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Review Article

Beyond Standard Urine Culture: Advanced Molecular Testing for Urinary Tract Infections

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Abstract: In this review article, it is emphasized that adopting advanced molecular diagnostics is essential for modern UTI management, offering a more comprehensive, accurate, and rapid approach compared to traditional methods. Standard urine culture's limitations include low sensitivity and failure to culture certain microorganisms, leading to undiagnosed cases and increased morbidity. Advanced molecular techniques, like multiplex-PCR and pooled antibiotic susceptibility testing have been shown to reduce empiric treatments and negative outcomes significantly. And advanced molecular methods like metagenomics (mNGS) offer comprehensive pathogen detection without prior knowledge of target organisms, improving diagnostic yield. These methods also detect antibiotic resistance genes, aiding in precise treatment strategies and improving patient outcomes. Adopting advanced molecular diagnostics is essential for modern UTI management, offering a more comprehensive, accurate, and rapid approach compared to traditional methods. These technologies are vital in improving patient care and combating antibiotic-resistant infections.
Keywords: Urinary tract infections (UTIs), Polymicrobial Infections, Antibiotic Resistance, Urinary Microbiome, Metagenomics, Precision Medicine, Chronic UTI.

INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections, particularly affecting women. Nearly 50% of people will experience a UTI at least once in their lifetime, with adult women being disproportionately impacted due to anatomical differences in the lower urinary tract [1]. Moreover, as the population ages, the prevalence of UTIs is expected to rise. The post-menopausal population is particularly affected due to low estrogen levels, which can alter vaginal tissue and trigger bacterial changes that increase the risk of UTIs [2]. Women with complicated UTIs, especially those with functional or anatomical risk factors such as postmenopausal status, tend to exhibit higher rates of antibiotic resistance to both narrow- and broad-spectrum oral antibiotics. Preventive care is crucial for managing these patients, yet estrogen cream, a valuable preventive measure [3], is underutilized. This increased resistance complicates treatment and highlights the need for more effective diagnostic and therapeutic strategies tailored to this high-risk population. In the United States alone, over 1 million individuals suffer from difficult-to-treat or chronic UTIs annually. UTIs are a leading cause of antibiotic prescriptions in adults, which can disrupt the urinary tract microbiome and contribute to antimicrobial resistance, a significant public health challenge in recent years [4, 5].

Uncomplicated UTIs occur in otherwise healthy individuals with no structural or functional urinary abnormalities and are routinely diagnosed using standard urine culture (SUC) techniques. These infections are generally treated effectively with a course of antibiotics, resulting in high rates of resolution and patient satisfaction. However, a subset of patients experiences recurrent or complicated UTIs, characterized by factors such as structural abnormalities, comorbid conditions, or antibiotic-resistant pathogens. These cases are more challenging to diagnose and treat. Traditional diagnostic methods and empiric antibiotic therapies often prove insufficient, leading to persistent infections, increased morbidity, and a higher risk of severe complications such as sepsis. Indeed, approximately 20-30% of sepsis cases originate from the urogenital tract [6]. However, the effectiveness of urine culture as a diagnostic tool is often compromised by factors such as prior antibiotic use, low sensitivity, and the presence of microorganisms that are difficult or impossible to culture. Consequently, SUC techniques fail to identify pathogens in up to 50% of symptomatic women [7]. Therefore, there is a pressing need for additional laboratory techniques that can promptly and accurately detect uropathogens.

Challenges of Standard Urine Culture

Standard urine culture, which involves agar-based clinical culture of urine specimens, has been the primary method for diagnosing UTIs since the 19th century, with minimal technical advancements over the years. However, the American Urological Association (AUA) has recently highlighted a significant limitation of this method. According to their latest guidance, recent studies have shown that a substantial portion of urinary bacteria cannot be cultivated under standard conditions [8, 9]—underscoring the need for more advanced diagnostic techniques to accurately identify uropathogens that traditional methods might miss.

One significant issue with SUC is that results showing more than two microorganisms are often dismissed as contaminated. Yet, recent studies have found that up to 72% of recurrent, complicated, or persistent UTIs are polymicrobial [10]. Certainly, polymicrobial interactions can play a role in urologic infections [11]. Additionally, the timing of standard cultures can pose challenges, especially for complex UTI cases. SUC results typically take 24-72 hours to be available, which can be too long for certain patients who require immediate treatment. It is well accepted in the literature that inadequate therapy for infections in critically ill, hospitalized patients is associated with poor outcomes, including greater morbidity and mortality as well as increased length of stay [12]. As a result, clinicians often resort to broad-spectrum antimicrobial agents as initial empiric therapy while waiting for SUC results. This practice can contribute to the development of antibiotic resistance within patient populations. A notable example is the increased use of levofloxacin as initial UTI therapy resulting in a rise in fluoroquinolone resistance among urinary Escherichia coli isolates [13]. This highlights the need for more rapid and accurate diagnostic methods to improve patient outcomes.

Where Advanced Molecular Testing could be Helpful

A recent study [14] conducted in urology and urogynecology settings evaluated the effectiveness of Advanced Microbiological Testing (AMT), specifically using a combination of multiplex-PCR and pooled antibiotic susceptibility (P-AST™), against standard urine culture. The results showed that PCR/P-AST led to over a 50% reduction in the use of empiric treatments and a 37% decrease in negative outcomes compared to traditional urine culture methods. This research highlights the benefits of AMT in specialized settings for improving the management of complex UTIs. Rapidly identifying the appropriate antibiotic is crucial for healthcare providers, who often have to depend on empiric therapy when standard diagnostic methods are too slow. AMT offers a faster and more accurate alternative, ensuring timely and effective treatment.

Advanced UTI testing plays a significant role in clinical settings, particularly in managing recurrent urinary tract infections and cases with complicating factors. Treatment algorithms are crucial in these unique situations to ensure effective management. However, it is clear that these advanced testing modalities should not be used for uncomplicated and isolated infections, where standard cultures remain the primary method for therapy [15]. For urologists, AMT can be an invaluable tool. After an initial treatment failure, it is important to utilize specialized testing to identify the underlying issue. Urologists are encouraged to employ advanced diagnostic methods to improve patient outcomes and ensure accurate treatment.

For urologists, AMT is a valuable tool. When initial treatments fail, specialized testing is crucial to identify underlying issues. Using advanced diagnostic methods can improve patient outcomes and ensure accurate treatment. Advanced testing allows for more focused urinary assessment in cases of recurrent or complicated UTIs. It is also beneficial for assessing individuals with chronic urinary syndromes, such as interstitial cystitis or painful bladder syndrome, where symptoms may vary due to bacterial infections. Effective and targeted therapy based on advanced testing significantly benefits these common scenarios in both specialized and general urologic practice. Combining conventional cultures with advanced diagnostic testing ensures precision in both diagnostics and interventions. This dual approach stresses the importance of accuracy in both diagnostic and therapeutic methods, ultimately improving patient care.

An Argument for Metagenomics Next-Generation Sequencing

Over the past thirty years, numerous next-generation sequencing (NGS) platforms have emerged, enabling the high-throughput, massively parallel sequencing of thousands to billions of DNA fragments [16]. This technological advancement has revolutionized our understanding of UTIs. Traditionally, it was believed that urine was sterile in healthy individuals. However, this paradigm has shifted as we now recognize the presence of bacterial communities within the urinary tracts of healthy people. The term 'microbiota' refers to the microorganisms inhabiting specific niches such as the kidney, bladder, and prostate. In contrast, 'microbiome' describes the collective microbial community within a well-defined habitat, characterized by distinct physicochemical properties. These microbial communities play a crucial role in the pathophysiology of infections and are currently a major focus of both basic science and clinical research. Despite this, their function within urological medicine has not been extensively studied.

Metagenomics, a key component of NGS, allows for the simultaneous analysis of large quantities of DNA strands, providing extensive information about the presence of numerous species [16, 17]. This capability is particularly attractive to clinicians as it offers a more comprehensive understanding of a patient's urinary tract microenvironment compared to traditional culture and sensitivity methods [18]. This comprehensive data enables medical practitioners to more accurately target disease-causing organisms, exemplifying a precision medicine approach. One significant advantage of NGS over techniques like polymerase chain reaction (PCR) is its ability to detect and identify pathogens without prior knowledge of the target organisms or the need for specific primers [19]. NGS can generate sequences for multiple pathogens within a single run, facilitating the reliable identification of numerous organisms in a single specimen. Recent advancements have significantly reduced the cost and complexity of NGS instrumentation, enhancing its applicability in clinical settings [20].

Although AMTs like PCR methods can quickly detect pathogens directly from clinical samples, including those that are uncultivable, they are limited to amplifying preselected species [21]. The adoption of metagenomics next generation sequencing (mNGS) in the diagnostic workflow for urology patients experiencing persistent or recurrent symptoms represents a transformative advancement in the field. Traditional diagnostic methods, including SUC, AMT and PCR techniques, often fall short due to their limited scope and inability to detect a comprehensive range of pathogens. In contrast, mNGS offers a target-agnostic approach, sequencing all nucleic acids in a sample and thereby providing a broad-spectrum analysis capable of identifying bacteria, viruses, fungi, and parasites without prior knowledge of their presence [22].

Recent studies highlight the utility of mNGS in clinical settings. For instance, research [23], demonstrated the effectiveness of mNGS in identifying pathogens in UTIs that eluded conventional diagnostic methods. This comprehensive detection capability is particularly crucial for patients with polymicrobial infections, where multiple pathogens coexist and are often missed by standard tests. The precision and breadth of mNGS not only enhance diagnostic yield but also provide a detailed overview of the microbial landscape, crucial for accurate and timely treatment. One significant advantage of mNGS over traditional methods is its ability to bypass the biases inherent in PCR and culture techniques, which require specific primers or are unable to culture certain microorganisms. mNGS addresses these limitations by sequencing all genetic material present in a sample, thus offering a more inclusive and unbiased diagnostic tool. Furthermore, advancements such as precision metagenomics enhance mNGS capabilities through hybridization capture-based targeted sequencing. This method improves diagnostic yield and ensures that clinically significant data is not diluted, effectively addressing one of the main criticisms of standard mNGS approaches [18].

Clinical case studies have consistently shown the advantages of mNGS in providing accurate diagnoses. For example, recent research [18], highlighted the ability of precision metagenomics to detect fastidious, obligate anaerobic, and non-culturable microorganisms directly from clinical samples, displaying its potential to revolutionize UTI diagnostics. Despite the initial prohibitive costs associated with mNGS implementation, the long-term benefits are substantial. Accurate and timely pathogen identification can prevent the progression of infections, reduce hospital readmissions, and lead to more targeted and effective treatments, resulting in overall cost savings for healthcare systems. Moreover, mNGS plays a critical role in addressing the global health threat posed by antimicrobial resistance (AMR). By accurately identifying causative pathogens and their resistance profiles, mNGS enables clinicians to prescribe targeted antibiotics, thereby reducing the misuse of broad-spectrum antibiotics and mitigating the development of resistance. As AMR continues to challenge global health, the integration of advanced diagnostic tools like mNGS into clinical practice is essential for guiding appropriate antimicrobial therapy and preserving the efficacy of existing antibiotics.

Indeed, the integration of mNGS in urological diagnostics for patients with persistent or recurrent symptoms and inconclusive conventional test results is an imperative advancement. The comprehensive detection capabilities, enhanced diagnostic yield, and ability to overcome the limitations of traditional methods make mNGS an invaluable tool in modern urological practice. By adopting this technology, urologists can improve diagnostic accuracy, optimize treatment strategies, and ultimately enhance patient outcomes, while also contributing to the broader effort to combat antimicrobial resistance—ensuring testing methodologies are not siloed [24]. The consensus is clear; advanced molecular testing plays an integral role in managing patients with complex UTI.

Selecting the Right Test for UTI

Once providers have determined the right clinical scenario for using AMT, how do they go about choosing the right test? There are multiple options. PCR testing alone is quite common and can detect pathogens as well as antibiotic resistance genes with high levels of accuracy. However, this method only detects DNA. It cannot determine which genes are being expressed. In fact, by using PCR-resistance gene data alone, providers are significantly limiting their treatment options. Studies show up to 40% discordance between resistance gene detection and phenotypic resistance [25].

One key consideration in choosing advanced UTI tests is the ability to identify specific genotypes and phenotypes of pathogenic organisms. This capability can significantly improve antibiotic selection criteria and potentially reduce the duration of antibiotic therapy. For healthcare practitioners, the ability to phenotype certain organisms and match them to the patient's clinical presentation is of paramount importance. Implementing appropriate antimicrobial testing (AMT) algorithms is expected to not only enhance patient outcomes and reduce healthcare costs but also expedite research [Table 1]. Focused use of advanced testing, as guided by diagnostic testing algorithms, will likely improve patient outcomes and have a lasting effect on reducing healthcare spending associated with incomplete or poorly focused management of unique symptom presentations. Moreover, algorithmic-based reflex ordering can increase the appropriateness of care and provide evidence for payment coverage for these unique testing modalities. It is anticipated that guideline-based management of infections will incorporate the appropriate use of advanced testing where supported by evidence and improved outcomes. There is a call for more data to guide decision-making when choosing among AMT options. Long-term clinical studies are necessary to determine whether these advanced tests truly lead to improved outcomes, cost savings, and changes in clinical decision-making.

Table 1: ***Diagnostic and Therapeutic Approach to Urinary Tract Infections***

Steps	**Actions**	**Reference**
Step 1: Initial Assessment and Diagnosis	Symptoms: Dysuria, frequency, urgency, hematuria, lower abdominal pain. History: Recurrent UTIs, recent antibiotic use, postmenopausal status, anatomical or functional risk factors. Consider rapid diagnostic tests (e.g., dipstick urinalysis) for initial assessment. Consider clinical decision support systems to differentiate between uncomplicated and complicated UTIs. Order Standard Urine Culture (SUC). Use rapid nucleic acid amplification tests (NAATs) for faster diagnosis.	Giesen *et al*., 2021 [27]
Step 2: Interpretation of Initial Results	Positive SUC with Single Pathogen: Proceed with targeted antibiotic therapy based on culture results. Negative SUC or Contaminated Results (More than Two Microorganisms): Evaluate for potential contamination and consider patient's symptoms and history. For inconclusive results, consider using enhanced quantitative urine culture (EQUC).	Hilt *et al*., 2014 [28]
Step 3: Advanced Diagnostic Testing	Criteria for Advanced Microbiological Testing (AMT): Persistent or recurrent UTI symptoms despite initial treatment. Negative or inconclusive SUC results. Presence of risk factors: Postmenopausal status, anatomical abnormalities, history of complicated UTIs. AMT Implementation: If cystoscopy is performed and bladder inflammation is observed, collect catheterized urine samples. Send samples for standard urine culture. Schedule follow-up for reassessment. Employ advanced molecular diagnostics (e.g., PCR) for pathogens not identified by conventional methods. Consider urine proteomics and metabolomics for deeper insights into recurrent UTI cases. Introduce metagenomics next generation sequencing (mNGS)**:** For patients with persistent or recurrent symptoms and negative/inconclusive results from conventional tests, use mNGS to identify a broad range of potential pathogens, including bacteria, viruses, fungi, and parasites.	Almas *et al*., 2023 [18]; Gupta *et al*., 2017 [29]
Step 4: Follow-Up and Reassessment	Persistent Symptoms or Inflammation: Perform a repeat cystoscopy to assess bladder condition. Include AMT in the repeat panel of tests. Use AMT to identify polymicrobial infections or uncultivable microorganisms. Use imaging techniques (e.g., ultrasound, CT scans) to identify anatomical abnormalities.	Foxman, 2010 [30]

Steps	Actions	Reference
Step 5: Treatment Adjustment	Based on Advanced Test Results: Tailor antibiotic therapy to the specific pathogens identified through AMT. Consider combination therapy if polymicrobial infection is detected. Avoid broad-spectrum antibiotics if specific pathogens are identified to minimize resistance. Monitor Patient Response: Reassess symptoms and bladder condition during follow-up visits. Adjust treatment plan as necessary based on patient response and lab results. Consider personalized medicine approaches, tailoring antibiotic therapy based on genetic and phenotypic profiles of pathogens.	Flores-Mireles *et al.*, 2015 [31]; Foxman, 2010 [30]
Step 6: Preventive Measures and Education	Patient Education: Discuss preventive strategies such as increased hydration, proper hygiene, and potentially estrogen therapy for postmenopausal women. Educate about the importance of completing prescribed antibiotic courses. Long-term Management: For patients with recurrent UTIs, consider prophylactic antibiotics or other preventive measures. Promote non-antibiotic prophylactic measures such as cranberry products, D-mannose, and probiotics. Educate on behavioral modifications and lifestyle changes to reduce UTI risk. Schedule regular follow-up visits to monitor and manage any recurrent infections promptly.	Gupta *et al.*, 2011 [32]; Hooton, 2012 [33]

Several advantages are gained by introducing mNGS in the UTI diagnostic workflow. First, mNGS provides comprehensive pathogen detection, identifying a broad spectrum of pathogens, including those that are difficult to culture or typically missed by traditional methods [34]. This is particularly beneficial for detecting polymicrobial infections, which are often associated with recurrent and complicated UTIs [35]. Additionally, mNGS can provide insights into antibiotic resistance genes present in the pathogens, aiding in the development of more effective treatment strategies [36]. In cases where SUC results are negative or inconclusive, mNGS offers a faster and more accurate diagnosis, leading to better-targeted treatments and improved patient outcomes [37]. Therefore, integrating mNGS at this stage ensures thorough investigation of complex and persistent UTI cases, enhancing diagnostic accuracy and treatment efficacy.

CONCLUSION

Advancements in NGS have significantly transformed the landscape of UTI diagnostics, offering unique insights into the microbial ecosystems of the urinary tract. The shift from the conventional understanding of urine sterility to recognizing the presence and role of bacterial communities within healthy urinary tracts stresses the importance for more sophisticated diagnostic tools. The integration of metagenomics and other AMT methods represents a valuable step forward, enabling the comprehensive detection and characterization of uropathogens that traditional methods often lack.

Metagenomics, in particular, stands out due to its ability to analyze large quantities of DNA simultaneously, providing a detailed and inclusive view of the urinary microbiome [18]. This capability is especially crucial for identifying polymicrobial infections and pathogens that are difficult to culture, which are common in recurrent and complicated UTIs. By employing a target-agnostic approach, metagenomics overcomes the limitations of PCR and traditional urine culture, offering a broader and more accurate diagnostic tool. The application of metagenomics further enhances these capabilities, ensuring epidemiological and clinically significant data is not overlooked, thus improving diagnostic yield and treatment efficacy [38]. The clinical benefits of integrating advanced diagnostic techniques like mNGS into routine practice are substantial. Rapid and accurate pathogen identification can lead to more targeted antibiotic therapies, reducing the misuse of broad-spectrum antibiotics and mitigating the development of antimicrobial resistance. This not only improves patient outcomes by ensuring timely and appropriate treatment but also has the potential to reduce healthcare costs associated with prolonged hospital stays and recurrent infections. Moreover, the ability to detect antibiotic resistance genes through metagenomics provides critical information for tailoring effective treatment strategies, further enhancing the precision of UTI management.

As the body of evidence supporting the efficacy of AMT grows, it is clear that these advanced testing modalities are essential for managing complex UTI cases. Long-term clinical studies are necessary to fully understand the impact of these technologies on clinical decision-making, patient outcomes, and healthcare costs. Nevertheless, the integration of metagenomics and other AMT methods into clinical practice represents a significant advancement in urological diagnostics. By embracing these technologies, healthcare providers can improve diagnostic accuracy, optimize treatment plans, and ultimately enhance patient care, addressing the challenges posed by recurrent and complicated UTIs in a more effective and efficient manner.

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