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Review Article

Molecular and Imaging Diagnostic Techniques for Urinary Tract Infections: Modern Approaches

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Abstract: In developing countries, the frequent failure of the available phenotypic approaches for laboratory diagnosis of urinary tract infections in providing results at the point where medical care is mostly required, becomes a major barrier to efficient antibiotic treatment and management of urinary tract infections in the public health sector. This review therefore focuses on molecular and imaging diagnostic techniques for urinary tract infection as rapid and effective modern approaches requires in health care delivery. Currently, available laboratory diagnoses of urinary tract infection in developing countries are mostly phenotypic approaches, and takes not less than two-four days before completion and result made available for appropriate treatment. From literature, it is apparent that these old-century approaches produce portion of patients' result that does not fit the true picture; and the techniques had been found with more disadvantages than advantages. Molecular approaches are now emerging as modern laboratory test techniques which enable rapid and effective diagnosis of urinary tract infection with Biosensor, Microfluidics, Polymerase Chain Reaction (PCR) and other integrated platforms technologies. These emerging technologies could improve urinary tract infection diagnosis via direct pathogen detection from urine samples, rapid antimicrobial susceptibility testing, high precision and point-of-care testing in public health sector. Imaging techniques have also been so useful in identifying risk factors and abnormalities that can be modified; to decrease likelihood of recurrent (upper) UTI; and to reduce risk of renal scarring. These approaches however, had proved so successful that seems they will replace old-century testing methods, and hence, provides efficient antibiotic treatment and management; therefore, saving health care costs and valuable diagnosis time.

Keywords: UTI, Molecular, Imaging, Laboratory, Diagnostic Techniques, Point of Care

1. Introduction

Urinary tract infection (UTI) refers to the presence of microorganisms in the urinary tract or a significant bacteriuria in the presence of symptoms [1]. Urinary tract infections are the most common diseases encountered in the practice of medicine today [2], major cause of patient death and health care expenditure for all age groups, and it is estimated to account for more than seven million clinic visits and more than one million hospital admissions per year. The total cost of urinary tract infections to the United States health care system in 2000 was approximately 3.5 billion dollars. Urinary tract infections can be community or hospital acquired. It is the most prevalent infection in hospital and commonly associated with catheterization or instrumentation of the Urinary tract. The community acquired Urinary tract infections are prevalent in susceptible patient group including both pregnant and non-pregnant women of childbearing age, children and elderly people of both sexes [3].

Urinary tract infection (UTI) associated with the urinary tract are hospital and community acquired; and the major etiologic agents implicated include *Escherichia coli*, *Proteus*

mirabilis Pseudomonas aeruginosa, Klebsiella pueumoniae, Staphylococcus saprophyticus, Nasseria gonorrhoeae, Hemolytic streptococci, Schistosoma haematobium, Trichomonas vaginalis Candida albican and *Adenovirus* [4].

Urine dipsticks are fast and amenable to point-of-care testing, but do not have adequate diagnostic accuracy or provide microbiological diagnosis. At present, microscopy and culture remain the most important techniques for laboratory diagnosis of urinary tract infections in developing countries. In microscopy, urine is examined as a wet preparation to detect significant pyuria i.e white blood cells (WBC), red cells, casts, yeast cells, *Trichomonas vaginalis*, motile trophozoites, *Schistosoma haematobium* egg, and bacteria. In culture, appropriate number of bacteria in urine sample is estimated and urine from a person with untreated acute urinary tract infection usually contains 10^5 cfu/ml or more bacteria. Biochemical examination techniques are also available, and these include protein reagent strip test, leucocyte esterase strip test and nitrite reagent test to determine proteinuria, specific polymorphonuclear neutrophils [pus cell] and nitrate-reducing pathogens respectively [4, 5].

Manual methods for diagnosing urinary tract infections have been found to require more scrutiny. In current laboratory practice, pathogens in clinical specimens are grown on culture petri dishes until they can be visually identified. Major draw-back of this century-old technique is the two-day time lag between specimen collection and bacteria identification. As a result, physicians must decide whether to prescribe antibiotic therapy and, if so, which type of bacteria to treat-all without knowing the cause of the infection, if any [3]. The major barrier to efficient antibiotic management of the urinary tract infections is that the standard diagnostic methodologies do not provide result at the point where medical care is mostly required [6].

The standard culture-based diagnosis of UTI has a typical delay of two to three days. This delay is due to the need for sample transport to centralized laboratories and the time required for bacteria to grow on artificial media for phenotypic identification. The delays between sample collection, bacterial culture and antibiotic susceptibility reporting have led to empirical use of antibiotics, contributing to the emergence of drug resistance pathogens. The emergence of drug resistant pathogens is an increasing problem worldwide, driven by the injudicious use of antibiotics and few new antibiotics [6].

There is a considerable interest in decreasing overall health care cost by providing smarter medicine, when laboratory quality testing can be rapidly performed anywhere and the result made available in real- time for tremendous improvement in patient care [3]. Identifying urinary tract infection pathogens in a short time frame will enable physicians to make dramatically superior clinical decisions, since the ability to obtain rapid, definitive point of care (POC) diagnosis of UTI will have an enormous favorable impact on its management: timely antibiotic treatment could be initiated and imprecise empirical treatment obviated [6].

The use of specific probes or the biosensor eliminates the need to separate pathogens of interest from potential contaminants such as normal skin and genital flora, and allows for the determination of antibiotic susceptibility profile of multiple pathogens in a single sample [6]. Manual bench top assay for urinary tract infections using biosensor pathogen identification and biosensor antimicrobial susceptibility test are currently available and can provide culture and susceptibility information directly from clinical sample within three and half hour [7]. The new generation of biosensors based on micro- and nanotechnologies offer the possibility of highly sensitive molecular diagnosis within a compact platform and low power consumption suitable for POC applications [6].

Urinary tract infection (UTI) is a heterogeneous condition ranging from mild cystitis, easy to treat with oral antibiotics, to life-threatening bacteremia with shock and multiple organ failure. Most studies provide recommendations for imaging of patients with urosepsis in order to detect urological complications that need intervention, as well as conditions that predispose to renal infection. The term urosepsis signifies bacteremia with a urinary tract focus. Patients with urosepsis and a suspected upper UTI need special attention because these patients may require radiological evaluation in order to discover urological complications, such as renal abscess or pyonephrosis, or conditions that predispose to renal infection, such as structural malformations. Radiological findings may lead to treatment adjustment or urgent interventions to drain the infectious focus or prevent permanent loss of renal function [8].

This review therefore focuses on molecular and imaging UTI diagnostic techniques as modern and effective diagnostic platforms at patients point of care applications, aiming at replacing old-century testing methods, saving health care costs, and valuable diagnosis time.

2. Molecular (Nucleic Acid) – Based Techniques for UTI Diagnosis

2.1. Emerging Biosensing Techniques

Biosensors are emerging as a powerful diagnostic platform for infectious diseases. They are poised to significantly improve UTI diagnosis; and are amenable to integration with microfluidic technology for point-of-care applications [6]. A biosensor is any device or system capable of detecting a biological entity, ranging from lateral flow test strips for pregnancy testing [9] to cell-based sensors using B-lymphocytes to detect pathogens [10]. In the simplest sense, a biosensor is composed of a *recognition element* and a signal *transducer*. Binding of the target (*analyte*) to the recognition element leads to generation of a measurable signal (e.g. electrons, light, mass effect) that is then detected by the transducer. For quantitative detection, the magnitude of the signal is proportional to the analyte concentration. Common examples of recognition elements include antibodies, enzymes, receptors, nucleic acids, aptamers, and other synthetic

molecules. Common transducers include electrodes for electrochemical sensors and CCD cameras for optical sensors [11, 12, 13, 14, 15]. Specifically for UTI, a successful biosensor needs to meet the following criteria:(i) The ability to definitively rule out infection; (ii) The assay needs to be fast, within the POC time frame to effect treatment planning; (iii) Automation of the sample preparation with minimal intervention from the end-user ('plug and play'); (iv) Robust assay protocol compatible with urine matrix effect; (v) Incorporation of pathogen identification with antimicrobial susceptibility testing; and (vi) Versatile to be adaptable for the different pathogen profiles in different clinical scenarios [6].

2.1.1. Principles

The biosensors use sequence specific hybridization of bacterial 16s rRNA for the molecular identification of pathogens. The identification of pathogens is by:

i. Hybridization of specific capture and detector probes to bacterial 16s rRNA at the sensor surface, follow by,
ii. Electrochemical signal amplification with an enzyme tag, and then,
iii. Transmission of a molecular recognition event, i.e. DNA-RNA hybridization into a quantitative electrical signal.

2.1.2. Method

This method involves:

1. Functionalizing the electrochemical sensor with capture oligonucleotide targeting 16s rRNA of bacteria such as *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia, etc.* and negative control.
2. Collecting a cellular fraction of the urine sample for the identification of pathogens by centrifugation.
3. Performing the biosensor assay using specific probes for specific pathogen identification.

Pathogens identification takes 1 hour and can be performed from urine without target purification or amplification and if pathogens are identified, level of 16s rRNA from sample incubated is quantified on biosensor providing susceptibility data within 3½ hours of urine sample collection [5].

A typical example of a biosensor for uropathogen identification is the UTI Sensor Array [16, 17, 18, 19]. The sensor-platform is based on an electrochemical sensor array customized with bacterial specific DNA probes as recognition elements. Each of the 16 sensors is modified with a surface layer step called self-assembled monolayer to allow versatility in surface modification and reduce background noise [20, 21]. A library of the DNA probes targeting the most common uropathogens is immobilized on the sensor surface [16, 18]. The detection protocol is based on conversion of hybridization events into quantifiable electrochemical signals. In this method of analysis, the urine sample is first lysed, then the bacterial 16S rRNA is detected by sandwich hybridization of capture and detector oligonucleotide probe pairs with the 16S rRNA as an ideal target for pathogen identification because it is one of the most abundant molecules in bacteria and has sequences that are highly conserved as well as sequences unique to individual species [22, 23]. The relative abundance of the 16S rRNA (approximately 10,000 copies per cell) precludes the need for nucleic acid amplification [22]. The capture probe is immobilized to the sensor surface and the detector probe is free in solution. A multiplex assay uses universal probe targeting 16S rRNA sequences conserved in all bacterial species, as well as genus- and species-specific probes, allowing for detection of all bacteria via hybridization to the universal probe and refined typing of the most common causative agents. This uses an array of 16 electrochemical biosensors with each sensor composing of three (3) electrodes (working, reference and counter) (Figure 1) [24]. In this technique, lysis of pathogens in urine samples releases the 16S rRNA target. The UTI sensor array currently has an overall detection limit of 10^4 cfu/ml, which is within the clinical cutoff compared to urine culture [18]. In testing with patient urine samples, the UTI sensor array had 92% overall sensitivity and 97% specificity for pathogen detection compared to urine culture. The UTI Sensor Array offers a promising technology platform without the need for nucleic acid amplification [18, 19].

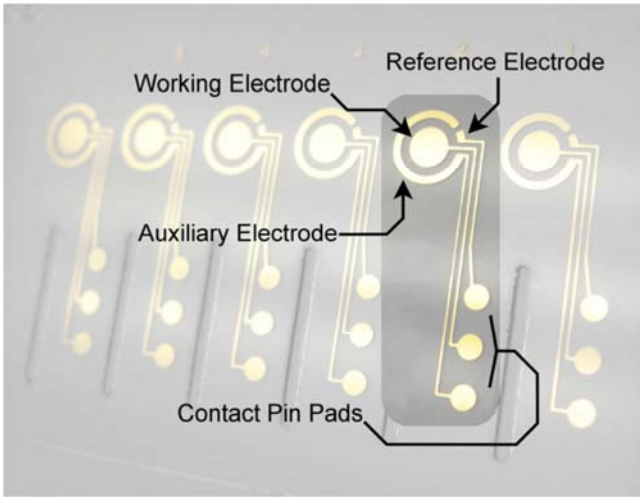

Figure 1. *Electrochemical sensor array.*

2.2. Polymerase Chain Reaction (PCR) Technique

PCR-based approaches to pathogen identification also shows promise for rapid molecular diagnosis for infectious diseases [25]. Recently, Lehmann *et al.* [26, 27] demonstrated direct real-time PCR for identification of pathogens from patient urine samples. Using real-time, the PCR primers designed for uropathogen detection assay had a sensitivity and specificity of 90% and 87%, respectively. In a study by Anneke *et al*. [28] for development of a semi-quantitative real-time PCR to detect uropathogens. Two multiplex PCR reactions were designed to detect *Escherichia coli*, *Klebsiella spp*., *Enterobacter spp*., *Citrobacter spp*., *Proteus mirabilis*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. 16S based PCR was performed in parallel to detect Gram-positive and Gram-negative bacteria. For the detection of uropathogens, PCR was found to be more sensitive than culture. This indicated that it is feasible to detect and identify

uropathogens by multiplex real-time PCR assay

2.3. Emerging Biosensor Antibiotic Susceptibility Assay Technique

A biosensor-based Antimicrobial Sensitivity Test (b-AST) exist, which combines the versatility of the phenotypic assay with genotypic specificity of the 16S rRNA probes [19]. In this assay, following pathogen identification, 16S rRNA is utilized as a bacterial growth marker for phenotypic Antimicrobial Sensitivity Test (AST). Each electrode on the UTI Sensor Array biosensor is tethered with an oligonucleotide probe specific to the bacteria of interest and used to measure growth of the pathogen under different antibiotic conditions. Biosensor signals from samples incubated with an antibiotic are comparable in magnitude to biosensors signals from samples incubated without antibiotic, indicating comparable growth and thus antibiotic resistance. In laboratory testing with patient samples, this biosensor based has 94% overall sensitivity [19]. The main advantage of the biosensor approach over standard AST is time. Since the biosensor specifically detected the pathogen of interest, then, pathogen isolation through overnight plating is not necessary. The overall assay time currently is at 3½ hours, compared to over 18 hours for old-century standard AST [19]. The b-AST approach is inherently compatible with microfluidics, which facilitates the implementation at the POC and allows on-chip sample preparation. The antibiotic susceptibility assay is carry out using the following procedure:

1. Bacteria are inoculated into Mueller-Hinton broth, grown at OD600 = 0.2, then dilute to OD600 = 0.02 in Mueller-Hinton broth with or without antibiotic.
2. Incubate at 37°C with shaking.
3. Aliquot samples are then collected for OD600, biosensor assay and/or determination of cfu at regular intervals during the incubation.
4. Equal volume of urine and Mueller-Hinton is usually mixed for antibiotic susceptibility from urine.
5. Fifty microliters (50 µl) of the mixture is pipetted into the wells of a sensitive plate containing dehydrated antibiotic and control well without antibiotic.
6. The mixture of the plate is then incubated at 37°C with shaking for 2½ hours.
7. Biosensor assay can then be performed immediately after incubation or frozen at -8°C for later assay.

2.4. Emerging Automated Genefluidics' Biosensor (Microfluidic) System

This laboratory device is small in size and uses crude, unseparated samples to reduce the processing time and in turn lower volume of sample in hospital laboratory, reduce health care cost, and increase specificity. It has 16 sensor chips per cartridge coated with bacteria species-specific genetic probes. The device detects bacterial pathogens in clinical fluids samples using a microfabricated electrochemical sensor array. The genes probes transcription-mediated amplification technology involves multiple rounds of synthesis of RNA and DNA copies of the target. Each of the newly synthesized molecules serves as a template for a new round of replication, leading to exponential expansion of amplicon. This expansion can result in the production of billions of amplicons in less than one hour. Incorporated to the system are several features such as random asses sample loading that makes it more user-friendly for laboratory technicians and technologists. Clinical samples are directly loaded into the system and the electrochemical signal subsequently measured by the genefluidics' multichannel reader. Urinary tract infection pathogens are identified by examining which signal on the sensors are elevated. The automated genefluidics' biosensors system has been reported to process successfully 275 samples in 8 hours with up to 120 tubes being loaded at the same time [29].

To enable automated sample preparation at POC, microfluidics technology, or fluidic manipulation at the micron scale, is the core technology that will integrate the reagent transfer, target isolation, and sample mixing steps in a multi-layered cartridge containing channels, valves, and reagent reservoirs [30, 31, 32, 33, 34].

Biosensors integrated with microfluidic cartridge have recently been described [24, 32]. These disposable cartridges have majority of the necessary components for sample handling with dimensions comparable to a credit card. The cartridge can be inserted into a portable reader device and electrochemical sensors are well suited for integration with microfluidic systems [6].

3. Imaging Diagnostic Techniques

The aim of imaging in UTI is to enable early identification of risk factors and abnormalities that can be modified; to decrease likelihood of recurrent (upper) UTI; and to reduce risk of renal scarring [1]. The imaging techniques are used for the following cases in urinary tract infections:

i. Serious and recurrent cases of pyelonephritis.
ii. When structural abnormalities are suspected.
iii. If infection do not respond to treatment.
iv. If physician suspect obstruction or an abscess.
v. After a first urinary tract infection in children two to twenty- four months to detect possible obstruction or vesicoureteral reflux.

4. Potentials/Prospects of Molecular Diagnostic Techniques for Urinary Tract Infection

1. Cartridge-based microfluidics is a highly promising technology for clinical diagnostics.
2. Reagent and specimen volume is minimized along with the size of the system.
3. Automated microfluidic system capable of performing six multiplexed genomic and proteomic analyses simultaneously, by means of an integrated electrochemical sensor and embedded controls.

4. The process provides for improvement in the sensitivity, specificity, and the total processing time required for biochemical analysis.
5. By introducing advanced bio- nanotechnology and innovative transduction principles, a microfluidic-based platform has the potential to greatly expand the scope of point-of-care testing and other resource-limited applications.
6. A key advantage of a microfluidic-based system is its capability in automating molecular analysis.
7. Benefits include reducing errors associated with manual processing and dramatically reducing the quantity of reagents and samples required.
8. With continuous improvement in production cost, scalability, and reliability, microfluidic-based systems can revolutionize current practices in molecular analysis.
9. This system produces quantitative results and performs most assays in under an hour.
10. Better detection and species-specific identification of pathogens in clinical urine specimens using sensors.

Table 1. *Describes the summary of the techniques, purposes and comments on imaging methods of UTI diagnosis.*

S/N	Techniques	Purpose	Comment
1	Ultrasound	used to screen for hydronephritis i.e. obstruction of the flow of urine, kidney stone that predisposes to infection, abscess of the prostate gland and kidney abscesses, detection of vesico ureteral reflux in children with urinary tract infections. In combination with x-rays for accurate detection of incomplete emptying of bladder which is a common cause of urinary tract infection in men over 50 years	It is non-invasive and not accurate as voiding cysto urethrogram
2	Nuclear scan	Useful in complicated issue such as detecting kidney scarring after pyelonephritis in children	They produce better image and expose the patient to far less radiation than x-rays do.
3	Magnetic Resonance Imaging (MRI) and computed tomography (CT) scan	Useful for ruling out kidney stones or obstruction in women with recurrent urinary tract infections.	It is non-invasive and sometimes used when nuclear scan is inconclusive
4	X-rays i. Voiding cystourethrogram ii. Intravenous pyelogram (IVP)	Used to screen for structural abnormalities such as urethral narrowing, or incomplete emptying of the bladder which can cause stagnation of urine and predispose to infection	X-rays are not performed on pregnant women due to the possible risk posed to the fetus.
5	Cystoscopy	Used to detect structural abnormalities, intestinal cystitis, or masses that may not show up, on x-rays during intravenous pyelogram (IVP)	The procedure uses a cystoscope, flexible tube-like instrument that the urologist inserts through the urethra into the bladder

Table 2. *Advantages and Disadvantages of Diagnostic Imaging in Evaluation of Urinary Tract Infections.*

S/N	Imaging study	Advantages	Disadvantages
1	Ultrasound	Measures renal size and shape Identifies hydronephrosis, structural or anatomic abnormalities and renal calculi No radiation	Not reliable to detect vesicoureteral reflux, renal scarring or inflammatory changes
2	Intravenous urography	Precise anatomic image of the kidneys Estimates renal function	Not as reliable to detect renal scarring or pyelonephritis High radiation dose Risk of reaction to contrast medium Poor detail in infants
3	Renal cortical scintigraphy	Detects pyelonephritis and renal scarring even in early stages Useful in neonates Little radiation	Does not evaluate collecting system Cannot detect obstruction
4	Computed tomography	Useful in patients with poor renal function Provides both anatomic and functional information about the kidney. Possibly more sensitive in diagnosing pyelonephritis	Expensive High radiation
5	Voiding cystourethrography	Assesses the size and shape of bladder Detects and grades vesicoureteral reflux Evaluates posterior urethral anomalies in boys	Few clinical or experimental data to support its use at present Gonadal radiation Catheterization

5. Conclusion and Recommendations

5.1. Conclusion

Major laboratory diagnostic techniques available for detection of urinary tract infection pathogens are phenotypic approaches, whereby clinical samples are grown on culture media and the test takes 48 to 96 hours to complete. Thus, leading to patients potentially missing their appropriate treatment points. This approach causes accumulation and increase in volume of patient samples in the diagnostic laboratories. Most often, these diagnostic approaches are on presumptive basis; and more over the disadvantages of these techniques overweighs their advantages in practice. Treatment from these diagnoses are mostly on empirical basis, with a consequence of emergence of drugs resistance pathogens. UTI is a common infection that affects all patient demographics. There is a significant need for improved diagnostics, including pathogen identification and antimicrobial susceptibility profiling. Evidence-based treatment plans can therefore be implemented and judicious use of antibiotics applied. As a growing concern over this, nucleic acid based techniques, specifically the biosensing diagnostic techniques being molecular (genotypic) approach have been developed and are available as manual bench top assay biosensors. They provide result within 1 to 3 ½ hours and are specific and sensitive. Most importantly is the emerging automated genefluidics' systems, a molecular laboratory diagnostic device developed and tested in Europe to process two hundred and seventy-five (275) crude urine sample in 8 hours with up to 120 tubes loaded at the same time. This automated device is target to reduce and eliminate the problems of phenotypic laboratory diagnostic techniques of urinary tract infections. The versatility of the biosensors and the potential for multiplexing also raises the possibility that the future UTI diagnostics based on biosensors, in addition to being faster, will be more informative than the current old- century approach. For biosensor diagnosis of UTI—or any POC molecular diagnostic tests for infectious diseases—to succeed, stakeholders including patients, clinicians, and third party payers will need to carefully assess the utility and the cost effectiveness of such technology. If mass produced, UTI biosensors may only be modestly more expensive than glucose strips or pregnancy test strips due to increased complexity and likely less expensive than standard urine culture. Additional cost savings will likely come from decrease utilization of broad spectrum antibiotics in both inpatient and outpatient settings. Biosensors offer a promising approach to deliver highly sensitive molecular diagnostics testing in POC settings. With continuing technology advancements and clinical acceptance, they will potentially lead to a paradigm-shift for UTI diagnosis and treatment and serve as a model for other common infectious diseases. Imaging techniques, in other hands, are increasingly valuable tools for assessing the urinary tract in adults and children. However, their imaging capabilities, while overlapping in some respects, are considered as complementary, as each technique offers specific advantages and disadvantages both in actual inherent qualities of the technique and in specific patients and with a specific diagnostic question.

5.2. Recommendations

From the point of this review, recommendations are hereby made as follows:

i. There is a need for extreme care in use of the available phenotypic approaches in order to avoid misdiagnosis of UTI.
ii. There is a need for collaborated and concerted effort by both government, international organizations, and non-governmental organization toward providing nucleic acid based diagnostic facilities and equipment such as the manual bench top biosensors, PCR, the automated genefluidics' system and modern imaging machines to our medical diagnostic laboratories.
iii. There is a need for government, and non-governmental organizations to collaborate and establish modern imaging and molecular research laboratory centers for the purpose of manpower training in areas of laboratory diagnosis of urinary tract infection using modern imaging and molecular approaches.

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