JOURNAL OF INTERNATIONAL CRISIS AND RISK COMMUNICATION RESEARCH
ISSN: 2576-0017
2024, VOL 7, NO S10

Advances in Diagnostic Laboratory Techniques for Urinary Tract Infections

Sakher Hamed Alzahrani [1], Waleed Jameel Alshehri [2], Awatif Mohammed Alattas [3], Mohammed Ousi Asiri [4], Saad Abdullah Alshehri [5], Ali Ayed Aljunaid [6], Yousef Ali M Aboud [7], Othman Ali Ojaybi [8], Abdulrahman Hassan Otayn [9], Mana Saleh Ali Alhermas [10], Ruqayyah Munji Alghubayshi [11], Areej Sultan Halawi [12], Mohammed Mahmoud Abdullah Almuntashiri [13], Mohammed Gharm Mohsan Al Alshiq [14], Naif Mohammed Ali Muswadi [15].

[1]Laboratory Specialist, Riyadh Regional Laboratory, Ministry of Health, Kingdom of Saudi Arabia. Sakherhamed@hotmail.com
[2]Laboratory Specialist Riyadh, Regional Laboratory, Ministry of Health, Kingdom of Saudi Arabia. Waleedjameelsh@gmail.com
[3]Senior Laboratory Specialist, Riyadh Regional Laboratory, Ministry Of Health, Kingdom Of Saudi Arabia. awamo.2007@gmail.com
[4]Laboratory Technical, Riyadh, Ministry of Health, Kingdom of Saudi Arabia. Mohammedoa@moh.gov.sa
[5]Laboratory Specialist, Riyadh Regional Laboratory, Ministry of Health, Kingdom of Saudi Arabia. Saad_0909@hotmail.com
[6]Laboratory Technician, Riyadh Regional Laboratory, Ministry of Health , Kingdom of Saudi Arabia. Juned.nejaz@gmail.com
[7]Laboratory Specialist, Ministry of Health, Kingdom of Saudi Arabia. Yabood@moh.gov.sa
[8]Laboratory lSpecialist, Riyadh Regional Laboratory, Ministry of Health, Kingdom of Saudi Arabia. Othman_322@hotmail.com
[9]Laboratory Specialist, AlQuwayiyah General Hospital, Ministry of Health, Kingdom of Saudi Arabia. Otayn.Abdulrahman@gmail.com
[10]Specialist Laboratory, King Khaled hospital at Najran, Ministry of Health , kingdom of Saudi Arabia. Msalhermis@moh.gov.sa
[11]Laboratory Technician ،King Fahad Hospital Jeddah, Ministry of Health, Kingdom of Saudi Arabia. Roqaia.m@hotmail.com
[12]Laboratory Technician. Abha Hospital Maternity and Children, Kingdom of Saudi Arabia. ahalwai@moh.gov.sa
[13]Laboratory Technician, Aseer Regional Lab, Ministry of Health, Kingdom of Saudi Arabia. mohd.monta@gmail.com
[14]Laboratory Specialist, Aseer Regional Laboratory, Ministry of Health, Kingdom of Saudi Arabia. gharm-2009@hotmail.com
[15]Laboratory, Environmental Health Management and Occupational Health, Ministry of Health, Kingdom of Saudi Arabia. naifmasswadi@hotmail.com

ABSTRACT

Urinary tract infections (UTIs) pose a significant global health burden, affecting millions annually. Conventional diagnostic methods, such as dipstick testing and urine

culture, face limitations in accuracy and timeliness. However, recent advancements in diagnostic techniques have revolutionized UTI management. This review explores the epidemiology, risk factors, and emerging biomarkers for UTIs, focusing on the development of innovative diagnostic tools. Metabolomic and enzymatic markers, including trimethylamine, acetate, xanthine oxidase, and myeloperoxidase, have shown promise in UTI diagnosis. Biofilm-based and quorum sensing-based diagnostic strategies offer novel approaches to detect biofilm-associated UTIs. The evolution of diagnostic methods, from conventional culturing to molecular techniques like MALDI-TOF and PCR, has improved pathogen identification. Nanotechnology-based biosensors, particularly electrochemical and spectroscopic approaches, have demonstrated high sensitivity and specificity in UTI detection. Microfluidic integration has further enhanced point-of-care diagnostics. Artificial intelligence-based methodologies, such as artificial neural networks and random forests, have emerged as powerful tools for predictive diagnosis and personalized medicine in UTI management. Despite these advancements, further research and validation are necessary to confirm the robustness and applicability of these innovative diagnostic techniques across diverse clinical settings. This review highlights the transformative potential of emerging diagnostic tools in improving UTI management and patient outcomes.

Keywords: UTI Diagnosis, Diagnostic tests, Urinary Tract Infections Diagnosis

Introduction

Urinary tract infections (UTIs) represent a significant public health challenge, ranking among the most frequently encountered bacterial infections in both community and hospital settings. In the United States alone, UTIs account for approximately 2–3 million critical care visits annually, with associated healthcare expenditures estimated at $3.5 billion (Flores-Mireles et al., 2015). Globally, UTIs affect nearly 150 million individuals each year, with approximately half of the global population experiencing a UTI at least once in their lifetime (Davenport et al., 2017). Although UTIs can occur in both men and women, they are considerably more prevalent in women. This heightened susceptibility is primarily due to anatomical factors, such as the proximity of the urethral orifice to the vaginal and anal orifices (Foxman, 2010). Among women, UTIs are the second most common community-acquired infection, affecting half of the female population at least once in their lives. Additionally, statistics indicate that one in three women will experience a UTI before the age of 24. These infections significantly contribute to morbidity across all age groups, particularly among infants and the elderly. The conventional diagnostic approach for UTIs involves dipstick testing followed by urine culture, but these methods face limitations, especially considering rising antimicrobial resistance. However, advancements in molecular diagnostics, biofilm-based technologies, point-of-care testing (POCT), and AI-driven solutions have transformed UTI management by enabling early and accurate detection, as discussed in subsequent sections.

2. Epidemiology

UTIs are caused by a range of microbial agents, including gram-positive and gram-negative bacteria, fungi, and yeast. Among these, gram-negative bacteria,

particularly uropathogenic Escherichia coli (UPEC), are the predominant causative agents, accounting for approximately 80% of UTIs (McLellan & Hunstad, 2016). Clinically, UTIs are categorized as uncomplicated or complicated. Uncomplicated UTIs typically occur in otherwise healthy individuals without structural or functional abnormalities, instrumentation such as catheters, prior UTIs, or pregnancy. In contrast, complicated UTIs encompass all other cases. Uncomplicated UTIs primarily affect the lower urinary tract, causing inflammation of the urethra or bladder and resulting in conditions such as urethritis and cystitis. Complicated UTIs, however, are more concerning and include infections of the upper urinary tract, such as pyelonephritis, which may progress to urosepsis. Urosepsis, a form of sepsis originating from UTIs, accounts for 8.6% to 36% of sepsis cases in the United States and has a mortality rate ranging from 20% to 50% (Sekine et al., 2021). Factors contributing to complicated UTIs include urinary obstruction, retention, and severe conditions linked to neurological disorders, renal failure, transplantation, and indwelling catheters, particularly those associated with catheter-associated urinary tract infections (CAUTIs) (Ricardo et al., 2020). These complications substantially increase morbidity and mortality, with approximately 1 million cases in the United States alone and an estimated global burden of 404.61 million cases (95% CI: 359.43–446.55) and 236,790 (95% CI: 198,430–259,030) deaths in 2019 (Yang et al., 2022). Although anatomical factors contribute to the higher prevalence of UTIs among women, socio-demographic variables such as age, region, catheter use, hygiene practices, and socioeconomic conditions also influence UTI epidemiology.

The widespread occurrence and recurrence of UTIs have led to an increase in broad-spectrum antibiotic prescriptions, which, in turn, have fueled the rise of antibiotic-resistant uropathogens. The primary obstacle in antibiotic stewardship is inaccurate diagnosis, as emphasized by Goebel et al. (Goebel et al., 2021). Consequently, developing robust diagnostic tools capable of accurate UTI identification is critical. These tools are essential for effective infection control and advancing therapeutic strategies, ultimately mitigating the impact of antibiotic misuse and enhancing patient outcomes.

2.1. History of Epidemics Based on UTI

During the late 1980s, a community-wide outbreak of multidrug-resistant E. coli serotype O15 was reported in South London, UK. In the subsequent decades, outbreaks of community-acquired UTIs caused by broad-spectrum β-lactam-resistant E. coli strains were documented. For instance, a significant outbreak involving broad-spectrum-resistant E. coli O78:H10 occurred in Copenhagen in 1991, while New Zealand experienced similar outbreaks in 2004 and 2006. In India, studies have highlighted the high prevalence of resistant E. coli strains (Olesen et al., 2012). An analysis by Yang et al. evaluated UTI trends from 1990 to 2021 across 204 countries and territories, revealing an increasing burden, particularly in higher-income regions and aging populations over 60 years of age. Countries such as Armenia, Portugal, Taiwan, Kuwait, Uruguay, Argentina, Turkmenistan, Georgia, Costa Rica, Mauritius, Belgium, Turkey, the UK, and Germany have reported rising UTI trends. Factors such as greater catheter use among the elderly, advanced diagnostic facilities, and comprehensive documentation may explain this increase. Conversely, in lower-middle-income countries, the significantly higher prevalence of UTIs is exacerbated

by limited surveillance and access to healthcare. This often leads to treatment without accurate diagnosis, further contributing to antimicrobial resistance (AMR). To address this concern, Majigo et al. developed a protocol for tracking AMR in UTIs within lower-middle-income countries (Majigo et al., 2024).

3. Risk Factors for UTI

Several factors elevate the risk of UTIs. Contaminated food and water, sexual activity, and contraceptive methods—particularly spermicide-coated contraceptives such as diaphragms, condoms, cervical caps, and unlubricated condoms—can alter the vaginal pH and disrupt the microbiome, promoting the colonization of uropathogens (Dienye & Gbeneol, 2011). These are recognized as significant sources of infection. Additionally, hospitalization, catheterization, the use of indwelling and drainage devices, as well as host-related factors such as genetics and immune response, contribute to UTI susceptibility. Among host-related factors, the female gender presents a heightened risk due to anatomical differences in the urogenital tract. Vaginal infections and dysbiosis further exacerbate this risk, as the vaginal microbiome, predominantly composed of Lactobacillus species, plays a critical role in preventing uropathogen adherence to uroepithelial cells and mitigating UTI pathogenesis (Stapleton, 2016). Pregnancy is another significant risk factor. Furthermore, comorbidities such as diabetes mellitus can further increase the likelihood of UTIs (Wagenlehner et al., 2020). These multifaceted risk factors underscore the necessity of a comprehensive understanding to effectively prevent and manage UTIs.

4. Biomarkers for UTI

4.1. Conventional biomarkers in practice

Leucocyte esterase and urine nitrate initially served as essential biomarkers for diagnosing UTIs, predominantly employed in dipstick tests (Deusenbery et al., 2021). Nevertheless, research utilizing the commercially available Chemstrip-10 demonstrated that these markers alone are insufficient for the accurate diagnosis of UTIs. Semeniuk and Church emphasized the need for additional markers to enhance diagnostic accuracy. One such marker is lactoferrin (LF), which is secreted by polymorphonuclear leukocytes (PMNs) in response to urinary tract inflammation. Arao et al. developed an immunochromatography (IC) test strip for LF detection. Later, Pan et al. designed a sandwich amperometric immunoassay for simultaneous detection of LF and bacterial 16S rRNA in a conjugated array. Mohan et al. advanced this approach with a next-generation multiplexed detection method combining bacterial 16S rRNA and the host immune marker LF using an electrochemical sandwich assay, achieving specificity and sensitivity rates of 97% and 89%, respectively (Mohan et al., 2011).

Additionally, Flenker et al. devised a nuclease assay using a fluorescent probe to detect E. coli, employing Endonuclease I as a diagnostic biomarker for UTIs, with a sensitivity of 95.3%. Further expanding the diagnostic arsenal, Ghrera developed a procalcitonin (PCT)-specific immunosensor. PCT, a protein produced primarily by the

thyroid gland and lungs but overexpressed during UTIs—particularly by hepatocytes—leads to elevated circulating PCT levels. This immunosensor employed a combination of PCT-specific monoclonal antibodies and cadmium selenide quantum dots capped with zinc sulfide on an indium-tin-oxide-coated glass substrate, utilizing cyclic voltammetry. Xu et al. demonstrated that PCT and C-reactive protein (CRP) could differentiate between upper and lower UTIs, with PCT showing superior sensitivity and specificity in identifying pyelonephritis. Shi et al. further investigated the correlation between PCT and CRP as markers of UTI severity, revealing a positive correlation ($r = 0.646$, $P < 0.001$), albeit with limited efficacy in distinguishing between upper and lower UTIs. Instead, this study indicated a higher efficacy in detecting ureteritis.

Markers such as interleukins (IL-8, IL-6, and IL1-β) are widely employed to diagnose febrile UTIs in adults and children. Elevated serum and urinary IL-6 levels can differentiate pyelonephritis from acute cystitis in children and indicate UTI severity (Ching et al., 2018). Other markers, including neutrophil gelatinase-associated lipocalin (NGAL) and matrix metalloproteinase 9 (MMP-9), are utilized for clinical UTI diagnostics. However, NGAL, IL-6, and IL1-β consistently demonstrated specificity and sensitivity above 90% under the consensus criteria.

4.2. Developing biomarkers

4.2.1. Metabolomic & enzymatic markers

The study of metabolites has substantially advanced the identification of UTI biomarkers, with improvements in analytical techniques driving the discovery of metabolic biomarkers. These biomarkers provide critical insights into disease pathogenesis and facilitate early, accurate diagnoses. The metabolic profile of uropathogens has yielded numerous metabolomic compounds, while enzymatic markers reflect the activity of specific enzymes, enabling precise indications of disease states. Key metabolomic and enzymatic markers pertinent to UTIs are elaborated below:

4.2.1.1. Trimethylene amine (TMA)

Trimethylene amine (TMA) is an ideal biomarker for UTIs, particularly those associated with E. coli (Lussu et al., 2017). TMA presence in urine indicates bacterial colonization of the urinary bladder. In the human body, TMA is primarily produced in the gut and derived from dietary intake of trimethyl quaternary amines, which facilitate TMA generation. This volatile tertiary amine, categorized as a biogenic amine, is a bacterial byproduct absorbed in the gut and metabolized in the liver.

Under normal conditions, TMA is nearly entirely converted into trimethylamine-N-oxide (TMAO) by the flavin-containing monooxygenase enzyme. However, certain bacteria, particularly E. coli, utilize TMAO reductase to reduce TMAO back to TMA for bacterial respiration. This microbial-host co-metabolism results in elevated urinary TMA, serving as a potential biomarker for E. coli-related UTIs. NMR-based studies by Lussu et al. and Lam et al. validated TMA's diagnostic potential. However, urinary TMA levels may fluctuate based on sample collection time, temperature, and patient gender. Lam et al. quantified TMA levels in E. coli and non-E. coli UTI samples, reporting significant differences with diagnostic cut-off

ratios ≥ 0.0117 mmol/mmol and an optimal TMA concentration threshold of 3.4 μg/mL (Karlsen & Dong, 2015).

4.2.1.2. Acetate (Ac)

Acetate, the conjugate carboxylate base of acetic acid under physiological conditions, has emerged as a significant component in the metabolomic profile of uropathogens. Studies have identified acetate, succinate, lactate, and formate as potential UTI biomarkers, with acetate demonstrating high specificity and sensitivity. Grochocki et al. measured acetate-to-creatinine ratios in urine, revealing substantially elevated levels in UTI-infected samples compared to healthy controls (Grochocki et al., 2017).

Bacteria in the Enterobacterales group utilize heterofermentative fermentation pathways to produce acetate as a metabolic byproduct, often reducing TMAO to TMA and acetate. Despite acetate's potential as a biomarker, further research is required due to overlapping metabolic pathways among different bacterial species.

4.2.1.3. Xanthine oxidase (XO)

Xanthine oxidase (XO), a tissue-derived enzyme, has been proposed as a UTI biomarker due to its activity in the presence of pathogenic bacteria in urine at concentrations exceeding 10^5 bacteria/mL. While most bacteria lack purine oxidizing systems conducive to UTIs, exceptions like E. coli and P. aeruginosa display significant XO activity. The relationship between XO activity and specific bacterial strains necessitates further investigation.

4.2.1.4. Myeloperoxidase (MPO)

Myeloperoxidase (MPO), primarily found in neutrophils, has shown potential as a UTI biomarker. Ciragil et al. reported significantly elevated MPO levels in UTI cases, particularly those caused by E. coli. MPO activity demonstrates 100% specificity and 87% sensitivity in UTI diagnosis. Moreover, MPO levels can also indicate the effectiveness of UTI treatment, as Bai et al. observed decreased MPO-to-creatinine ratios in patients responding to antibiotics (Bai et al., 2018). Proper storage conditions are critical, as Steinhoff et al. noted substantial MPO concentration reductions in improperly stored samples.

4.2.2. Biofilm-based diagnosis

Conventional diagnostic methods, including culture-dependent techniques, microscopy, and genotypic approaches, primarily target planktonic bacterial forms and fail to detect biofilm-associated states. Since biofilms enhance bacterial resistance to host defenses and antimicrobials, innovative biofilm-based diagnostic strategies are critical. Antypas et al. developed a fluorescence-based diagnostic approach using cellulose as a biofilm biomarker. Their method employs luminescent-conjugated oligothiophene to selectively bind with target molecules, inducing a fluorescence spectrum indicative of biofilm presence (Antypas et al., 2018).

Building on this, recent studies have employed colorimetric and electrochemical detection techniques. For example, tyrosine-capped gold and silver nanoparticles have been utilized for cost-effective, rapid detection of biofilm cellulose, presenting significant advancements in the clinical diagnosis of biofilm-associated UTIs.

4.2.3. Quorum Sensing-Based Diagnosis

The interactions among various microbial species play a critical role in enhancing the virulence associated with urinary tract infections (UTIs), with quorum sensing emerging as a pivotal mechanism in this context. This biological process involves the secretion of specific signaling molecules by bacteria to enable both inter- and intra-species communication. Notably, Gram-negative uropathogens predominantly secrete Acyl Homoserine Lactone (AHL). A study conducted by Vasudevan et al. introduced a diagnostic methodology that utilizes AHL as a biomarker. This approach incorporates a nano-optical detection technique based on photoluminescence (PL), leveraging zinc oxide (ZnO) nanoparticles (Vasudevan et al., 2020). The sensing capability of this technique demonstrated an efficiency exceeding 90%, highlighting its potential as a robust diagnostic tool for UTIs.

4.2.4. Other Promising Developing Biomarkers

Research by Chromek et al. and Abedi et al. identified an elevation in the levels of matrix metalloproteinase 9 (MMP-9) and its inhibitor, tissue inhibitor of metalloproteinase 1 (TIMP-1), in the urine of children suffering from acute pyelonephritis. These markers have been used to monitor the severity and progression of the condition. Similarly, Watson et al. evaluated antimicrobial peptides, specifically human neutrophil peptides (HNP) 1–3 and human α-defensin 5 (HD5), as novel biomarkers in pediatric urine. These cationic peptides from the α-defensin family are expressed in response to pathogen entry into the urinary tract. Heat shock protein-70 (HSP-70) has also been proposed as a predictive biomarker for UTIs in children. Additionally, recent investigations have identified urine Heparin Binding Protein (HBP) as a superior biomarker for differentiating bacterial-associated UTIs, exhibiting a sensitivity and specificity of 87%. Biomarkers such as bone morphogenic protein 2 (BMP-2), cystatin C, and lipopolysaccharide binding protein (LBP) have shown high accuracy in diagnosing UTIs in pediatric patients. However, further research is necessary to confirm their clinical applicability.

5. The History of Diagnosis

Conventional diagnostic methods for UTIs involve the collection of urine specimens, which are subsequently cultured on specialized media for phenotypic identification. This diagnostic process includes two primary methodologies: culturing and non-culturing approaches. Gram-negative uropathogens are typically cultured on MacConkey agar and blood agar. However, the conventional culture method has notable limitations. This process is time-intensive, requiring 24–48 hours for result generation. Additionally, it is labor-intensive, involving diverse preparation protocols to optimize media for different pathogens. Another limitation, the great plate count anomaly, can obscure actual viable counts, leading to underestimated colony-forming units (CFUs). Given that clinicians often rely on a threshold of $>10^5$ CFU/mL in urine

to diagnose UTIs, the plate count method may inaccurately report lower CFU values, particularly on selective media like MacConkey agar.

Non-culturing techniques for UTI diagnosis include urine Gram staining and microscopic urine analysis, although these methods are time-consuming and have reduced sensitivity. The application of Gram staining to non-centrifuged urine for bacteriuria detection gained traction in 1968. While intermittently employed for UTI diagnosis, this method has stimulated interest in advancing point-of-care diagnostics. The 1980s saw the introduction of the urine dipstick test as a popular point-of-care diagnostic tool. This test assesses leukocyte esterase and nitrate levels to enhance bacteriuria detection. However, despite its simplicity and rapidity, the dipstick test exhibits suboptimal sensitivity, achieving only 48% sensitivity for clinically significant bacteriuria. Moreover, the test's poor specificity often results in misdiagnosing asymptomatic bacteriuria as UTI.

To address these limitations, researchers have developed innovative diagnostic technologies. A key advancement occurred in 2015 with the development of the antibody-based lateral flow assay known as RapidBac. This assay uses monoclonal antibodies to identify pathogens and demonstrates notable sensitivity and specificity, as evidenced by prior studies. However, further validation is required to confirm the reliability of this methodology.

Flow cytometry systems, initially designed as rapid screening tools based on light scattering principles in liquid environments, have become essential in UTI screening since their introduction in the 1980s. These systems have evolved significantly, with innovations such as the FDA-approved UF-1000i by Sysmex, which integrates light scattering and fluorescence to identify bacterial cells within 45 minutes. This system stains urine components with fluorescent dyes, allowing categorization of bacterial cells alongside blood cells (RBCs, WBCs, epithelial cells) and demonstrates a sensitivity of 97% and specificity of 94% (Manoni et al., 2009). The UF-5000, also by Sysmex, enhances performance by eliminating negative urine samples and achieving 81.6% sensitivity and 93.3% specificity, offering slight improvements in accuracy for diagnosing UTIs in women (De Rosa et al., 2018). Additional devices, such as Uro-Quick (Alifax) and BacterioScan model 216, focus on antimicrobial susceptibility testing (AST). While recent automated platforms detect bacteria, yeast, blood cells, spermatozoa, epithelial cells, and crystals, they do not provide antibiotic susceptibility information. To address this gap, integrated systems combining bacterial identification and AST, like Flow UTI, have emerged. Flow UTI, an interactive web application, utilizes flow cytometer data to detect UTIs by analyzing multiple variables, representing a step toward comprehensive diagnostic solutions.

Over the past two decades, molecular and proteome-based diagnostic technologies have advanced significantly, with methods like mass spectrometry gaining widespread acceptance. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) is a prominent technology in this domain, widely employed in

microbiological laboratories for rapid microbe identification through mass-to-charge ratio analysis. This method provides results within 1–3 hours using various sample types, from culture plates to clinical specimens. MALDI-TOF generates highly specific peptide mass fingerprints for each organism, achieving sensitivity levels of 67–86%. However, its utility in small clinics and remote areas is limited due to the high cost of instrumentation. One notable limitation of MALDI-TOF is its requirement for substantial sample sizes for accurate quantification, as bacterial strain-specific variability can affect quantification thresholds. Burillo et al. proposed addressing this issue by integrating Gram staining with MALDI-TOF to identify UTIs. This method involves using Gram staining to preclude samples selectively, followed by a sequential algorithm that identifies the etiological agent within one hour. Further evaluation by Íñigo et al. highlighted MALDI-TOF's efficacy in identifying UTI pathogens in urine specimens, supported by UF-1000i and SediMax urine analyzers for bacteriuria assessment, with Gram-negative bacteria identified as predominant UTI pathogens. Additionally, Horká et al. evaluated Nonionogenic Tenside Labeling combined with MALDI-TOF, employing cellulose-based preparative isoelectric focusing for rapid pathogen identification (Horká et al., 2019). The development of multiplex lateral flow systems integrated with mass spectrometry has also been proposed to minimize diagnostic inaccuracies and facilitate efficient point-of-care applications (Patil et al., 2023).

Fluorescence in situ hybridization (FISH) is a microscopy-based technique that uses nucleic acid probes labeled with fluorophores for hybridization, with 16sRNA commonly targeted due to its high organism-specific specificity. Almeida et al. developed a peptide nucleic acid (PNA) probe for Proteus spp. detection in urine, achieving 98% specificity and 100% sensitivity. Commercially available kits, such as hemoFISH by miacom diagnostics GmbH and QuickFISH by AdvanDX, utilize DNA and PNA probes respectively. These FDA-approved kits deliver rapid assays (20 minutes) with sensitivity and specificity exceeding 96% for bacterial identification. Additionally, FISH can be integrated with microfluidic platforms. For example, Barbosa et al. designed a silicon-based organic polymer to detect Candida spp (Barbosa et al., 2022). using hydrodynamic cell trapping for PNA FISH procedures, enhancing efficiency and reducing analysis time for point-of-care diagnostics. Similarly, Liu et al. developed μFlowFISH, a microfluidic device integrated with FISH for detecting E. coli, Desulfovibrio vulgaris, and Pseudomonas spp (Liu et al., 2011).

Polymerase chain reaction (PCR) remains a highly sensitive and specific technique for the identification of bacterial pathogens, offering species-specific detection of uropathogens. Hansen et al. introduced a semi-quantitative assay that facilitates rapid bacterial load detection in urine while identifying uropathogens within 4 hours, achieving a sensitivity and specificity of 97% and 80%, respectively. The SeptiFast real-time PCR system (Roche) has been shown to possess a sensitivity of 82% and specificity of 60% for detecting various Gram-positive and Gram-negative bacteria within a timeframe of 43 hours. Additionally, advancements in diagnostic platforms, such as GeneXpert and SeptiFast, are driving innovation in bacterial identification. For instance, the Gene Ohm Staph SR assay—a PCR-based diagnostic tool for multidrug-resistant Staphylococcus aureus—demonstrated impressive sensitivity and specificity rates of 100% and 98%, respectively. Similarly, the

GeneXpert real-time PCR system has exhibited sensitivity rates exceeding 98% for the detection of Staphylococcus. Despite these advancements, PCR-based diagnostic methods face persistent challenges within the context of UTI diagnosis.

Real-Time Microscopy Solutions

Phillips BioCell has pioneered innovations in microbiology with the introduction of the oCelloScope system, which employs digital time-lapse microscopy to detect bacterial cultures within an impressive 6-hour window. This platform not only enables detection but also facilitates the determination of minimum inhibitory concentrations (MIC) of antibiotics in under 3 hours. Similarly, the Accelerate ID/AST system, developed by Accelerate Diagnostics, utilizes dark-field time-lapse microscopy to expedite pathogen identification within 1.5 hours and antibiotic susceptibility testing within 5 hours. While these advancements represent significant strides, the application of such sophisticated methods in clinical laboratories entails complexities, particularly due to the intricate and time-intensive nature of sample processing. These challenges are compounded by the variable composition of the urine matrix, including fluctuations in pH, electrolytes, and other components even within samples from the same patient, necessitating assay optimization.

Nanotechnology-Based Diagnostic Tools

Emerging nanotechnology-based diagnostic tools, such as biosensors, represent a transformative innovation in UTI diagnosis. These compact, quantitative analytical devices operate on the principle of analyte binding to recognition elements, triggering a measurable signal. Recognition elements are often defined using optical and electrochemical techniques, enabling rapid, selective, and sensitive analysis. Such characteristics render biosensors ideal for point-of-care diagnostics, with signal magnitude directly proportional to analyte concentration. Recent advancements have integrated sensors with microfluidics and smartphone interfaces, enabling portable, low-cost diagnostics using small sample volumes. For instance, the UTI sensor array—a type of electrochemical sensor—uses sandwich hybridization to capture bacterial 16s RNA and achieves a detection limit of 10^4 CFU/mL, along with sensitivity and specificity rates of 90% and 87%, respectively.

Electrochemical Biosensors in UTI Diagnosis

Electrochemical biosensors have played a pivotal role in UTI detection since the 1970s. Nakamura et al. pioneered the application of cyclic voltammetry with basal-plane pyrolytic graphite (BPG) electrodes for bacterial detection and antibiotic susceptibility testing based on electron transfer mechanisms between bacterial coenzymes and the electrode. Subsequently, Liao et al. developed an electrochemical DNA biosensor array for species-specific uropathogen identification. This technique employed fluorescent probes targeting bacterial 16s RNA through oligonucleotide capture and detector probes. Despite initial challenges related to bacterial lysis and probe-target hybridization, subsequent modifications by Liao et al. improved signal intensity, demonstrating that the detection system's current output depended on the

proximity of hybridization sites (Liao et al., 2007). Pan et al. later developed an integrated self-assembled monolayer (SAM)-based electrochemical biosensor system, enhancing signal response while minimizing interference from clinical sample matrices. This biosensor achieved meticulous pathogen quantification and host immune response delineation, demonstrating a detection limit of 145 pg/mL for bacterial 16s RNA.

Mohan et al. devised an integrated biosensor assay targeting bacterial 16s RNA and protein using a sandwich assay combined with a horseradish peroxidase (HRP)-based redox reaction, yielding a detection limit of 2–20 pM with sensitivity and specificity rates of 89% and 97%, respectively. Altobelli et al. further developed a biosensor employing electrokinetic hybridization, enhancing probe rRNA binding through joule heating in a microfluidic preparation technique (Altobelli et al., 2017). This method optimized the signal-to-noise ratio and assay time, integrating pathogen identification and antibiotic susceptibility testing (AST) for Enterobacterales with ciprofloxacin, achieving sensitivity and specificity rates of 98.5% and 96.6%, respectively. Jijie et al. introduced a quantitative immunosensor for detecting uropathogenic E. coli using a gold electrode modified with polyethyleneimine-reduced graphene oxide nanosheets (Jijie et al., 2018). This sensor achieved a detection limit below 10 CFU/mL through differential pulse voltammetry (DPV).

Electronic Nose (eNose) Technologies

Electronic noses (eNoses) represent a novel biosensor class capable of rapid detection of volatile organic compounds produced by bacteria. Roine et al. evaluated a commercial eNose (ChemPro 100i) for detecting common uropathogens such as E. coli, Klebsiella spp., and S. saprophyticus through smell prints, achieving sensitivity and accuracy rates of 95% and 97%, respectively, within 15 minutes. Then et al. explored the potential of eNoses to detect E. coli strains using ethanol as a biomarker. Pavlou et al. employed eNose technology to identify bacterial contaminants in urine by analyzing volatile production patterns. Similarly, Martinez et al. designed an eNose prototype utilizing support vector machines (SVM) to detect prevalent UTI pathogens.

Spectroscopic Approaches in UTI Diagnosis

High-resolution nuclear magnetic resonance (H-NMR) spectroscopy and related spectroscopic techniques have enabled comprehensive analyses of urine metabolic compositions in UTI diagnostics (Gupta et al., 2012). Lam et al. introduced a diagnostic model using acetic acid as a biomarker, demonstrating a sensitivity and specificity of 91% and 95%, respectively. Building on this, Lam et al. investigated trimethylamine (TMA) as a co-metabolite biomarker for microbial and mammalian sources, achieving sensitivity and specificity rates of 66.7% and 97%. In a separate study, Lussu et al. evaluated H-NMR for diagnosing E. coli-associated UTIs, identifying acetate and TMA as robust biomarkers with 100% sensitivity and specificity. Collectively, these advancements underscore the potential of spectroscopic methodologies, particularly NMR, in fostering precision diagnostic strategies for UTIs.

Various colorimetric sensors have been innovatively designed to detect UTIs, leveraging changes in color that can be analyzed through UV-spectrophotometry or an

enzyme-linked immunosorbent assay (ELISA) plate reader. For instance, Raj et al. utilized cysteine-capped gold nanoparticles for this purpose, where the electrostatic interaction between the positive charge of –NH2 receptors on cysteine-capped gold nanoparticles and the negative charge of E. coli acts as a detection signal (Raj et al., 2015). Similarly, Zagorovsky et al. developed a smartphone-integrated point-of-care diagnostic platform utilizing gold nanoparticles conjugated with a DNA-based multicomponent nucleic acid enzyme specifically tailored for UTI detection (Zagorovsky et al., 2022). The detection mechanism relied on a wavelength shift induced by DNA crosslinking, which caused the aggregation of gold nanoparticles and resulted in a shift in the surface plasmon resonance (SPR) due to particle coupling.

The combination of antimicrobial susceptibility testing (AST) biosensors with microfluidics has unveiled promising advancements in UTI diagnostics. Notably, Michael et al. introduced an innovative point-of-care diagnostic tool known as Dx-FS, a centrifugal force-based hand-powered device (Michael et al., 2020). This device enables colorimetric detection of pathogens within 50 minutes and performs AST for ciprofloxacin and cefazolin using clinical urine samples in an impressive timeframe of 120 minutes. A two-sided Student's t-test conducted on this tool yielded ***P = 6.0064 × 10−21. Characterized by simplicity, speed, and cost-effectiveness, Dx-FS demonstrates potential for deployment in remote areas where advanced facilities are unavailable.

Noiphunng and Laiwattanapaisal devised a paper-based analytical device (PAD) for single-step, enzyme-based UTI detection using the Griess test (Noiphung & Laiwattanapaisal, 2019). This innovation integrates bacterial cultivation, identification, and nitrite determination within a remarkably efficient timeframe of six hours, significantly faster than conventional culture methods. The effectiveness of PADs is attributed to their affordability, rapid functionality, and portability, making them ideal for point-of-care applications. Among the substrates used, Whatman paper stands out due to its chromogenic substrate facilitation, allowing quick visual detection. Complementarily, Ohnishi et al. introduced the "diaper UTI test," a unique screening approach leveraging common diapers as diagnostic tools for UTIs. This method employs an enzyme-based diagnostic strategy targeting leukocyte esterase, achieving sensitivity and specificity rates of 90.5% and 93.2%, respectively. While initially designed for pediatric use, the approach shows promise for application in elderly populations, expanding its utility across diverse demographic groups. Additionally, Liu et al. developed an economical POCT paper-based sensor to rapidly detect β-lactamase-producing bacteria in UTIs. This system incorporates optical probes on custom-designed paper chips specifically targeting ESBL/AmpC and carbapenemases, employing intramolecular charge transfer effects of CCepS-N+(CH3)3 and CCS-N+(CH3)3, with a detection limit of 5×10^4 CFU/mL.

A contemporary molecular diagnostic technique, the Loop-Mediated Isothermal Amplification (LAMP) assay, has emerged as a dependable and cost-efficient tool for diagnosing UTI pathogens and antibiotic resistance genes. This approach exhibits remarkable sensitivity and specificity, ranging from 96–100% and

95–100%, respectively, and obviates the requirement for costly equipment like thermal cyclers, as emphasized by Rivoarilala et al.. Saengsawang et al. designed a cost-effective and straightforward paper-based device for the rapid detection of E. coli in urine, utilizing the fluorescent migratory distance of the LAMP amplicon-SYBR™ Safe dye complex. Similarly, Chen et al. presented the Loop-Mediated Isothermal Amplification and Centrifugal Disk (LCD) platform, enabling expedited identification of urinary pathogens and antibiotic resistance genes within 1.5 hours of sample collection. This platform delivers high sensitivity (90.4–100%) and specificity (98.8–100%), making it a reliable and economical diagnostic solution for UTIs.

In the context of UTI diagnosis, the emergence of artificial intelligence (AI) technologies signifies a transformative development. In the domain of personalized medicine, AI models can analyze extensive datasets and predict disease risk and early detection based on individual patient information, including genetic predisposition, genome data, lifestyle, and antibiotic history. AI's computational proficiency and multidimensional clinical data access have facilitated effective predictions for certain cancers and cardiovascular diseases. However, its utility in personalized medicine for UTI diagnosis requires parameter refinement and validation due to challenges such as data bias, socio-environmental factors, and concerns surrounding data safety and privacy.

Four principal AI-based methodologies have gained prominence in clinical diagnostics: Artificial Neural Network (ANN), Random Forest (RF), Decision Tree (DT), and Support Vector Machines (SVM). These models analyze large datasets, identify patterns, and perform predictive diagnoses, which are further verified through statistical analysis of specificity, sensitivity, and accuracy. Data collection involves patients' details and electronic health records, including symptoms, medical reports, and imaging documents. Pre-processing standardizes the data for subsequent analysis. Among these methods, ANN demonstrates the highest accuracy at 98.3% and sensitivity at 97.7%. RF follows closely, achieving 96.6% accuracy and 95.5% sensitivity, underscoring their potential to support clinicians in non-invasive and cost-effective diagnostic applications. Building on these advancements, Jeng et al. introduced machine learning models like DT, logistic regression (LR), and RF for predicting recurrent UTIs. The authors emphasized the necessity of further validation to enable seamless field application. Expanding AI's scope in UTI diagnostics, Gadalla et al. combined RF and SVM models to predict optimal immunological markers. This innovative approach shows promise not only for effective UTI diagnosis but also for the development of point-of-care diagnostic solutions.

Nevertheless, extensive research and validation are essential to confirm the robustness and applicability of these AI-based models across diverse clinical settings. Comprehensive developments in UTI diagnostics between 1990 and 2024 encapsulating the progression from 1990s culture-based tests and traditional dipsticks to contemporary integrated sensors and AI-driven techniques.

Conclusion

The advancements in diagnostic laboratory methodologies have significantly transformed the landscape of urinary tract infection (UTI) diagnosis. Traditional techniques, such as culture-based methods and dipstick tests, though foundational, are

often limited by time constraints and suboptimal sensitivity. Emerging technologies—including molecular diagnostics, biosensors, and AI-driven platforms—are bridging these gaps by providing rapid, accurate, and personalized diagnostic solutions. The integration of innovative tools such as Loop-Mediated Isothermal Amplification (LAMP), microfluidic biosensors, and colorimetric assays underscores a commitment to improving diagnostic accuracy, reducing antimicrobial resistance, and fostering point-of-care applicability. Moreover, artificial intelligence and machine learning models are redefining diagnostic paradigms by enabling predictive and real-time analytics, thereby tailoring diagnostic and therapeutic strategies. While challenges such as data validation, instrument accessibility, and matrix variability persist, the synergistic evolution of laboratory diagnostics and emerging technologies heralds a promising future for effective UTI management.

References

Altobelli, E., Mohan, R., Mach, K.E., Sin, M.L.Y., Anikst, V., Buscarini, M., Wong, P.K., Gau, V., Banaei, N., Liao, J.C. Integrated biosensor assay for rapid uropathogen identification and phenotypic antimicrobial susceptibility testing, Eur. Urol. Focus 3 (2017) 293–299, https://doi.org/10.1016/j.euf.2015.12.010.

Antypas, H., Choong, F.X., Libberton, B., Brauner, A., Richter-Dahlfors, A. Rapid diagnostic assay for detection of cellulose in urine as biomarker for biofilm-related urinary tract infections, npj Biofilms Microbiomes 4 (2018), https://doi.org/10.1038/s41522-018-0069-y.

Bai, M., Feng, J., Liang, G. Urinary myeloperoxidase to creatinine ratio as a new marker for monitoring treatment effects of urinary tract infection, Clin. Chim. Acta 481 (2018) 9–11, https://doi.org/10.1016/j.cca.2018.02.018.

Barbosa, V.B., Rodrigues, C.F., Cerqueira, L., Miranda, J.M., Azevedo, N.F. Microfluidics combined with fluorescence in situ hybridization (FISH) for Candida spp. detection, Front. Bioeng. Biotechnol. 10 (2022), https://doi.org/10.3389/fbioe.2022.987669.

Ching, C.B., Gupta, S., Li, B., Cortado, H., Mayne, N., Jackson, A.R., McHugh, K.M., Becknell, B. Interleukin-6/Stat3 signaling has an essential role in the host antimicrobial response to urinary tract infection, Kidney Int. 93 (2018) 1320–1329, https://doi.org/10.1016/j.kint.2017.12.006.

Deusenbery, C., Wang, Y., Shukla, A. Recent innovations in bacterial infection detection and treatment, ACS Infect. Dis. 7 (2021) 695–720, https://doi.org/10.1021/acsinfecdis.0c00890.

Dienye, P.O., Gbeneol, P.K. Contraception as a risk factor for urinary tract infection in Port Harcourt, Nigeria: a case-control study, African J. Prim. Heal. Care Fam. Med. 3 (2011) 1–4, https://doi.org/10.4102/phcfm.v3i1.207.

Flores-Mireles, A.L., Walker, J.N., Caparon, M., Hultgren, S.J. Urinary tract infections: epidemiology, mechanisms of infection, and treatment options, Nat. Rev. Microbiol. 13 (2015) 269–284, https://doi.org/10.1038/nrmicro3432.

Foxman, B. The epidemiology of urinary tract infection, Nat. Rev. Urol. 7 (2010) 653–660, https://doi.org/10.1038/nrurol.2010.190.

Goebel, M.C., Trautner, B.W., Grigoryan, L. The Five Ds of Outpatient Antibiotic Stewardship for Urinary Tract Infections, 2021. https://journals.asm.org/journal/cmr.

Grochocki, W., Markuszewski, M.J., Quirino, J.P. Simultaneous determination of creatinine and acetate by capillary electrophoresis with a contactless conductivity detector as a feasible approach for urinary tract infection diagnosis, J. Pharm. Biomed. Anal. 137 (2017) 178–181, https://doi.org/10.1016/j.jpba.2017.01.032.

Gupta, A., Dwivedi, M., Mahdi, A.A., Khetrapal, C.L., Bhandari, M. Broad identification of bacterial type in urinary tract infection using 1H NMR spectroscopy, J. Proteome Res. (2012) 1844–1854, https://doi.org/10.1021/pr2010692.

Horká, M., Salplachta, J., Růžička, F., Slais, K. Utilization of red nonionogenic tenside labeling, isoelectric focusing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in the identification of uropathogens in the presence of a high level of albumin, ACS Infect. Dis. 5 (2019) 1348–1356, https://doi.org/10.1021/acsinfecdis.9b00045.

Jijie, R., Kahlouche, K., Barras, A., Yamakawa, N., Bouckaert, J., Gharbi, T., Szunerits, S., Boukherroub, R. Reduced graphene oxide/polyethylenimine-based immunosensor for the selective and sensitive electrochemical detection of uropathogenic Escherichia coli, Sensors Actuators, B Chem. 260 (2018) 255–263, https://doi.org/10.1016/j.snb.2017.12.169.

Karlsen, H., Dong, T. Biomarkers of urinary tract infections: state of the art, and promising applications for rapid strip-based chemical sensors, Anal. Methods 7 (2015) 7961–7975, https://doi.org/10.1039/c5ay01678a.

Liao, J.C., Mastali, M., Li, Y., Gau, V., Suchard, M.A., Babbitt, J., Gornbein, J., Landaw, E.M., McCabe, E.R.B., Churchill, B.M., Haake, D.A. Development of an advanced electrochemical DNA biosensor for bacterial pathogen detection, J. Mol. Diagnostics 9 (2007) 158–168, https://doi.org/10.2353/jmoldx.2007.060052.

Liu, P., Meagher, R.J., Light, Y.K., Yilmaz, S., Chakraborty, R., Arkin, A.P., Hazen, T.C., Singh, A.K. Microfluidic fluorescence in situ hybridization and flow cytometry (μFlowFISH), Lab Chip 11 (2011) 2673–2679, https://doi.org/10.1039/c1lc20151d.

Lussu, M., Camboni, T., Piras, C., Serra, C., Del Carratore, F., Griffin, J., Atzori, L., Manzin, A. 1H NMR spectroscopy-based metabolomics analysis for the diagnosis of symptomatic E. coli-associated urinary tract infection (UTI), BMC Microbiol. 17 (2017), https://doi.org/10.1186/s12866-017-1108-1.

Majigo, M.V., Mshana, S., Komba, E., Moremi, N., Matee, M. Protocol for surveillance of antimicrobial-resistant bacteria causing community-acquired urinary tract infections in low-income countries, PLoS One 19 (2024) e0304388.

Manoni, F., Fornasiero, L., Ercolin, M., Tinello, A., Ferrian, M., Hoffer, P., Valverde, S., Gessoni, G. Cutoff values for bacteria and leukocytes for urine flow cytometer Sysmex UF-1000i in urinary tract infections, Diagn. Microbiol. Infect. Dis. 65 (2009) 103–107, https://doi.org/10.1016/j.diagmicrobio.2009.06.003.

McLellan, L.K., Hunstad, D.A. Urinary tract infection: pathogenesis and outlook, Trends Mol. Med. 22 (2016) 946–957, https://doi.org/10.1016/j.molmed.2016.09.003.

Michael, I., Kim, D., Gulenko, O., Kumar, S., Clara, J., Ki, D.Y., Park, J., Jeong, H.Y., Kim, T.S., Kwon, S., Cho, Y.K. A fidget spinner for the point-of-care diagnosis of urinary tract infection, Nat. Biomed. Eng. 4 (2020) 591–600, https://doi.org/10.1038/s41551-020-0557-2.

Mohan, R., Mach, K.E., Bercovici, M., Pan, Y., Dhulipala, L., Wong, P.K., Liao, J.C. Clinical validation of integrated nucleic acid and protein detection on an electrochemical biosensor array for urinary tract infection diagnosis, PLoS One 6 (2011), https://doi.org/10.1371/journal.pone.0026846.

Noiphung, J., Laiwattanapaisal, W. Multifunctional paper-based analytical device for in situ cultivation and screening of Escherichia coli infections, Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-018-38159-1.

Olesen, B., Scheutz, F., Andersen, R.L., Menard, M., Boisen, N., Johnston, B., Hansen, D.S., Krogfelt, K.A., Nataro, J.P., Johnson, J.R. Enteroaggregative Escherichia coli O78:H10, the cause of an outbreak of urinary tract infection, J. Clin. Microbiol. 50 (2012) 3703–3711, https://doi.org/10.1128/JCM.01909-12.

Patil, A.A., Kaushik, P., Jain, R.D., Dandekar, P.P. Assessment of urinary biomarkers for infectious diseases using lateral flow assays: a comprehensive overview, ACS Infect. Dis. 9 (2023) 9–22, https://doi.org/10.1021/acsinfecdis.2c00449.

Raj, V., Vijayan, A.N., Joseph, K. Cysteine-capped gold nanoparticles for naked eye detection of E. coli bacteria in UTI patients, Sens. Bio-Sensing Res. 5 (2015) 33–36, https://doi.org/10.1016/j.sbsr.2015.05.004.

Ricardo, S.I.C., Anjos, I.I.L., Monge, N., Faustino, C.M.C., Ribeiro, I.A.C. A glance at antimicrobial strategies to prevent catheter-associated medical infections, ACS Infect. Dis. 6 (2020) 3109–3130, https://doi.org/10.1021/acsinfecdis.0c00526.

Sekine, Y., Kotani, K., Oka, D., Nakayama, H., Miyazawa, Y., Syuto, T., Arai, S., Nomura, M., Koike, H., Matsui, H., Shibata, Y., Murakami, M., Suzuki, K. Presepsin as a predictor of septic shock in patients with urinary tract infection, BMC Urol. 21 (2021) 1–7, https://doi.org/10.1186/s12894-021-00906-4.

Stapleton, A.E. The vaginal microbiota and urinary tract infection, Urin. Tract Infect. Mol. Pathog. Clin. Manag. (2016) 79–86, https://doi.org/10.1128/9781555817404.ch5.

Vasudevan, S., Srinivasan, P., Rayappan, J.B.B., Solomon, A.P. A photoluminescence biosensor for the detection of N-acyl homoserine lactone using cysteamine-functionalized ZnO nanoparticles for the early diagnosis of urinary tract infections, J. Mater. Chem. B 8 (2020) 4228–4236, https://doi.org/10.1039/c9tb02243k.

Wagenlehner, F.M.E., Bjerklund Johansen, T.E., Cai, T., Koves, B., Kranz, J., Pilatz, A., Tandogdu, Z. Epidemiology, definition, and treatment of complicated urinary tract infections, Nat. Rev. Urol. 17

(2020) 586–600, https://doi.org/10.1038/s41585-020-0362-4.

Zagorovsky, K., Fernandez-Argüelles, M.T., Bona, D., Elshawadfy, A.M., Syed, A.M., Kadhiresan, P., Mazzulli, T., Maxwell, K.L., Chan, W.C.W. Gold nanoparticle smartphone platform for diagnosing urinary tract infections, ACS Nanosci. Au 2 (2022) 324–332, https://doi.org/10.1021/acsnanoscienceau.2c00001.