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Advanced Technologies for Detection of Urinary Tract Infection

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Abstract: *Urinary tract infections (UTIs) are the most prevalent kind of infectious disease worldwide. It is the second most common type of infection in the urinary system. UTIs are mainly caused by bacterial infections. The most common uropathogens that cause UTIs are Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus spp, and Enterobacter spp. The diagnosis of the UTI infection is not straightforward. The colony morphology, sugar fermentation, and hemolytic properties are considered "gold standard" methods for the identification of uropathogens. All these methods mentioned above have some limitations in that they are expensive and less sensitive, and some of these methods also lead to false-positive results. Hence, this review intends to summarize the culture-based methods and emerging diagnostic techniques such as PCR, NGS, MALDI-TOF MS, and FISH for the diagnosis of urinary tract infection.*

Keywords: *Urinary tract infection, Urine Culture, Culture based detection, Uropathogens, Rapid detection*

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1. Introduction

Urinary Tract Infections (UTIs) is an infection caused by the presence and growth of bacteria anywhere in the urinary tract including ureters, bladder, kidney, and urethra [1]. UTIs can affect the upper or lower urinary tract, as well as both, and various factors like hygiene, socio-economy, nutrition, and immunity can influence their occurrence [2]. Every year worldwide 150 million people affected with UTIs [3]. It is a serious health problem affecting millions of people each year and the leading cause of gram-negative bacteriaemia. UTIs are also the leading cause of morbidity and health care expenditures in persons of all ages. Each and every women has a lifetime risk of developing UTIs of 60%, while men have a lifetime risk of only 13% [4]. The reported rate of UTIs in pregnant mothers is about 8% [5]. Premenopausal adult women are at particularly high risk of developing acute cystitis. Other at risk groups for UTI include patients with diabetes, neurogenic bladder, spinal cord injury, pregnancy, prostatic hyperplasia, or urinary tract related urinary disorders. The symptoms of UTI include frequent and strong urge to urinate, blood passing urine and more smell than typical smell of urine, burning sensation while passing urine, pain in lower abdomen even when passing urine and more smell than typical smell of urine, fever, itching and pyuria. Symptomatic UTIs are more frequent than asymptomatic [6]. As per reporting number of pathogens are responsible for bringing about UTIs. The most common pathogenic pathogen for UTIs, is *Escherichia coli,* being responsible for 70-80% of infecton [7]. Other common gram-negative and gram-positive bacteria associated with urinary tract infections include, *Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia, Salmonella paratyphi, Citrobacterfreundii, Vibrio cholera,* and *Cocci, like Group B streptococcus* and *staphylococcus species*[8].

Urinary tract infection occurs with increased frequency and severity in diabetes mellitus patients. General host factors that increase the risk factor of UTI in diabetics include age, metabolic control, and long-term complications [9]. It includes bacteriuria, or upper urinary tract infection, in diabetes mellitus patients, and the number of hospitalizations for the treatment of pyelonephritis, or bilateral renal infection, is

higher in diabetes mellitus patients than in non- diabetic patients. In addition, patients with diabetes mellitus are at increased risk for severe urosepsis, such as intra- or perirenal abscesses or emphysematous urinary tract infections [10]. At the time of occurrence, urine should be cultured and bacteriuria should be treated with antibiotics [11].

UTI causes the serious complications like low birth weight, hypertention, preeclampsia, pyelonephritis, amnionitis, stillbirths, anaemia, toxic septicaemia and bacteraemia [12]. An untreated UTIs can cause the pyelonephritis, and increases the risk of premature birth [13]. Conversely, pregnant woman may also harmed by overtreatment with antibiotics. Misuse and overuse of antibiotics lead to the antimicrobial resistance. In addition, unnecessary exposure of the unborn child to antibiotics may not be without risk [14].

UTI is challenging, not only because of the large number of infections that occur each year, but also because the diagnosis of UTI is not always straight forward [15]. Criteria for the diagnosis of UTI vary greatly depending on the patients and context [16]. For patients with symptoms of UTI and bacteriuria, the primary goal of treatment is to eliminate the infection bacteria causing any adverse effect of the treatment and preventing the recurrence of symptoms. It is well established that culture based methods are gold standard for the detection and identification of pathogens. However, evidence has been accumulating to support use of molecular methods such as PCR. With antimicrobial resistance becoming both more common and complex, effective treatment of UTIs is even more dependent on the accurate identification of pathogens. Some organisms can be fastidious, and therefore difficult to grow in culture. So, the rapid methods such as polymerase chain reaction (PCR), Next-generation sequencing (NGS), Matrix - assisted laser desorption/ionisation-time of flight (MALDI-TOF), and Fluorescence insitu hybridization (FISH) are reliable tool for the detection of UTI. These methods represent a significant reduction in detection time and have higher sensitivity and specificity than culture based methods. However, all these methods can be combined, allowing an accurate identification and precise evaluation of the pathogen's antibiotic susceptibility.

2. Culture Based Methods

Culture based method for the detecting urinary tract infection is highly effective

approach that involves the careful selection and utilisation of specific culture media. A lower urinary tract infection can be diagnosed using the standard urine culture, even through studies have indicated that the 10^5-cfu/ml threshold has limitations. The standard urine culture was first described to identify patient who were at risk for *pyelonephritis*. This fundamental approach to uropathogens detection has not changed, even though clinical attention has been directed toward different cut off thresholds [17].

The culture-based method for detecting urinary tract infections (UTIs) is a highly effective approach that involves the careful selection and utilization of specific culture media. Culture-based methods are considered the gold standard for diagnosing UTIs. Because they provide valuable information about the causative agents and their susceptibility to antibiotics. It takes 1 to 3 days to yield results. However, they typically take longer to produce results compared to rapid diagnostic tests, which can detect the presence of certain pathogens more quickly.

3. Polymerase Chain Reaction

Polymerase chain reaction based diagnostic testing is one of the numerous advancements in DNA-related laboratory techniques that are now widely accessible in a range of settings. Multiplex PCR testing has significantly decreased the cost and time associated with this type of test while also improving its usefulness in clinical medicine. Multiplex PCR testing uses multiple primers to detect multiple targets at once. This technique has been investigated for various types of infections, and has proven to be especially helpful in identifying pathogenic microorganisms. The appeal of PCR-based diagnostic testing for UTI lies in its high specificity and sensitivity, as well as the rapidity with which results can be obtained compared to standard bacterial cultures for the previously named infections [18]. The studies have reported higher detection rates using PCR compared to urine culture, most tested against single pathogens, which is clearly not sufficiently comprehensive for clinical use. [19-20]. A small number of studies have examined the performance of multiplex PCR, testing for between 9 and 20 pathogens. The multiplex PCR was able to detect polymicrobial infections than culture and was a better able to identify the pathogens which cause UTI. The Culture based method was rarely able to detect cases of >3 pathogens and was unable to identify

the bacteria. The multiplex PCR is able to detect polymicrobial infections and the detection rate for polymicrobial infections, is 2 or more pathogens. Multiplex PCR testing can give a more comprehensive picture of the antibiotic resistance of the pathogens that have been identified and increase access to the required technology, which results in sufficient cost savings to overcome the current logistical and financial obstacles [21].

4. Next Generation Sequencing

The use of next-generation sequencing is currently considered for the detection of UTIs. The culture-independent DNA-based identification of microorganisms was developed by microbial ecologists in order to detect bacterial species without the need for culture. One technique utilizes PCR amplification and high throughout sequencing of essential 16S rRNA genes, a form of NGS. More specifically, this technique takes advantage of nine known hypervariable regions of the otherwise highly conserved 16s rRNA gene amplicon to distinguish even closely related bacterial species through evolutionary polymorphism. This is a clinical application for the diagnosis of UTI [22]. The NGS testing can be used to identify other harmful, unculturable bacteria in addition to serving as a potential substitute for urine culture testing. However, the blood NGS testing might be helpful in cases of severe urosepsis in identifying pathogenic bacteria that are spreading widely. Furthermore, there are fewer pathogenic bacteria in the blood, it appears likely that our data from non-severe, non-sepsis cases represent the potential of NGS for detecting false-positive bacteria owing to contaminants or noise.

In most cases, it was found that urine culture-positive pathogenic bacteria showed the highest occupancy rates in urine NGS. The common UTI pathogens *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis,* and *Aerococcus urinae* were found in both the urine culture and NGS tests [23]. Many other bacteria detected in urine NGS tests were negative in the urine culture tests. *Aerococcus urinae*, which prior research indicates is most likely the actual causative bacterium, was detected by the urine NGS test, while *Streptococcus* species was detected by the urine culture test. *Aerococcus urinae* can be diagnosed through genome analysis because they are frequently challenging to isolate through urine culture [24]. Urine NGS testing

performs well in diagnosing acute cystitis cases and is useful in medical treatment.

5. Matrix-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectroscopy

In modern clinical settings, MALDI-TOF MS is a standard diagnostic procedure for infectious diseases. The quick, inexpensive, and easy-to-use approach is becoming more widely used in clinical microbiology labs to identify antimicrobial resistance in microbe. Two FDA-cleared MALDI-TOF MS system are available- the VITEK MS system (biomerieux Inc.) and the MALDI biotyper CA system (Bruker Daltonic Inc.). Despite the high initial cost of the instrument, MALDI-TOF-MS provides fast and accurate results while saving money on technical labor and reagent costs [25]. Urine sample pose a challenge because their urine matrix, which includes PH, electrolyte concentration, and cellular composition, varies not only person to person but also same person different time. Numerous groups have investigated various urine processing stage, such as dual filtration or differential centrifugation, with encouraging outcomes. Numerous studies have also looked at the combination of screening tools followed by direct MALDI-TOF testing of Urine sample in order to reduce workflow times [26].

As compared to conventional identification, the accuracy of the results from studies that prescreened samples using flow cytometry and then MALDI-TOF ranged from 74 to 94 percent. In comparison to either method alone, the combination of MALDI-TOF-MS and *urinalysis* (leucocyte esterase positive, nitrate positive, and bacterial counts of >500/µL) produced results for pathogen identification more quickly-within one hour [27]. This method provided identification in less than 4 hour directly from urine.

6. Fluorescence Insitu Hybridization

The Fluorescence Insitu Hybridization (FISH) method for the detection of urinary tract infections involves several key procedural steps. Initially, a urine sample is collected from the patient and centrifuged to concentrate any bacteria present. The concentrated sample is then fixed onto a glass slide to immobilize the bacteria. Next, fluorescently labelled DNA probes specific to the target bacterial species commonly associated with UTIs, such as *Escherichia coli, Enterococcus faecalis, or Klebsiella pneumoniae,* are applied to the slide. These

DNA probes are designed to complementarily bind to the bacterial DNA within the urine sample. After a period of hybridization, excess unbound probes are washed away to reduce background fluorescence. The slide is then examined under a fluorescence microscope equipped with appropriate filters to visualize the fluorescently labelled bacteria [28]. The presence of fluorescence indicates the presence of the target bacterial species in the urine sample, enabling rapid identification and diagnosis of UTIs. In the table comparison of culture based method with other advance method is discussed (Table1). FISH offers several advantages, including high sensitivity, specificity, and the ability to detect bacteria that may be missed by traditional culture-based methods, making it a valuable tool for diagnosing UTIs in clinical settings

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Table 1. Comparative of culture based and various advance methods for the detection of UTI

S. No.	Detection method	Working principle	Diagnosis time	References
1.	Standard Urine Culture	Urine culture on agar plates for growth, concentration, identification and isolation of pathogens	1-3 days	[17]
2.	PCR Method	Amplification of specific genes from the total genomic DNA extracted from urine sample	4-5 hours	[29]
3.	NGS	PCR amplification and next generation sequencing (NGS), or high throughout sequencing, of essential 16s ribosomal RNA genes.	3-4 hours	[22]
4.	MALDI-TOF-MS	Changed molecules are created by ionization, separated based on the mass/charge ratio and detected and measured using the TOF mass analyser	10-30 min	[30]
5.	FISH	Microscopic detection of microorganisms using fluorescently labelled nucleic acid probes Hybridized to complementary targets	20 min	[31]

7. Conclusion

Recently, advance technology have revolutionized for UTI detection, offering enhanced sensitivity, specificity, and rapidity. Molecular based techniques, including polymerase chain reaction (PCR) and next generation sequencing (NGS) have emerged as powerful tools for detecting uropathogens with high precision. Additional, matrix-Assisted laser desorption/ionization time-of-fllight mass spectroscopy (MALDI-TOF MS), Fluorescence In Situ Hybridization (FISH) has revolutionized microbial identification, enabling rapid and accurate species-level identification. In UTI detection, the incorporation of cutting-edge technologies into standard clinical practice signifies a paradigm change. Adoption of advance technology shows promise in improving patient outcomes and detection accuracy, even though conventional procedures are still essential for initial screening.

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