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Review Article

Systematic review–how do we identify urinary tract infections today?

Marina Čeprnja[1]*, Damir Oros[2], Andrea Janeš[3], Jurica Žučko[2], Karmela Barišić[4], Jasenka Škrlin[3] and Antonio Starčević[2]

[1]Special Hospital Agram, Polyclinic Zagreb, Biochemical Laboratory, 10000 Zagreb, Croatia

[2]Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, Zagreb University, 10000 Zagreb, Croatia

[3]Centar for Clinical Microbiology and Hospital Infections, University Hospital Dubrava, 10000 Zagreb, Croatia

[4]Faculty of Pharmacy and Biochemistry, Zagreb University, 10000 Zagreb, Croatia

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***Corresponding author:** Marina Čeprnja, Special Hospital Agram, Polyclinic Zagreb, Biochemical Laboratory, Dujšinova Street 1, 10000 Zagreb, Croatia,
E-mail: marinaceprnja@gmail.com

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Abstract

Standard urine culture is still considered a gold standard in the identification of Urinary Tract Infections (UTIs), but is time-consuming and in approximately 20% of patients with UTI symptoms produces false-negative results. Medical and scientific communities are in search of a faster, more accurate, yet affordable method with high clinical utility. As a supplement to standard culture in routine practice Urine Flow Cytometer (UFC) screening method is used, in order to detect negative urine culture samples. This allows for shortening issuing time for sterile urine culture reports and the cost of the analysis itself. In addition, urine dipstick tests and microscopic examinations of urine sediment can also be performed in biochemical laboratories but are usually preceded by urine culture. Nowadays, advanced methods such as proteomics and genomics are used to identify pathogens causing UTIs but are still used mainly for scientific purposes and rarely in clinical practice. From genomic methods PCR, 16S rRNA gene sequencing, and metagenome sequencing are being researched. PCR is great for targeted diagnostics, 16S RNA gene amplification can determine bacterial genera and their abundance, but is not good for in-depth species analysis, while metagenomics is the most comprehensive and unbiased method. The proteomics field also offers several methods for microbial identification, with MS as the leading one. Clinical applications of MS platforms usually imply MALDI-TOF MS analyzers which produce a characteristic spectrum called peptide mass fingerprint or more present for scientific purposes LC-MS/MS-based peptide sequencing.

Introduction

Urinary Tract Infections (UTIs) belong to the most common bacterial infections, and urinalysis is one of the most common examinations in biochemical and microbiological laboratories. This test includes physical and chemical urine analysis, microscopic analysis of urinary sediment; classic urine culture which includes methods of cultivation of microorganisms, their identification, and Antimicrobial Susceptibility Testing (AST) [1]. Usually, 36 - 72 h are needed for all these examinations [2]. The main disadvantage of these methods is the length of time required, but also a number of shortcomings in the standard urine culture protocol that limit the amount of potentially important information to clinicians, as demonstrated in Price et.al. study [3]. For these reasons, faster and more effective procedures for the identification of the number and types of microorganisms present in urine as well as their sensitivity to antibiotics are continuously explored. Despite these reasons, standard urine culture represents the gold standard method in the case of identification of uncomplicated Urinary Tract Infections (UTIs) [4]. The urine dipstick test and microscopic examination of urine sediment can be carried out in biochemical laboratories, although they are typically preceded by a urine culture. Currently, advanced techniques such as proteomics and genomics are employed to identify the pathogens responsible for urinary tract infections (UTIs), but they are primarily

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used for scientific research and not extensively applied in clinical settings. Among genomic methods, PCR, 16S rRNA gene sequencing, and metagenome sequencing are actively investigated. PCR is particularly useful for targeted diagnostics, while amplification of 16S rRNA genes can determine bacterial genera and their relative abundance, but it is less suitable for detailed species analysis. On the other hand, metagenomics represents the most comprehensive and unbiased approach. In the field of proteomics, multiple methods are available for microbial identification, with Mass Spectrometry (MS) being the leading one. In clinical practice, MS platforms such as MALDI-TOF MS analyzers are commonly utilized, generating a characteristic spectrum known as a peptide mass fingerprint. Additionally, liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based peptide sequencing is more prevalent in scientific research. Over the past decade, both MS platforms have received FDA approval for bacterial identification in clinical practice, leading to their increased popularity in this area.

Urinalysis

Urinalysis is one of the most common examinations in biochemical and microbiological laboratories. Also, point-of-care tests are often practiced at doctors' offices [5]. Furthermore, European guidelines for urinalysis were published in 2001: a collaborative document produced by European clinical microbiologists and clinical chemists under ECLM in collaboration with ESCMID [6]. This document classifies measurement methods into four levels of performance, based on the accuracy of the measurement. Level 1 is rapid procedures (dipsticks with ordinal scale reporting as screening procedure used to identify negative cultures and significant growth of Escherichia coli only). Level 2 includes quantitative procedures (a routine procedure in which 1 µL of urine is inoculated on CLED or blood agar aerobically followed by 24 h incubation). Level 3 covers qualified comparison procedures (10 µL of urine inoculated by pipette on CLED agar, hematin agar, and blood agar, aerobically and afterward cultured anaerobically under CO2 for 48 h) and finally, level 4 which includes primary reference measurement procedures also known as definitive methods, which are not available in microbiology5. Still, there is no general international standard for accreditation or validation of new technologies available, despite the fact that some of them are already in clinical and research practice, such as MALDI-TOF (MS1) or tandem Mass Spectrometry (MS2) methods [7-9]. Urinalysis, besides urine culture, encompasses UFC, a screening method used to detect negative culture samples, dipstick chemical tests, urine microscy particle counting, and proteomic and genomic analyses.

Factors such as pH, osmolality, and natural inhibitors that hinder bacterial adherence create an unfavorable environment for bacterial growth. Several risk factors contribute to the development of urinary tract infections, including gender (women have a shorter urethra), age, catheter use, pregnancy, vesicoureteral reflux, and various functional or anatomical abnormalities in the urinary system (such as prostate hypertrophy, neurogenic bladder, stones, tumors). Other risk factors include urinary and fecal incontinence, diabetes, immunosuppression, hospital treatment, kidney transplantation, and genetic factors [10]. Urinalysis is a crucial laboratory test for diagnosing urinary tract conditions, highlighting the need to carefully examine the workflow of urine testing and emphasizing the importance of minimizing preanalytical variability. Establishing standardized procedures for urine collection, transportation, sample preparation, and analysis is essential for an effective diagnostic approach to urinalysis. As advances in technology have significantly enhanced the reproducibility of urinalysis, greater attention has been given to the preanalytical requirements of the test, resulting in stricter protocols. Since patients themselves often provide urine samples, the preanalytical phase of urinalysis is particularly vulnerable to potential issues. Different collection methods and improper specimen transportation can lead to significant preanalytical errors. However, today's task of diagnosing urinary infections is becoming more complex due to our evolving knowledge that urine is not sterile and the existence of a balanced urinary microbiota has a noteworthy impact [11]. Furthermore, with the increasing availability of PCR (Polymerase Chain Reaction) and NGS (Next-Generation Sequencing) in the diagnosis of urinary tract infections, the pre-analytical requirements have also become more demanding due to their heightened sensitivity [12]. Also, we distinguish several ways of sampling different urine samples; urine samples collected by clean catch method, urine taken with a single catheterization, urine from a permanent catheter, urine obtained by cystoscopy, urine from urostomy, urine obtained by suprapubic puncture or urine of incontinent persons. For each of the methods, there are specific recommendations and notes that should be followed and the findings interpreted in accordance with them [10].

Urine flow cytometry screening method to detect negative culture samples

Different studies published different percentages for negative urine culture. These numbers depend on the definition of negative cultures. For example, the criterion may be; no growth, $<10^4$, or $<10^5$ CFU/ml. Most studies have reported a percentage of negative culture samples around 70 to 80 % [13,14], but also with a lower percentage between 40 and 60% [15,16]. Surely, a high number of negative urine cultures significantly increases the cost of diagnostics, so a cost-efficient screening method for the identification of negative urine samples would be a promising prospect that would reduce the ultimate cost of analyses and could reduce the overall turnaround time for negative urine cultures [15]. UFC analyzers are optical instruments that allow simultaneous analysis of chemical and/or physical urine characteristics [17]. Urinary cellular elements flow in a fluid stream through a laser beam for which scattering is measured by a series of detectors. The UFC can identify urinary particles such as Red Blood Cells (RBC), White Blood Cells (WBC), squamous epithelial cells, small round cells (renal tubular cells and transitional epithelial cells), hyaline casts, bacteria, yeast-like cells, spermatozoa, and crystals. UFC automatically mixes, aspires, and stained urine samples using a specific fluorescent dye in two different analytical chambers: one for

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bacteria and the second for other urine elements [18]. Each particle scatters light detected from two angles and reads the fluorescence intensities into the optoelectronic signals which are then analyzed. Individual bacterial examination channel is present for bacteria identification so interference with RBCs is prevented. All results are presented in the form of histograms and scattergrams by built-in software support [18-20]. Bacteria cut-off values should be adjusted by each laboratory, depending on their UTI prevalence and the pathology of the patients analyzed. Most review articles have agreed that UFC is a useful method for screening urine samples if the optimal cut-off value is established for each group of patients. Quick diagnostics of negative cultures will lead to a reduction in the number of bacterial cultures and ultimately in the reduction of unnecessary antibiotic prescriptions, but in subjects with a risk of UTI complications, its use is controversial, and the growth culture is still regarded as a necessity [21]. High sensitivity and a high threshold for the negative predictive value are required for the screening test for UTIs, in order to minimize the number of false-positive and false-negative results. Sensitivity is, therefore, more important than specificity [18], due to the fact that all samples that are labeled as positive in the screening test will be cultured, and properly categorized [16].

Urine dipstick test

The dipstick chemical test is essentially a strip with reagent pads for semiquantitative assessment of nitrite, a by-product of common urinary pathogens and leukocyte esterase, protein, and blood, as a good indication of inflammation [22-25]. Despite attempts to standardize the pre-analytical and analytical phases in the process of urine collection and analysis, there are still many differences that can lead to bad results. A meta-analysis "The urine dipstick test is useful to rule out infections" shows [26] sensitivity of urine dipstick test for nitrites and leukocyte esterase [24]. The sensitivity of the urine dipstick test for nitrites was estimated between 45 and 60 % in most research being performed, with significantly higher specificity ranging between 85 and 90 %. In the case of leukocyte esterase sensitivity was estimated between 48 and 86 %, and specificity is extremely variable and goes between 17 and 93 %. Sensitivity can be increased from 68 to 88 % by combining the results of these two dipstick tests, in cases when one or both show a positive result [23]. A negative dipstick test results are interpreted as excluded infection presence in most studies [22,25]. Numerous studies have shown that combining the results from nitrite and leucocyte esterase dipstick tests produces better diagnostic performances compared with just nitrite or just leucocyte esterase test performances. Also, it is demonstrated that, in the general population, the urine dipstick test alone can be useful to exclude the presence of infection if the results for nitrites or leukocyte-esterase are negative [26]. Although dipstick appeared as a good predictor of negative urine cultures [26], in definitive diagnostics, compared to a quantitative urine culture, final measurement results should not rely solely upon dipstick tests and should certainly encourage quantitative urine culture, especially in the primary healthcare settings [27-29].

The microscopic examination of urine sediment

Examinations of microscopic urine sediment play a very important role in the diagnostics of urinary tract infections and other diseases of the kidney and urinary system. This is an important type of non-invasive, repeatable morphological examination. This examination relies upon the proper morphological classification of urine components, such as epithelial cells, non-epithelial cells (blood cells), casts, salts/crystals, and microorganisms [30]. These urine components are being counted and reported according to the observed number [5]. In the interpretation of the findings, it is important to know which type of urine sample is being examined, but it is also important to record the time and method of collecting urine. Bacteria can be classified as bacilli or cocci via microscopic examinations under 400X magnification. The presence of bacteria in urine is essential for the diagnosis of common UTIs, such as cystitis and pyelonephritis. Because urine samples can be easily contaminated with inappropriate urine collection by non-symptomatic urogenital flora of surrounding tissues, midstream urine collection is emphasized in order to reduce the contamination. The reported count of bacteria in urinary sediment may not always agree with the culture result [30]. Microscopic examination of sediment is considered a subjective and time-consuming analysis with poor sensitivity and usually also specificity [31]. Interpretation errors are possible due to many diverse factors and the difference in results among laboratories is mostly due to interindividual variability. Today, automatic urine sediment examination is the new standard in routine urinalysis mostly based on Fluorescence Flow Cytometry and Digital Microscopic Image-based technology [31].

Urine culture as a gold standard

For more than 50 years, clinic trials and scientific studies considered urine culture as a gold standard in the identification of UTI causes. Edward Kass in his 1956 publication determined a strict line between contamination and infection and provided us with a definition of "positive" urine culture. He applied quantitative culture methods to urine specimens obtained from adults by catheterization, and urine collection and concluded that most, but not all acute pyelonephritis patients' samples contained $>10^5$ colony-forming units (CFU)/mL. So a count of 10^5 or more bacteria/mL of urine has been set as the dividing line between true bacteriuria and simple contamination [32,33]. Depending on the definition of negative urine culture and taking into account the prevalence of UTI in the patient cohort, the percentages of negative urine cultures among the studies varied from 40 to 80 %. At first, the Standard Urine Culture (SUC) was originally used to identify patients at increased risk of pyelonephritis. Since the 1950s, clinical practice has been expanded to detecting UTIs with a cutoff of $\geq 10^5$ CFU/mL of a known uropathogenic. Urine culture test results were generalized to also diagnose lower urinary tract infections [34]. Clinical studies consider different cut-off values, but the SUC method did not change, over a long period of time. However, more recent data suggest that bacteria are present in approximately 90 % of standard urine cultures with no bacterial

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growth. This is why enhanced quantitative urine culture (EQUC), a new protocol is finally recommended. The EQUC protocol was shown to achieve much higher uropathogenic detection rates than the standard urine culture (84 % *vs*. 33 %). This protocol was established as a result of a group of researchers' efforts to identify the optimal urine culture protocol for diagnosing UTI, the urinary symptoms most strongly associated with UTI, and the CFU threshold for diagnosing UTI [34].

The standard procedure used in SUC protocol uses 1 µl of urine, spread quantitatively onto 5% sheep Blood Agar Plate (BAP) and MacConkey agars, and incubated aerobically at 35 °C for 24 h. On the other hand, the EQUC protocol uses three urine volumes (1 µl, 10 µl, and 100 µl) and additional plating conditions. Each urine sample is been spread quantitatively onto media (BAP, chocolate Agar,) afterward plates are incubated in 5 % CO_2 at 35 °C for 48 h; BAP and MacConkey agars are incubated aerobically at 35 °C for 48 h. Only growth of a single organism, with a count of ≥10^5 CFU/ml is presumed to be significant growth. For EQUC, the significant colony count is calculated relative to inoculated volume [11,31]. Price, et al. [3], in their extensive research, conclude that an EQUC should be recommended as a supplemental test when individuals with UTI-like symptoms have "no growth" via standard urine culture and for individuals with persistent UTI-like symptoms [34]. Turnaround time for urine culture is about 24 h and another 24 h for Antimicrobial Susceptibility Testing (AST). Considering the overall duration until diagnosis, initial antibiotic therapy is mostly empirical [34]. Despite its shortcomings, culturing of the urine samples is still considered the standard diagnostic test for UTI in symptomatic patients, and a gold standard to exclude these infections in a number of studies [30,35,36]. This method puts emphasis on the number of viable microbial cells that grow rapidly on agar surfaces. However, some slow-growing organisms may need more time, some even several weeks to show their growth on the agar surfaces [15]. So, the lack of accuracy in pathogen identification, selective growth of microorganisms, inability to quantify innate immune responses, and time-consuming methodology are considered major shortcomings of SUC [18,36,37].

Several studies have demonstrated that employing automation systems such as Copan or BD Kiestra, along with closed incubators, can result in increased yield in a Sample Under Culture (SUC). This improvement is evident in both the Colony-Forming Unit (CFU) count and the diversity of bacterial species detected. One key advantage of automated systems is that the media plates used are digitized, allowing for the application of artificial intelligence (AI) techniques. For instance, Copan's PhenoMATRIX, which is already available commercially, utilizes AI algorithms to analyze digitized media plates. By combining the enhanced yield achieved through smart incubators, chromogenic media plates, and the utilization of PhenoMATRIX, exciting new possibilities emerge in the realm of SUC [38,39].

Proteomics in the identification of UTI causer

Proteomic-based methods are used as rapid, accurate, and cost-effective alternatives to "traditional" urine culture methods for the identification of bacterial pathogens. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) as a diagnostic tool has been adapted for routine detection and identification of cultured bacteria from human specimens in clinical microbiology laboratories [40]. Since 2010, commercially available MALDI-TOF MS platforms such as the Bruker MALDI Biotyper (Bruker Daltonics, Bremen, Germany) and VITEK MS (bioMerieux, Marcy l'Etoile, France) have been approved by the US Food and Drug Administration (FDA) [41,42]. Nowadays, MALDI is a common ionization technique widely used in combination with TOF-MS [31,32,43]. MALDI-TOF mass analyzers produce molecular masses of proteins by separating ions according to their mass/charge ratio (m/z) which gives a characteristic spectrum called Peptide Mass Fingerprint (PMF). For species and strain level identification of bacteria, a common m/z ratio falls between 2 and 20 kDa [44,45]. Recorded MS1 fingerprint spectra of highly abundant proteins, dominated by ribosomal protein spectra, need to be compared to the reference mass spectra in the database of known microbes [46-50]. Generally, individual proteins are not identified. Intact cell method ICM or short incubation technique in which samples are cultured for 3-5 h, using either intact cells or cell extracts, has been used for direct identification of microorganisms [44,51–53]. In diagnosing UTI by MALDI-TOF MS it is generally required between 1.5 x 10^5 and 5 x 10^6 CFU/ml of the uropathogens [46]. MALDI-TOF MS allows species-level identification directly from a single microbial colony grown on culture plates within minutes after the sample is mixed with matrix solution and placed on the MALDI plate [45,47,52]. It is estimated that the time from sample receipt to results is about 1 h. However, if bacteria from a mixed culture is not identified, additional tests lasting 18 h – 24 h may be required. It is clear that cultivation is a time-consuming step, so to bypass this step researcher suggest new culture-independent methods [54-59]. MALDI-TOF instrument is connected to the computer software and a database. The software automatically compares thousands of recorded spectra profiles with spectra in a reference databank of known microorganisms to obtain an identification [52,59–62]. The identification of subspecies and strains depends upon the availability of extensive microorganism databases [44,52]. There is a large number of existing studies examining the performance of MALDI-TOF MS for bacteria identification [53,63]. In a recent article, a meta-analysis of twenty-eight studies in the identification of clinically important anaerobic bacteria has shown a high overall identification accuracy, 92 % at the genus and 84 % at the species level [50]. Furthermore, the identification of bacterial pathogens directly from urine using MALDI-TOF MS carried out among different hospitals has resulted in total sensitivity of 86.6 % [64]. However, MALDI Biotyper correctly identified 86.4 % of the strains present in the Bruker database, while VITEK MS correctly identified 92.3 % of the strains present in the VITEK MS IVD database for a wide range of microorganisms [48]. Many studies have reported the advantages of the use of MALDI-TOF MS for clinically relevant bacteria identification over conventional methods [53,65]. The independence of factors, such as culture conditions, growth media, cultivation time, and amount of microbial biomass for

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analysis, have made MALDI-TOF MS the technique of choice in many laboratories [44,52]. This approach has been used based on its ease of operation, low operational costs, high-throughput analysis, and accuracy of the method [59,66,67]. In addition, there are significant advantages such as standard sample preparation, as well as rapid data acquisition and data analysis. More recently, numerous studies have reported the direct detection of pathogens from urine samples to the genus and in many cases to the species levels [53,65]. It is well known that the use of MALDI-TOF MS still has several limitations. First of all, the high initial capital investment necessary for acquiring the instrument, coupled with its maintenance costs, can be the most significant limitation of this technique for many laboratories [63,68]. Secondly, at present MALDI-TOF MS has the inability to differentiate taxonomically related microorganisms at the species level, for example closely related species such as *E. coli* and *Shigella* spp. The reason for this limitation is similarities in mass fingerprints, highly similar proteomic patterns, of a species that do not differ sufficiently in their ribosomal protein sequences [40,46,53,67,69-71]. Thirdly, the identification of a microorganism by MALDI-TOF MS relies on samples with a high concentration of well-isolated bacterial colonies [56]. Therefore, a preculture and isolation of pure colonies are necessary for successful analysis [56,72]. Furthermore, poor sample preparation can lead to low-quality spectra and failed identification of a bacteria [45,67]. Fourthly, the identification of more than one strain/species of bacteria directly from mixed or polymicrobial clinical specimens using MALDI-TOF MS remains a challenge [41,63]. MALDI-TOF MS was unable to identify the most abundant pathogen present in polymicrobial specimens [53]. Finally, MALDI-TOF MS pathogen identification depends on the coverage of the microorganism in the database, the quality of the reference library database, and the software for analysis [42,48,63,70].

Identification of urine bacteria by Genomic methods

Nowadays the main issue in UTI diagnostics using culture methods is its relatively high rate of false-negative results, with the inability to identify pathogens in almost 20 % of patients with symptoms [73,74]. For this reason, molecular methods using Polymerase Chain Reaction (PCR) and sequencing of DNA are beginning to enter clinical diagnostics. Clinical microbiology laboratories have already implemented PCR in routine work while Next-Generation Sequencing (NGS) technology has begun to slowly permeate from research and reference laboratories into the clinical practice [75-78]. With advances in sequencing technology, even hand-held sequencers might become a potential option for the average microbiology laboratory [79].

Multiplex PCR can be used directly on urine samples shortening the time to results making it a valuable option for diagnosing UTI, although still not used in clinical practice [73,74]. Wojno, et al. [73] study compared multiplex PCR applied directly to urine samples with standard urine cultures. Comparison of multiplex PCR and culture methods had shown concordance of 90 % when both methods yielded results, which is in agreement with previous studies [68,69]. When quantitative PCR (qPCR) was used to test urine samples of women with UTI symptoms but with the negative culture, it identified 95.9 % of samples positive for *E. coli*, while analysis on asymptomatic women showed the same percentage of positive results obtained both by qPCR and culture methods implying PCR really detects true pathogens not grown in culture and not contaminants or non-significant result caused by the higher sensitivity of PCR [75,80].

PCR methods gave false-negative results only if bacterial species were not included in the panel showing that the sensitivity of multiplex PCR is limited by the scope of its panel [68,69]. Another challenging field where PCR methods outperform culture is polymicrobial infections [73], although in a majority of cases questions of whether identified species that is most abundant is a causative agent of a UTI remains open [75]. In such more sensitive methods for detecting bacteriuria the quality of samples is of utmost importance with freshly catheterized urine being a more acceptable sample than midstream urine [81,82].

With the discovery of the urinary microbiome, there has been a considerable shift in the UTI paradigm. Since "everyone is bacteriuric", the pathophysiology of UTI is now explained in terms of urinary microbiome dysbiosis, rather than by intrusion of bacteria into a sterile space [77]. It is possible that chronic urinary tract conditions, which areas of now considered idiopathic or non-infectious, might have their own specific microbiome profiles, which might contribute to clinical presentation and acute worsening of symptoms [77,83].

Besides higher sensitivity in detecting pathogens, molecular methods have also downsides. One significant is the inability to inform clinicians about the phenotypic profile of antimicrobial resistance of relevant pathogens and distinguish it from residential flora [70,72,74]. In addition, the presence of the resistance genes does not correlate well with the phenotypic resistance [75,77,79,83]. Therefore, standard antibiotic susceptibility testing based on cultivation is still a golden standard. However, there are ambitious plans and developments of an NGS-based method that might not only accurately predict the resistance phenotype but could also give a Minimal Inhibitory Concentration (MIC) for an antibiotic of interest [79]. Some efforts have already been made in using NGS to detect resistance genes and use that information to personalize preoperative antimicrobial prophylaxis [83].

Additional downsides of NGS are more technical in nature, like relatively high costs, uncurated libraries with sometimes incorrect annotations, a non-existent or underdeveloped system of external quality control and proficiency testing, a general lack of validation and regulation in the field of microbiology, and the need to have staff educated in bioinformatics and big data analyses, or the need to outsource those analyses to external bioinformatic firms [75,77,79]. Another issue is that it is still unclear how results obtained by NGS actually affect the health outcomes of patients with UTIs. Research comparing urine culture and NGS in terms of patients' outcomes is still emerging, but results are promising [84]. More research is also needed in quantifying thresholds of UTI pathogens identified

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by NGS needed to cause an infection [75]. When using conventional culture to diagnose UTI it is accepted that every pathogen has its own corresponding cut-off value in order to meet microbiological criteria for infection, e.g. the accepted threshold for *E. coli* is $\geq 10^2$ CFU/mL and $\geq 10^4$ CFU/ml for other uropathogens [5]. Quantification is also possible when qPCR is used [80].

As already mentioned polymicrobial samples pose a challenge for defining and measuring markers of inflammation, especially in terms of the variation within a healthy urinary microbiome. A newly developed method combining metagenomic sequencing with information about the host is able to distinguish infection from infectious disease and assess its severity in kidney-transplanted patients. Relying on high throughput sequencing of bisulfite-treated cell-free DNA it is able to map cells and tissue types of origin of urinary cell-free DNA based on their methylation profiles and to quantify abundances of a wide array of viruses and bacteria in the sample [66].

Evidently, NGS metagenomics is a robust method, but it is clear that its strength over PCR and 16 sRNA gene sequencing lies not in its higher sensitivity, but in the fact that the method is unbiased. Multiplex PCR platforms, although useful and affordable can only detect the "expected" pathogens and already familiar antimicrobial resistance profiles. Using NGS we gather an incredible amount of information, but most of which we still have trouble interpreting. Regarding the clinical utility and cost-effectiveness, the balance probably lies in implementing stepwise protocols and selecting patients who would benefit from the multiplex PCR approach and those that clinicians suspect might be candidates for more in-depth analyses such as NGS [75]. For example, for patients who suffer from recurrent UTI, but repeatedly have a negative urine culture, it is reasonable to try EQUC or the multiplex PCR approach [77]. If these methods fail to yield conclusive results, NGS could be used in the next step [77,79]. Kidney transplant patients would most certainly benefit from the metagenomics approach when evaluated for urinary tract infection and nephropathy [66]. On the other hand, if it were proved that flares of chronic conditions, such as urinary incontinence/overactive bladder, interstitial cystitis, or neurogenic bladder, might be caused by microbiome imbalance, it would be justified to monitor those patients by NGS and treated accordingly during flares [75,77,85]. In addition, tailoring preoperative prophylaxis for certain urologic procedures on the basis of NGS results might prove to be cost-effective in the future and another step in the direction of personalized medicine [78,80]. And, lastly, if the dream of the NGS-based MIC came true, we would have a powerful method with a true clinical utility that would affect not only the treatment of a single patient but would also inform the global antimicrobial stewardship programs.

Conclusion

Despite advances in genomics and proteomics and the previously listed disadvantages of standard culture-based methods, the SUC method remains the gold standard for the diagnosis of UTIs. It is still considered clinically the most significant, informative method, and most widespread in clinical practice. Nevertheless, it is important to be aware of its limitations and shortcomings and, when required, consider additional protocols. The high costs, long turnaround time, and large percentage of negative urine cultures in symptomatic patients are reasons to make improvements in the existing protocols and implement new, easier, and faster method solutions. The introduction of primary screening of samples by flow cytometric methods can significantly contribute to savings and faster issuance of negative urine culture results. However, in symptomatic patients whose urine cultures are repeatedly sterile, additional urine sample processing with the EQUC protocol should be considered. Other methods are used sporadically and implemented according to clinical presentation, however, most are not routinely available in clinical microbiology laboratories. In the context of UTIs and significant bacteriuria not detected by SUC, genomic, proteomic, and metaproteomic methods provide us with a deeper knowledge of pathogen species, their abundance, and the body's inflammatory response elicited by those pathogens. In patients with UTI, it is of utmost importance to identify the actual causal agent. However, in the future, it is possible that in certain cases and clinical conditions, we will have to abandon our present paradigm of a single and conventional uropathogen as a dominating causal agent and shift our perspective in the direction of microbial dysbiosis which might better explain the pathophysiology and clinical presentation, especially in patients with urinary tract symptoms and repeatedly sterile urine cultures. Failing to provide clinicians with appropriate, relevant, and timely microbiological results leads to inappropriate therapy and patient management, which are the main drivers of bacterial resistance and decreased patient satisfaction. Therefore, it is becoming more common to supplement conventional urine culture with proteomics, PCR, and NGS in order to accurately identify the potential microbial cause of infection. To distinguish clinically insignificant and significant bacteriuria, the threshold of 10^5 CFU/mL as yielded by the SUC is no longer adequate, which is already reflected in clinical and laboratory UTI guidelines and repeatedly confirmed in studies utilising more sensitive diagnostic methods such as EQUC, proteomics, PCR and NGS. On the other hand, detecting and identifying microorganisms in urine does not render them a culprit, which means that using more sensitive methods does not always translates into clinically useful information. If the healthy urinary microbiome is ever discerned and if the characteristic microbial patterns related to certain urinary tract conditions emerge, genomics might become an attractive diagnostic tool for clinicians. In addition, once one is able to sift through the plethora of information generated especially by genomics, these proteomic and genomic methods could start to play a major significant role in clinical decision-making, notably for most complicated cases such as patients with recurrent UTIs with repeatedly sterile cultures, overactive bladder/urgency urinary incontinence, interstitial cystitis/painful bladder syndrome or those with a kidney transplant. Although it is clear that more studies on these methods are needed, hopefully, they will not remain confined only to the realm of research and will find their niche in clinical practice (Table 1).

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Table: The different method analysis of Urinary tract infections.

Method	Application	Shortcomings	Advantages	Utility
Flow cytometry	Screening method	Low sensitivity	Quickly (1-4 h) provides useful information about the presence of microorganisms; significantly decreases the cost of diagnostics reduces the turnaround time for negative urine culture	Quick selection of "negative" samples
Dipstick test	Bacteriuria screening method; an additional method in UTI diagnosing	Some authors consider it unreproducible; a semiquantitative method that should not be used to assess the presence and degree of leukocyturia; low sensitivity	The nitrite test determines the activity of nitrate reductase, which detects bacteriuria (≥ 105 CFU / ml urine); the leukocyte esterase test is a screening test for pyuria	Helpful in diagnosing UTI performed in biochemistry laboratories
Urine sediment	Used to further direct a diagnostic process	Subjectivity of the analyst; nonstandardized methods	Assistance in the interpretation of culture results, UTI diagnosis, assessing the quality of the sample and directing further diagnostic process	Helpful in diagnosing of urinary tract disease; performed in biochemistry laboratories
Standard Urine culture	At present considered the gold standard used for clinical decision making in conjunction with other relevant clinical and diagnostic parameters	Time-consuming; dependence of culture conditions and growth media; interpretation dependent on the clinical condition	identification of pathogens and determination of their susceptibility to antibiotics	routine application in microbiology laboratories
EQUC	More sensitive method applicable for screening symptomatic patients with sterile urine cultures	Time-consuming; dependence of culture conditions and growth media; interpretation dependent on the clinical condition	Identification of pathogens and determination of their susceptibility to antibiotics	Application in microbiology laboratories
16 sRNA sequencing	A more sensitivity method used to determine bacterial genera	Not optimal for indepth species analysis	Can determine bacterial genera and their abundance	Research and reference laboratories
NGS	Identification of pathogens in recurrent UTIs in which the urine cultures repeatedly sterile; potentially useful in conditions such as overactive bladder/urgency urinary incontinence and interstitial cystitis/painful bladder syndrome	Time to results about 3-5 days	The most comprehensive and unbiased genomics method of uropatogen identification.	Slow shift from academic and reference laboratories into clinical laboratories
(Multiplex) PCR	Direct targeting diagnostics	Sensitivity limited by the scope of the panel	Can be used directly on urine samples; shortens time to results; higher sensitivity; great for targeted diagnostics	Implemented in better equipped clinical microbiology laboratories
MALDI-TOF MS	Routine use in clinical laboratories; mostly used in the identification of cultured uropathogens; used directly on urine samples for research purposes	High initial capital investment; the problem of identifying multicultural pattern when used directly on urine samples	High identification accuracy, at the genus and species level; independence of culture conditions and growth media, shorter cultivation time; requires lesser amount of microbial biomass for analysis	Clinical laboratories
MALDI-TOF MS/MS	Direct identification from samples; identification from cultures; identification at the level of subspecies; identification of inflammatory and tumor markers	High initial capital investment;	Direct identification without cultivation	Research laboratories

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Author contributions

Writing: Review & Editing, Visualization: Marina Čeprnja, Damir Oros

Writing: Review & Editing: Andrea Janeš, Jurica Žučko

Project administration: Antonio Starčević

Supervision: Karmela Barišić, Jasenka Škrlin and Antonio Starčević

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