during passage through the birth canal.[34] There is no diagnostic threshold for GBS to be treated. The 2002 recommendation from the CDC stated that: "clinical microbiology labs should report any concentration of GBS detected in urine (of pregnant women)."

Pathogenesis of UTI

UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (*E. coli*) (UPEC). Following UPEC, causative pathogens of uncomplicated UTIs in decreasing order of prevalence include *Klebsiella pneumoniae* (*K. pneumoniae*), *Staphylococcus saprophyticus* (*S. saprophyticus*), *Enterococcus faecalis* (*E. faecalis*), Group B streptococcus (GBS), *Proteus mirabilis* (*P. mirabilis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*) and Candida species.[11] *S. saprophyticus* is a predominant cause of community acquired UTI, while *S. aureus* occurs more frequently in urinary-catheterized individuals and pregnant women.[3, 24]

In cases of complicated UTIs, UPEC is followed in prevalence by *Enterococcus* species, *K. pneumoniae*, Candida species, *S. aureus*, *P. mirabilis*, *P. aeruginosa*, and GBS.[11] Enterococci are rarely associated with community-acquired UTI but play a prominent role in the pathogenesis of catheter-associated UTI and are among the main pathogens isolated from polymicrobial infections on the surface of indwelling urinary catheters and biliary stents.[9, 10, 24]

There are also several emerging, rare, misclassified, and otherwise under-reported Gram-positive pathogens of the urinary tract including *Aerococcus*, *Corynebacterium*, *Actinobaculum*, and *Gardnerella*.[24]

Polymicrobial UTIs often involve one or more Gram-positive bacteria that have also been shown to influence the pathogenicity of co-infecting organisms; furthermore, many of these pathogens can be easily overlooked by routine diagnostic methods.[24]

Diagnosis of UTI

Standard Diagnostic Tests

A diagnosis of UTI based on clinical symptoms alone is often wrong. The traditional gold standard for the diagnosis of UTI is a combination of clinical symptoms and midstream urine culture. Many laboratories define 105 colony forming units (cfu)/mL urine as the threshold. However, because this threshold misses many relevant infections, a diagnosis of UTI based on a colony a count of 103 cfu/mL, depending on the types of bacteria detected, has also been recommended A diagnosis of ASB is made when an upper limit of ≥105 cfu/mL identified in a sample of midstream urine is exceeded on two consecutive occasions for women and one for men in someone without clinical symptoms of a UTI.[33]

Several other diagnostic methods have both advantages and disadvantages (Table 1).[36] These include: 1) dipstick, a quick method that helps identify UTIs caused by Gram-negative bacteria, but less useful for Gram-positive pathogens, especially in pregnant women and in the elderly[24] 2) Urine microscopy Gram-stain; 3) Immersion culture; and 4) Algorithms, where a diagnosis is based on meeting a certain number of criteria.[33] In addition, new and emerging technologies are under current development.[8]

Tests with low specificities that lead to a high rate of false positives contribute to unnecessary prescriptions and increase the risk of resistant pathogens.

PCR

PCR-based molecular testing has been shown to be non-inferior to urine culture for detecting bacterial pathogens in symptomatic patients. In a study evaluating 582 patients with symptoms of lower UTI, in 22% (130/582) of cases, PCR was positive while culture was negative, while in only 4% (21/582) was the culture positive when the PCR was negative. Of the 30% (175/582) of patients with polymicrobial infections, 166 were reported by PCR and 39 with culture. Furthermore, polymicrobial infections were identified in 67 patients (12%, 67/582) whose culture results were negative. Thus, PCR was more accurate than culture in detecting pathogens and identified bacteria in 36% of patients who had a negative traditional urine culture. PCR was also much more sensitive than culture in detecting polymicrobial infections. This study suggests that PCR testing is more accurate and faster than traditional urine culture and also has the potential to identify polymicrobial infections with complicated mechanisms of antimicrobial resistance sharing.[37]

Table 1 – Comparison of technologies for UTI pathogen detection[8]

Technology	Advantages	Disadvantages
Nitrite and leukocyte esterase (dipstick)	In office point of care	Poor specificity
Conventional culture	Standard of care, sensitive, inexpensive	Time consuming Not point of care
Urinalysis/microscopy	Fast, detects presence of bacteria	No pathogen detection
Immunological-based assays	Rapid, inexpensive	Poor specificity and sensitivity
PCR	Resistance probes available, specific, sensitive, rapid	Multiple probes for pathogens intensive initial processing

The Problem of Antimicrobial Resistance

Currently, antibiotics such as trimethoprim, sulfamethoxazole, ciprofloxacin, and ampicillin are the most commonly recommended drugs for treating UTIs.[11] However, increasing rates of antibiotic resistance and high recurrence rates are steadily increasing the burden on our healthcare systems.

Antibiotic resistance has been well documented for members of the family Enterobacteriaceae, including *E. coli* and *K. pneumoniae*. Both pathogens have acquired plasmids encoding extended-spectrum β-lactamases (ESBLs). These plasmids rapidly spread resistance to third-generation cephalosporins among other antibiotics. Other Enterobacteriaceae produce the class C β-lactamases (AmpC enzymes), enzymes that work against cephamycin and third-generation cephalosporins; they are also resistant to β-lactamase inhibitors. The expression of AmpC enzymes is also linked to carbapenem resistance in *K. pneumoniae* strains.

Multidrug resistance is also common among enterococci, which are naturally resistant to trimethoprim, clindamycin, cephalosporins, and penicillins. Enterococcus species have also developed a high-level resistance to glycopeptides, including vancomycin, one of the last lines of defense against multidrug-resistant organisms.[4, 6, 12, 15, 31, 32]

Even though antibiotic resistance in *S. saprophyticus* is uncommon, methicillin-resistance has been found in ~1–8% of urine isolates and appears to be due to the acquisition of a penicillin-binding protein that has low β-lactam affinity encoded by the mecA gene. The majority of *S. aureus* UTI isolates, which are more common in pregnant women and catheterized individuals, are methicillin-resistant.[17, 18, 22, 24, 28]

Antibiotic resistance is a global problem that is one of the biggest health challenges facing medicine today. In March 2020, the Center for Disease Control (CDC) and Prevention launched a National Action Plan to slow the emergence and spread of resistant bacteria (Tables 2 & 3). This multi-strategic plan includes heightened surveillance, education and preventive measures, the development of new therapeutics such as vaccines, as well as the development and use of rapid and innovative diagnostic tests to identify and characterize resistant bacteria. Improved diagnostic accuracy and characterization of antibiotic resistance of common UTI pathogens is an important strategy that will reduce the overuse of antimicrobial prescriptions and slow the emergence of resistant pathogens.

Table 2 – Examples of CDC-based approaches to reducing antimicrobial resitsant UTI pathogens

Strategy		References
PREVENTION **Patient Education**	Urinate after sexual activity. Stay well hydrated and urinate regularly. Take showers instead of baths. Minimize douching, sprays, or powders in the genital area. Teach girls when potty training to wipe front to back.	https://www.cdc.gov/antibiotic-use/community/for-patients/common-illnesses/uti.html
Nutrition	Cranberry	Juthani-Mehta et al 2016[21]
Physician antibiotic prescribing	Align prescribing practices with evidence-based recommendations	https://www.cdc.gov/antibiotic-use/core-elements/outpatient.html
New Therapies	Comination therapies. Vaccines targeting bacterial adhesions, toxins, and proteases. Small molecules targeting bacterial adhesions. Non-CDC natural options: Ascorbic acid Canephron N, an herbal medication	Flores-Mireles et al.2015[11] Ghouri et al. 2018
New Diagnostic Tests Under Development	MALDI-TOF mass spectrometry Fluorescent *in situ* hybridization Microfluidics Forward light scattering	Davenport et al.2017[8]

Table 3 – CDC's Antibiotic Resistance Threats in the United States, 2019[5]

URGENT THREATS	Year of Data	Deaths	Hospitalized Patients
Acinetobacter	2017	700	8,500
Candida auris	2018	323 clinical cases	
***Candida* species**	2017	1,700	34,800
***Enterbacteriaceae* (CRE)**	2017	1,100	13,100
ESBL-producing *Enterbacteriaceae*	2017	9,100	197,400
Vancomycin-resistant *Enterococcus* (VRE)	2017	5,400	54,500
Multidrug-resistant *Pseudomonas aeruginosa*	2017	2,700	32,600
Methicillin-resistant *Staphylococcus aureus* (MRSA)	2017	10,600	323,700
CONCERNING THREATS			
Erythromycin-resistant Group A streptococcus	2017	450	5,400
Clindamycin-resistant Group B streptococcus	2016	720	13,000
WATCH LIST			
Drug-resistant *Mycoplasma genitalium*			

Assurance Scientific Laboratories UTI Panel

At Assurance Scientific Laboratories, we provide accurate, comprehensive PCR assay for the detection of a wide range of UTI uropathogens. In addition, we offer selective antimicrobial resistance profiles to help clinicians better target therapies in an effort to reduce the use of broad therapeutic regimens that contribute to the growing threat of antibiotic resistance.

General Guidelines

Laboratory test results should always be considered in the context of clinical observations and epidemiological data (such as local prevalence rates and current outbreak/epicenter locations) in making a final diagnosis and patient management decisions. With any test, the possibility of false positive and false negative results should always be considered, and the impact on patient management decisions and clinical outcomes should be carefully weighed.

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