New and developing diagnostic technologies for urinary tract infections

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Abstract

Timely and accurate identification and determination of the antimicrobial susceptibility of uropathogens is central to the management of UTIs. Urine dipsticks are fast and amenable to point-of-care testing, but do not have adequate diagnostic accuracy or provide microbiological diagnosis. Urine culture with antimicrobial susceptibility testing takes 2 3 days and requires a clinical laboratory. The common use of empirical antibiotics has contributed to the rise of multidrug-resistant organisms, reducing treatment options and increasing costs. In addition to improved antimicrobial stewardship and the development of new antimicrobials, novel diagnostics are needed for timely microbial identification and determination of antimicrobial susceptibilities. New diagnostic platforms, including nucleic acid tests and mass spectrometry, have been approved for clinical use and have improved the speed and accuracy of pathogen identification from primary cultures. Optimization for direct urine testing would reduce the time to diagnosis, yet these

technologies do not provide comprehensive information on antimicrobial susceptibility. Emerging technologies including biosensors, microfluidics, and other integrated platforms could improve UTI diagnosis via direct pathogen detection from urine samples, rapid antimicrobial susceptibility testing, and point-of-care testing. Successful development and implementation of these technologies has the potential to usher in an era of precision medicine to improve patient care and public health.

Subject ontology terms

Health sciences / Diseases / Urogenital diseases / Urinary tract infection; [URI / 692/699/2768/1865]; Physical sciences / Materials science / Materials for devices / Sensors and biosensors; [URI /639/301/1005/1009]; Biological sciences / Microbiology / Bacteria / Bacterial physiology / Antibacterial drug resistance; [URI /631/326/41/1969/2038]; Physical sciences / Nanoscience and technology / Nanomedicine / Diagnostic devices; [URI /639/925/352/1060]

UTIs are among the most prevalent community-acquired and hospital-acquired infections, affecting almost 50% of the population at least once in their lifetime, accounting for considerable morbidity and health-care expenditure with an estimated annual cost of US$3.5 billion in the USA[1–3]. Complicating factors, such as obstructing urinary stones, indwelling catheters, and urinary tract surgery increase the risk of urosepsis, which has an associated mortality as high as 20%[4,5]. The majority of UTIs are caused by Gram-negative pathogens, primarily from the *Enterobacteriaceae* family including *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae*, and *Enterobacter* species[1–3,6]. Fungal UTIs are not as common as bacterial UTIs, but patients with indwelling catheters, diabetes, or recent antibiotic use are at increased risk of fungal infection[7,8]. Urogenital tuberculosis and parasitic organisms such as *Schistosoma haematobium* can cause UTIs, although these infections are not common in the USA[9]. Technologies capable of rapidly identifying these pathogens and their antimicrobial susceptibility have the potential to improve and expedite diagnosis, enabling personalized treatment.

The emergence of drug-resistant and multidrug-resistant pathogens — recognized as a healthcare threat of global proportions — is further driving the need for expeditious diagnosis and prudent use of antibiotics[10]. Drug resistance occurs via intrinsic or acquired mechanisms that enable bacteria to evade antimicrobial agents. Antimicrobial resistance can be acquired through mutation or horizontal gene transfer. For example, resistance to β-lactams (such as penicillins and cephalosporins) is conferred by β-lactamase genes that naturally evolved in many bacteria[11,12]. Genes are often transferred between cells via bacteriophage transduction or plasmid conjugation between bacteria (such as *mecA* that leads to methicillin resistance in *Staphylococcus aureus*)[13,14]. Many mutations and genes that confer antimicrobial resistance can be detected by clinical microbiology using genetic assays. However, the absence of specific genes or mutations does not ensure sensitivity to a given antibiotic; thus phenotypic antimicrobial tests remain the diagnostic standard when testing a variety of pathogens with different antimicrobial resistance mechanisms.

Key points

- UTIs are increasingly caused by multidrug-resistant organisms as a result of the overuse of empirical, broad-spectrum antibiotic therapy

- Antimicrobial susceptibility, determined by the phenotypic response to antibiotic exposure, is key for clinical decision making for treating the wide variety of uropathogens and identifying resistance markers

- Existing technologies (such as PCR, fluorescence *in situ* hybridization, and mass spectrometry) and new technologies (such as droplet microfluidic and biosensor platforms) need to focus on direct urine testing to expedite objective diagnoses

- Integrated biosensor–microfluidic platforms have the most potential for point-of-care testing, as they facilitate direct urine analysis and can encompass all assay steps in a compact device

- New technologies are a key step towards improved antimicrobial stewardship

The acquisition of resistance is in part caused by selective pressure from injudicious use of antibiotics, as up to half of the antibiotics prescribed in the USA are not needed or not optimally prescribed[10]. Resistance is also fuelled by the widespread use of antibiotics in agriculture to prevent infection and promote animal growth[15]. The increasing resistance to ciprofloxacin and trimethoprim-sulfamethoxazole observed in urinary isolates from the outpatient setting directly affects UTI treatment, reducing options for oral antimicrobial therapy[16–19]. In this setting, nitrofurantoin has emerged as a first-line therapy for uncomplicated cystitis and is highly effective against *E. coli* and *Enterococci* that cause ~75% of UTIs; however, species such as *P. mirabilis* and *Pseudomonas aeruginosa*, are intrinsically resistant to this antimicrobial[20]. Subsequently, objective knowledge of the causative pathogen is necessary to provide timely, effective treatment for the large number of patients with nitrofurantoin-resistant pathogens.

In patients seen in the urological setting, antibiotic resistance is problematic and can be the result of over-treatment of asymptomatic bacteriuria during chronic catheterization or urinary diversion[21–23]. Transrectal ultrasonography (TRUS)-guided prostate biopsy is an invasive urological procedure that is commonly performed in the clinical setting and puts patients at increased risk of UTI and urosepsis. The rate of sepsis after TRUS-guided prostate biopsy ranges from 0.3 to 3.5%[24,25] and a 2016 population-based study found that infectious complications after biopsy are increasing[26]. This increase might be in part caused by an increase in bacterial resistance to fluoroquinolones, which are commonly given as prophylaxis, thereby prompting some clinicians to shift to using broader-spectrum antibiotics such as gentamicin and ceftriaxone, or combinations of antibiotics[24,27]. However, shifting to more potent antibiotics is inconsistent with the goal of improving antimicrobial stewardship and could further increase resistance. One approach to provide targeted prophylaxis is to identify the resistance patterns of bacteria from a preprocedure rectal swab culture. Providing targeted prophylaxis based on this analysis resulted in a decrease in the

rate of postbiopsy infection with a 4.55% infection rate for men receiving empirical antibiotics compared with a 0.72% infection rate for men receiving targeted prophylaxis[27]. Targeted prophylactic antibiotics can decrease the rate of postbiopsy infection, therefore, a rapid assay for antibiotic susceptibility has the potential to benefit patients in this clinical setting.

Standard diagnostic examination for UTI begins with presentation of clinical symptoms, which commonly include dysuria, urinary frequency, and urgency. Clinicians often order screening by colorimetric dipstick testing for nitrites and leukocyte esterase, which detect bacteriuria and pyuria, respectively (FIG. 1). However, urine dipsticks can give false-negative results in the case of non-nitrite-producing pathogens, such as *Enterococcus* and *Staphylococcus* spp., or in dilute urine samples[28]. Thus, in many health-care settings, urine dipstick tests are no longer performed at the point of care but in the clinical laboratory, where standardized readouts and further microscopic urinalysis can be undertaken.

In the clinical microbiology laboratory, urine is cultured on agar plates for growth, concentration, identification, and isolation. Voided urine samples that grow $\geq 10^4$ cfu/ml of a single or predominant species of uropathogenic bacteria are considered culture positive[29]. Use of chromogenic agar enables direct identification of *E. coli, Staphylococcus saprophyticus* and *Enterococcus* species after overnight incubation[30]. Definitive identification of other pathogens or speciation of non-*E. coli Enterobacteriaceae* requires further phenotypic characterization of the isolated bacteria. This analysis is usually performed with automated systems based in the clinical microbiology laboratory (TABLE 1). Urine collection to pathogen identification typically takes 18–30 hours (FIG. 1a).

After pathogen isolation and identification, antimicrobial susceptibility testing (AST) takes an additional 24–48 hours. AST is typically conducted as a phenotypic assay that measures bacterial growth in the presence of specific antimicrobial agents. AST results for each pathogen–antimicrobial combination are interpreted according to standardized guidelines and reported as sensitive, intermediate, or resistant. Manual AST methods, including broth dilution, disk diffusion, and gradient diffusion, require manual sample preparation steps and an incubation period of 16–24 hours[31,32]. High-throughput, automated instruments, such as the Microscan Walkaway (Beckman Coulter), Phoenix Automated Microbiology System (BD), and Vitek 2 (bioMérieux) (TABLE 1) have been developed to provide readouts of increased sensitivity, resulting in a modest reduction in turnaround time to approximately 10–16 hours[33,34].

The development of new diagnostic tools for identifying infectious diseases has been recognized as an integral part of the overall strategy to combat the rise of drug-resistant pathogens[10]. Beyond improving individual patient management, new diagnostic tools will influence epidemiological surveillance, infection control, antimicrobial stewardship, facilitation of clinical trial enrolment, and the codevelopment of drugs and diagnostic tests[35,36]. The ability of new diagnostic technologies to work directly with urine samples without compromising the sensitivity and specificity of standard methods is paramount, as the initial overnight urine culture is often the most time-consuming step in the diagnostic paradigm. Screening tests should deliver pathogen-positive or pathogen-negative results in

minutes and integrated platforms should enable pathogen identification and AST within a few hours of sample collection. This efficiency would obviate initiation of empirical antibiotics in the absence of pathogens, and facilitate pathogen-specific (antibiogram-based) antibiotic selection or, ideally, individually tailored antibiotics on the day of clinical presentation. To promote the adoption of new tests, they should be easy to use, cost-effective, and amenable to point-of-care testing. This Review will discuss some of the promising technologies for UTI diagnostics including emerging new tests that are at various stages of development, screening assays to improve the diagnostic yield of downstream analysis, approved molecular and proteomic technologies that could be adapted for UTI identification, and integrated platforms that have the potential to deliver combined pathogen identification and AST.

Detecting UTIs

Screening assays

The majority of urine samples sent for microbiological analysis are negative for pathogens[37,38]; thus, an initial screen for bacteriuria could improve laboratory work-flow and reduce costs. Current screening assays for bacteriuria include urine dipstick tests for detecting nitrites, microscopic urinalysis, and urine Gram stain. Urine dipsticks are fast and simple to use, but they have inadequate sensitivity[6,39]. A meta-analysis of 34 studies assessing the accuracy of the nitrite dipstick test in a variety of clinical settings found a mean sensitivity of 48% for the detection of clinically significant bacteriuria of $>10^5$cfu/ml (REF. 28). Microscopic urinalysis and urine Gram staining are more laborious methods of detection than urine dipsick tests and are performed in a clinical laboratory, but have also been shown to lack sensitivity for samples with $<10^5$ fu/ml and to have poor specificity[6,40]. To address these shortcomings, new screening technologies are in development for rapid and direct screening of urine samples.

Lateral flow immunoassay—Lateral flow assays are a good choice for point-of-care screening tests; they are inexpensive and easy to use, as the sample and reagents are mixed on a paper support with liquid transport driven by capillary action and a colorimetric readout. Dipstick tests for urine nitrite and leukocyte esterase are widely used lateral flow assays, but they are limited by shortcomings of poor sensitivity[6,39]. In 2015, a new antibody-based lateral flow assay (RapidBac), which is currently approved for veterinary use, was evaluated in a human study for rapid detection of bacteriuria[41]. This test is comprised of two monoclonal antibodies, one specific for *Enterobacteriacea* and a broader-spectrum antibody against Gram-negative and several Gram-positive species including *Staphylococcus*, *Enterococcus*, and *Actinomyces* spp.; however, rare bacteria and fungi would not be detected. This lateral flow immunoassay had a sensitivity of 86% for samples with $\geq 10^3$cfu/ml bacteria with a specificity of 94% when tested in 966 human urine samples compared with standard culture (set at 100% sensitivity and specificity) and a sensitivity of 96% for Gram-negative bacteria present at $\geq 10^4$cfu/ml (REF. 41). These data are promising, but further testing of this assay in a multicentre prospective study is needed to confirm the accuracy of the test.

Flow cytometry—Rapid screening based on the detection of cells in solution by light scattering has been employed in many devices and can detect most bacterial species as well at fungi[42–44]. Flow cytometry systems, such as the FDA-approved UF-1000i (Sysmex), use a combination of light scattering and fluorescence to rapidly screen for the presence of bacteria in urine[45–47]. Flow cytometry is a good system for selecting samples for further analysis, and has been used to identify pathogen-positive urine for further complex testing, such as mass spectrometry analysis. Initial screening of urine samples by flow cytometry might improve clinical laboratory workflow by reducing the number of samples sent for further analysis; however, flow cytometry is only a screen for bacteriuria as it does not provide species identification or AST for definitive diagnosis[48–50].

Systems for the detection of bacterial growth that are based on changes in forward light scattering have been used since the 1980s for the direct testing of urine samples for viable bacteria[51,52]. For this assay, a small volume of urine is used to inoculate culture medium and changes in forward light scattering indicative of bacterial growth are measured over time. Improvements in system integration and automation have resulted in the development of systems that can identify bacteriuria quickly, in as little as 45 minutes, such as Uro-Quick (Alifax) and BacterioScan model 216 (BacterioScan Inc.). These systems do not provide pathogen identification, but they can include AST. The measurement obtained is the change in forward light scattering over time, so comparison of cultures with or without antibiotic treatment can, therefore, be used to determine the susceptibility profile of the bacteria. Thus, these methods cannot be used for definitive diagnosis, but they might provide additional objective guidance for antibiotic prescription. Further attempts are being made to incorporate some speciation information into light scattering techniques, such as differential lysis of Gram-positive and Gram-negative bacteria with sodium dodecyl sulfate[53] and algorithms to distinguish the light-scattering patterns of bacilli from cocci[54], but these techniques are still experimental.

Adapting clinical molecular platforms

Inherent shortcomings of phenotypic identification based on biochemical characteristics include the dependency on bacterial growth in a laboratory setting and overlapping phenotypic characteristics among similar bacteria. Advances in molecular biology have substantially expanded our understanding of microbial genetics enabling detection of pathogens based on their molecular signature. The wide application of sequencing technologies has transformed taxonomic classifications that were previously based on phenotypic similarities with comparison of evolutionarily conserved sequences[55,56]. Additionally, the genetic basis for resistance to specific antimicrobials has been elucidated for many clinically significant pathogens[57]. These advances enable the identification of pathogens by targeting pathogen-specific molecular signatures. Furthermore, genome sequence analysis is being used to understand mechanisms and patterns of antibiotic resistance[58] as well as provide tools for discovery of new antimicrobials[59].

Molecular and proteomic technologies including mass spectrometry (such as matrix-assisted laser desorption ionization–time of flight (MALDI–TOF) mass spectrometry), fluorescence *in situ* hybridization (FISH), and PCR have been approved in the past 20 years and improve

the diagnosis of bacterial infections[60,61]. Currently, these technologies are performed in a clinical laboratory and require initial isolation of bacteria or considerable sample processing to isolate the bacterial cells from the urine matrix before analysis[62,63]. Repurposing these technologies for diagnosing UTI directly from urine is possible and could result in substantially improved efficiency and clinical outcome over current culture methods. However, direct urine testing will require further assay optimization, as the urine matrix can vary widely, not only from person to person but also between different samples from the same patient in terms of pH, electrolyte concentration, and cellular composition.

MALDI–TOF mass spectrometry—Mass spectrometry is a technique in which charged molecules are created by ionization and their identity is determined based on the mass:charge ratio. Specifically, matrix-MALDI–TOF mass spectrometry has been approved by regulatory agencies for pathogen identification from culture. The technique generates a peptide-mass fingerprint that is unique to a specific organism and can be compared against a database of reference spectra[64,65]. For application in UTI identification, the urine sample is first cultured to isolate the bacteria and a colony from the culture plate is analysed.

Several groups have explored direct analysis of urine samples using MALDI–TOF to decrease the time needed for pathogen identification[48,50,62,66]. For direct analysis of urine, initial sample preparation steps are necessary to remove cellular debris, leukocytes, and mucus, and to collect bacteria. Common procedures include differential centrifugation and washing, or dual filtration to enrich the bacterial fraction for MALDI–TOF[48,50]. The entire process can deliver results within 1–3 hours. In initial studies that combine upstream urine-screening methods (such as Gram staining and flow cytometry) with direct application of MALDI–TOF to bacteriuria-positive samples, the sensitivity for direct pathogen identification ranged from 67 to 86%[48,66–68]. These results mirror those of similar studies that also show success in pathogen identification from pathogen-positive blood culture[48,69].

MALDI–TOF has improved the workflow for pathogen identification from isolates in clinical laboratories. The cost per sample of MALDI-TOF analysis of pathogens could be about half that of culture analysis[70], but the high initial purchase price of the instrument (over $250,000 (REF. 71)) might present a considerable obstacle for adoption in smaller clinics limiting use of this platform to high-volume laboratories. Additionally, whether MALDI–TOF can meet the demands of UTI diagnostics is unclear, given the need for upstream screening to improve the diagnostic yield of positive samples and the need for initial sample processing, although efforts are underway to simplify sample preparation[62]. In its current iteration, analysis of MALDI–TOF results is confounded by poly-microbial samples. Up to 77% of catheter-associated UTIs are polymicrobial, therefore, improved algorithms for interpreting the spectra of combinations of bacteria are needed for direct-from-urine testing of these samples[72–74]. Furthermore, the assay does not provide information regarding antimicrobial susceptibility. Indirect approaches to AST with MALDI–TOF are in development and include the measurement of bacterial metabolic by-products in the presence of antibiotics to assess susceptibility[75]. Pathogen identification by MALDI-TOF mass spectrometry is being used in many large clinical laboratories; however the limitation of sample preparation and complex sample interpretation will likely prevent adoption for direct-from-urine testing in the near future.

Fluorescence in situ hybridization—FISH assays are based on microscopic detection of fluorescently labelled nucleic acid probes that are hybridized to complementary targets[76]. FISH is widely used in research laboratories and has been translated into the clinical diagnostic arena for infectious diseases. A common target for detection of bacteria by nucleic acid hybridization such as FISH is 16S ribosomal (r)RNA, an integral component of the bacterial ribosome that contains both evolutionarily conserved sequences and regions that are unique to each bacterial species[77,78]. Furthermore, 16S rRNA is present in abundant quantities, with >10,000 copies per cell[79]. Detection is dependent on the development of specific probes, but the abundance of these species-specific sequences facilitates pathogen detection via molecular methods[80].

Assay kits such as hemoFISH (miacom diagnostics GmbH) and QuickFISH (AdvanDX) have been approved by the FDA for the identification of pathogens from positive blood cultures[81–83]. These assays can be used to reliably identify a number of bacterial species including *S. aureus, Enterococcus faecalis, E. coli, P. aeruginosa, K. pneumoniae* and multiple *Candida* species[84,85]. hemoFISH uses DNA probes, but the QuickFISH assay is based on peptide nucleic acid (PNA) probes, which replace the carbohydrate backbone of the nucleic acid with a neutral peptide[86,87]. This electric neutrality facilitates faster hybridization than DNA probes by eliminating electrostatic repulsion between the probe and the target. The rapid FISH assays can be processed in as little as 20 minutes with sensitivity and specificity of >96%[81]. The relatively high concentration of bacteria in infected urine samples might enable direct testing of a patient's urine sample with a rapid FISH assay. The available data suggest that FISH is a powerful tool in the detection of bacterial pathogens, but is limited by an inability to incorporate AST analysis[63]. FISH techniques can be used for rapid, accurate identification of pathogens; however translation of FISH to a point-of-care diagnostic could be challenging, limiting widespread application.

Multiplex PCR—Nucleic acid amplification by PCR has been a mainstay of molecular biology and genetic research since its inception in the late 1980s[88]. The sensitivity and specificity of PCR that enables the detection of rare targets has assisted its adoption in to clinical diagnosis applications. A role for PCR for direct-from-urine UTI diagnostics is being investigated by several companies that have developed systems for PCR-based detection of pathogens.

SeptiFast real-time PCR (Roche), is currently approved for identification of pathogens from whole-blood samples[89]. This multiplex panel targets both Gram-positive and Gram-negative bacteria in addition to a number of fungal species. To explore the possible adaption of this platform to UTI diagnosis, SeptiFast was directly compared with standard culture of 82 urine samples from 81 patients with suspected UTI. Urine samples were processed without prior incubation and DNA was extracted from cells in urine sediment for PCR analysis. Concordance between the two methods of positive and negative findings in the Gram-positive, Gram-negative and fungi were 90%, 97%, and 97%, respectively. The SeptiFast test had a sensitivity and a specificity of 82% and 60%, respectively, for the detection of infection. SeptiFast identification was available at least 43 hours before culture results[89].

To facilitate clinical adoption of new technologies, sample preparation and the detection assay should be integrated into a single platform capable of 'sample-in, answer-out'. Tests such as FilmArray (bioMérieux) and GeneXpert (Cepheid) have integrated nucleic acid extraction and multiplex PCR, so the end user only needs to mix the sample with buffer and apply it to the test cartridge. FilmArray is currently used for bacterial identification from blood and stool cultures that are positive by either Gram stain or initial culture[90,91]. Once identified as positive, samples are taken directly from the culture bottle or resuspended in buffer and injected into a cartridge containing all the necessary reagents to process the samples, extract the genetic material, and perform PCR analysis. From a positive clinical specimen, the system can detect as many as 19 bacterial pathogens, five species of yeast, and three antimicrobial-resistance markers. Furthermore, the assay can be completed in under 1 hour and the reported sensitivity for pathogen identification is >90%[90,92,93]. This system is not currently approved for UTI detection, but adaptation of the processes for positive urine cultures is feasible. GeneXpert is a fully integrated multiplex-PCR platform approved for a variety of infectious diseases and haematological assays. This test is not tailored for detection of UTI pathogens, but it is capable of detecting bacterial pathogens causing sexually transmitted infections (STIs), such as *Chlamydia trachomatis* and *Neisseria gonorrhoea,* within 90 minutes directly from urine samples[94,95]. The sensitivity and specificity of detection of these STI pathogens were both >97% in samples from men and women. However, like the SeptiFast system, the current GeneXpert assay is qualitative in nature and does not quantify bacterial load directly from urine samples, which is important for UTI diagnostics, as bacterial loads <10^3 cfu/ml might not be clinically significant.

These PCR assays have proven to be successful for identification of pathogens and potential resistance genes, but challenges remain for their use in UTI diagnostics. The current PCR assays only provide qualitative data indicating the presence of bacteria not concentration. Qualitative assays are useful for identifying pathogens that have been previously quantified using standard culture methods or diagnosing infection from fluids in which the presence of bacteria is itself pathological, such as blood or cerebro-spinal fluid[96]. However, in urine, the most common means of specimen collection, clean catch, increases the potential for contamination by urethral flora. Thus, quantification of bacteria within a urine sample is necessary to differentiate between true infection and contamination and guide clinical decision making. For UTI diagnostics based on direct urine testing, real-time PCR could be configured to provide quantitative results by inclusion of a quantitative measure of a conserved sequence, such as rDNA[97]. Furthermore, PCR can provide information regarding specific antimicrobial resistance genes present in bacteria, but it cannot provide comprehensive or definitive phenotypic information about antibiotic susceptibility. Quantitative PCR could be incorporated into a rapid diagnostic test for UTI for detection of pathogens and as a tool to measure bacterial growth in the presence of antibiotics.

Real-time microscopy systems for AST

Accurate pathogen identification is key to diagnosis, but assessing pathogen antimicrobial susceptibility is critical to identifying the appropriate antibiotic treatment for each patient. PCR assays are limited to pathogen identification and detection of known resistance genes

and are unable to assess all potential pathogen-resistance mechanism combinations. Particularly for UTI, phenotypic AST remains the approach of choice.

Phenotypic AST relies on monitoring cell growth over time in the presence or absence of antibiotic. By employing technologies that monitor each cell division, new AST systems can reduce the time needed to assess antimicrobial sensitivity. The oCelloScope (Phillips BioCell) is a small detection platform in which growing bacterial cultures are scanned using digital time-lapse microscopy. In a pilot study, urine samples were collected from pig models with indwelling catheters to simulate catheter-associated UTI[98]. The samples were processed by centrifugation to remove host cells and the bacteria were incubated for 2 hours to initiate exponential growth. The active bacteria were then plated into wells containing 23 different antibiotic conditions and growth was measured for 6 hours using time-lapse microscopy. The system was able to detect an initial change in growth rate in response to antibiotics — such as levofloxacin or combination ticarcillin and clavulanic acid — in only 30 minutes, and determine the minimum inhibitory concentration (MIC) in <3 hours. In a second study, nine clinical isolates from patient blood cultures, including multidrug-resistant strains, were compared with reference strains to evaluate the oCelloScope system AST[99]. The study results showed 96% agreement between the real-time microscopy and standard AST methods. Additionally, the average time to a result was 108 minutes, which is considerably faster than the 2–3 days required for standard culture methods. The sample number these studies was small, but the efficiency is markedly improved compared with standard AST methods, and these tools are promising future options for AST in human urine samples.

Another system based on real-time microscopic analysis of bacteria is the Accelerate ID/AST system (Accelerate Diagnostics). For this system, the sample matrix is replaced with an electrokinetic buffer of low ionic strength either by centrifugation and resuspension or gel electrofiltration. The sample is then applied to a multichannel fluidic cassette in which a low-voltage electrical field is used to concentrate and trap the bacteria on a poly-L-lysine-coated surface. The immobilized bacteria are cultured and bacterial growth monitored by time-lapse, dark-field microscopy. Analysis of the image data is used to characterize organisms based on relative mass, shape, geometric growth pattern, clone surface features, and growth rate over time. Pathogen characterization can be completed in 1.5 hours, and introduction of antibiotics to the culture medium can be used to assess susceptibility, with results available in approximately 5 hours (REF. 100). The assay has been demonstrated to be successful at both pathogen identification and antimicrobial susceptibility assessment from blood culture[101]. Further preliminary tests indicate that this system is capable of direct detection of pathogens from patient blood, urine, and sputum samples[100,102,103]. The phenotypic measures used for pathogen identification are not precise, but the system is also amenable to process samples for species identification methods such as FISH. This approach incorporates a basic pathogen classification and AST, therefore, it has the potential for clinical adoption as a UTI diagnostic tool. However, the true ease of use of this system is unclear and incorporation of the system into the workflow of a clinical lab might be impractical.

Emerging diagnostic platforms

Biosensors, microfluidics, and lab-on-a-chip technology

Emerging diagnostic platforms, such as biosensors, microfluidics, and lab-on-a-chip technology, demonstrate the potential for expedited UTI diagnosis using enhanced screening, molecular pathogen identification, and rapid AST. Most developments to date have been targeted toward decreasing the turnaround time and enhancing automated processing in the clinical laboratory, either to improve the yield of the screening assays, thereby reducing the workload of processing negative urine samples, or to improve the throughput for batch processing, as urine is the most common clinical sample[6]. Molecular technologies such as MALDI–TOF are highly sensitive but are largely limited to clinical laboratories with considerable resources. Integrated multiplex-PCR platforms (such as GeneXpert Omni, Cepheid) are amenable for point-of-care testing but are limited by the lack of quantitative pathogen identification and integrated phenotypic AST. Next-generation sequencing platforms are emerging as promising tools for challenging clinical scenarios (such as encephalopathy[104]) in which infectious agents (including bacteria, viruses, fungi, and parasites) and noninfectious aetiologies are among the differential diagnosis and a traditional culture-based approach has largely been ineffective. Integrated technology platforms (FIG. 1) that can be deployed at either point of care or in a clinical laboratory and can provide timely diagnostic information (within 3 hours) to direct personalized antibiotic treatment are highly desired.

Advances in microtechnologies and nanotechnologies have resulted in the development of biosensors with integrated microfluidic handling systems capable of performing the complex molecular assays that are required for the detection of pathogens in biological matrices. Biosensors are miniaturized analytical tools characterized by fast response, high sensitivity, high selectivity, and the capacity for multiplexed detection. A biosensor is typically composed of a recognition element (such as antibodies or nucleic acids) and a signal transducer (FIG. 2a). Binding of the target analyte to the recognition element results in a specific signal that can be detected by a variety of techniques including optical, electrochemical, conductance, or mass-based[105,106]. Detection strategies are divided into label-free and labelled assays. Label-free assays directly measure analyte binding on a transducer surface[107]. For labelled assays, the analyte is sandwiched between the recognition element, such as oligonucleotides or antibodies, and a detector agent, typically a second oligonucleotide of antibody, with a specific label for signal output, such as a fluorophore for optical detection[108,109]. Most biosensors are capable of quantitative detection in which the magnitude of the signal is proportional to the analyte concentration. Biosensors are an excellent option for integration into diagnostic platforms as they enable manipulation of small fluid volumes, short assay time, low energy consumption, high portability, high throughput, and multiplexing[105]. Furthermore, smartphones could be integrated into these technologies as the controller unit and readout instrument to enable low cost, point-of-care applications for biosensors[110,111].

For clinical translation of molecular diagnostics, biosensor-based platforms must integrate sample preparation steps. Examples of sample preparation include enrichment of target

analytes, removal of sample matrix inhibitors, and sample volume reduction. Strategies for sample preparation depend on the type of biological sample, the sample volume, and the target analyte concentration. Microfluidics is a broad discipline that involves the manipulation of reagents and analytes at the micron scale. Appealing features of microfluidics for molecular diagnostics include low reagent and substrate volume, laminar fluid flow, fast thermal relaxation, reduced assay time, and low power consumption, which make these techniques ideal for sample preparation[112].

Various biosensor applications in research settings have been investigated for UTI diagnostics[113]. An example of a label-free biosensor that shows promise as a screening tool for bacteriuria is an electronic nose. Electronic noses mimic the olfactory system and detect a specific signature of volatile organic compounds (VOCs) produced by bacteria. The eNose (Specific Technologies), a hand-held system, uses ion mobility spectrometry to assess a VOC profile in 15 minutes (REF. 114). Tests of the eNose system with cultured uropathogens isolated from patient samples achieved 95% sensitivity and 97% specificity[114]. A simpler approach than the eNose to detection of VOCs uses colorimetric sensor arrays rather than ion mobility spectrometry[115]. These arrays consist of a thin film printed with variety of dyes that change colour on the binding of compounds such as amines, fatty acids, alcohols, sulfides, and aldehydes. An agar-filled petri dish is inoculated with the sample and the array is placed the in a petri dish lid; as the bacteria grow, the VOCs produced cause a distinctive pattern of colour changes that can be read by scanner or smartphone camera for analysis[116]. Testing of colorimetric sensor arrays with blood agar plate culture of clinical isolates or blood culture showed ~91% sensitivity and ~99% specificity[117,118]. However, for point-of-care screening, systems for detecting bacteriuria based on VOCs will have to be tested directly with urine samples and interpretation of results could be confounded by the variability of VOCs in urine. The simplicity of VOC-based tests could make this approach an excellent option for low-resource settings.

Electrochemical biosensors have a proven track record for use in point-of-care diagnostics; portable glucose sensors for monitoring blood sugar levels are the most established biosensors in wide clinical use. An electrochemical biosensor platform for UTI diagnostics has been extensively investigated, including investigating its direct application to clinical samples[119,120] (FIG. 2b,c). This labelled assay detects uropathogens based on sandwich hybridization of bacterial 16S rRNA with a capture DNA oligonucleotide as the recognition element and a labelled DNA probe as the detector. Application of unprocessed urine lysate to the sensor surface can yield quantitative detection of uropathogens based on the amperometric readout (a measure of ions in a solution based on the electric current produced). Given the high copy number of 16S rRNA, PCR amplification is not required and direct detection down to 10^3 cfu/ml has been achieved[121]. This sandwich hybridization electrochemical biosensor platform consists of an array of individually addressable sensors that can be used to detect different analytes. Development of a panel of probes enables identification of a wide variety of uropathogens in a 1 hour assay[122]. Validation of this biosensor with patient-derived samples has demonstrated robust uropathogen detection and speciation from single-species and polymicrobial samples. Specifically, for pathogen identification, clinical validation studies using patient urine samples have demonstrated a sensitivity and specificity of 92% and 97%, respectively[120].

The same biosensor platform has been adapted to detect phenotypic antimicrobial susceptibility with genotypic specificity[109]. For a biosensor-based AST, urine samples are mixed with culture medium and incubated with or without antibiotics and the level of 16S rRNA is measured. A lower amperometeric signal from sample cultured in the presence of antibiotics than from the no-antibiotic control indicates susceptibility to the respective antimicrobial. Genotypic detection based on the level of 16S rRNA provides discrimination of uropathogens from potentially contaminating skin flora. Furthermore, owing to the sensitivity of the biosensor assay, AST can be completed after only 3 hours of culture. Using this approach, the biosensor-based AST achieved an overall accuracy of 94% directly from patient samples using standard culture as the gold standard for comparison[109]. A UTI biosensor negates the need for isolation of bacterial species by overnight plating to identify the pathogens and reduces the time for determination of AST from the time of sample collection from >18 hours for standard culture to <4 hours for the biosensor assay.

One of the advantages of the electrochemical biosensor is its adaptability to different assays and functions. For example, the basic biosensor-based AST was adapted and validated with clinical samples to include pathogen identification and AST with determination of MIC for ciprofloxacin[119]. Urine samples positive for *Enterobacteriaceae* ($n = 84$) and culture-negative samples ($n = 23$) were tested using an electrochemical biosensor array for pathogen identification consisting of probes for universal bacterial detection, *Enterobacteriaceae*-pathogen-specific probes, and a common probe for determining ciprofloxacin MIC. Analysis of all probes used for pathogen identification showed an overall sensitivity of 98.5% and specificity of 96.5%. Categorical and essential agreement with clinical microbiology of 97.6% for each factor were achieved for ciprofloxacin MIC[119]. In a variation of this biosensor-based AST, the level of precursor rRNAs (pre-rRNAs), an intermediate state in the formation of mature rRNA and a marker for cell growth, is assessed. In this assay, a decrease in pre-rRNAs indicates reduced growth and sensitivity to antibiotics in the culture media[123].

An electrochemical biosensor-based assay, such as the platform described above, has great potential for integration in point-of-care diagnostics, as this assay can combine genotypic pathogen identification with phenotypic AST for comprehensive diagnosis. Additionally, this platform has the potential to be modified for detection of nonbacterial uropathogens. For example, detection of Schistosomes, a parasitic infection of the urogenital tract that is endemic in East Africa, has been demonstrated[124]. However, further system integration is necessary to integrate the biosensor into a device that is of practical clinical use[125].

Most of the assays for UTI diagnosis have used oligonucleotide recognition and detector elements, but this versatile electrochemical biosensor can also be adapted to detect proteins. For example, the recognition element can be an antibody paired with a labelled detector antibody for detection of proteins. This approach was used for host immune marker testing to measure the degree of pyuria[126]. Capture antibodies against lactoferrin, an iron-binding protein that is secreted by white blood cells as part of the innate immune response, were integrated into a biosensor. A significant positive correlation between biosensor-measured lactoferrin concentration and white blood cell count ($P < 0.001$), or presence of leukocyte esterase ($P < 0.001$) by urinalysis was reported. Bacterial concentration and lactoferrin concentration, white blood cell count, and presence of leukocyte esterase were also

significantly positively correlated (all $P < 0.001$)[126]. Additionally, for both nucleic acid and protein assays, the electrodes used for electrochemical detection can be used to facilitate fluid motion. When current is applied across the electrode, a temperature gradient is formed and the electric field and temperature gradient combine to create a bulk electrical force resulting in fluid motion[127]. This functionality was used to incorporate a 16S rRNA biosensor into a microfluidic prototype that integrates on-chip lysis, electrolytic pumping, electrothermal mixing, and electrochemical detection[128]. Combining pathogen identification with biomarkers of pyuria would provide a key tool for differentiating infection from asymptomatic bacteriuria. This ability would be especially useful for clinical assessment of young children and patients with neurogenic bladder in which symptoms can be difficult to assess[129–131]. Lactoferrin is a good marker of pyuria, but might not be the ideal marker for distinguishing true infection that necessitates antibiotic treatment and further research is necessary to elucidate improved biomarkers.

Considerable advances have been reported regarding growth-based AST in microfluidic devices including microchambers[132–134], microchannels[135,136], and microdroplets[137–142]. In these devices, small populations of bacteria, sometimes down to a single cell, are cultured under different antibiotic conditions and cell growth, even a small number of cell divisions, can be monitored via various methods such as microscopy and fluorescence. Encapsulating a single cell or a small number of cells in nanolitre or picolitre volumes results in a high effective concentration of the bacterium and improved local culture conditions. Furthermore, accumulation of released biochemical products in the small volume shortens the detection time compared with the conventional bulk culture system.

For UTI diagnostics, microfluidic approaches are promising not only as adjuncts for fluid handling in electrochemical biosensor assays[128,143], but also as independent devices that can be coupled with optical detection methods. The potential of droplet microfluidic devices for pathogen detection has been demonstrated[144]. This microfluidic technology facilitates genotyping of single cells by isolating cells in picolitre-sized drops, offering a simple and fully integrated approach for cell isolation, lysis, probe–target binding, and fluorescent detection[144]. In one such droplet microfluidic device, a fluid mixture containing sample and dual-labelled detector PNA beacon to 16S rRNA are combined and single cells in the sample are isolated with the detector probe in picolitre-sized droplets formed by flowing the aqueous solution containing the sample into a micro-channel with a more viscous oil solution. The droplets then flow along the microfluidic channel and are thermally lysed. At this point, the concentration of bacterial 16S rRNA within the droplet is extremely high owing to the small volume, enabling efficient hybridization to the homologous PNA beacon. The PNA beacons are labelled with both a fluorophore and a quencher such that fluorescence is only detectable upon target binding. Thus, fluorescence can be detected in droplets that contain bacteria by confocal fluorescence spectroscopy. The initial experiments described for this type of droplet microfluidic system were successful in the detection of cultured *E. coli*[144]. Droplet microfluidic devices are currently restricted to research laboratories, but have a high potential for translation for urine diagnostics as these systems have fluid handling and detection capacity. One challenge for clinical translation of droplet microfluidic systems is to produce a compact system that is simple for a laboratory technician to operate.

A microfluidic device has been used for confinement of single cells with drugs in nanolitre droplets that flow from the polydimethylsiloxane-based mircrofluidic device into attached Teflon tubing for incubation and imaging[138]. This system is capable of analysing the MIC of cefoxitin for *S. aureus* within 7 h by measuring the fluorescent viability indicator[138] (FIG. 3a). This time frame is comparable with MIC analysis in standard automated systems in use in clinical laboratories for isolated bacteria. However, the droplet system has the potential to be used for direct-from-sample testing, obviating the need for an initial overnight culture. A microfluidic device that integrates droplet generation, incubation, and in-line fluorescent detection on a single chip was developed for AST[145] (FIG. 3b). Measuring the growth of single cells incubated in a small droplet size (20 pl) resulted in a reduction of the turn-around time to 1–2 hours for assessment of antimicrobial susceptibility[145]. Another approach used imaging-based measurement for a single-cell AST in which bacteria were cultured with or without antibiotic in microchannels. Individual uropathogenic *E. coli* cells were confined to bacterium-width micro-channels (0.5–10.0 μm wide) with dielectrophoresis, an electrokinetically driven short-range particle trapping force, applied using an integrated microelectrode[136]. In this system each cell division was observed, enabling rapid identification of antibiotic susceptibility.

Microfluidics has transformative potential for accelerating AST, but adoption of these devices in the clinic is still in its infancy. Implementing microfluidic AST devices in clinical settings requires developments to improve accessibility and automation. Several micro-fluidic designs have been proposed to perform on-chip serial dilutions of drugs by using either parallel channels as a sink-and-source system for gradient generation[146], microvalve-based multiplex channels for mixing[147], or magnetofluidic droplet fusion[142]. On-demand preparation of comprehensive combinations of drug concentrations to simultaneously determine MIC are in development using synchronized droplet generation and mixing[148,149] and interfacing with a multiwell plate compatible that is with conventional sample-handling robots[143]. In 2014, a microfluidic chip was adapted to the 96-well-plate format to enable morphological analysis of single cells under various antimicrobial conditions[132] (FIG. 3c). The system was tested with 189 clinical isolates of several pathogens including *E. coli, K. pneumonia*, and methicillin-resistant *S. aureus*. The result was obtained within 4 hours with 91.5% categorical agreement with the gold-standard broth microdilution test[133].

Most of these emerging technologies have not been rigorously tested in large-scale clinical settings. Indeed, many are not in a format that facilitates use on a large scale in a clinical laboratory and further system integration is needed for clinical adoption. Moreover, different assays and platforms might prove to be optimal for different clinical settings. For example, the improved diagnostic potential of immunological-based lateral flow assays over conventional nitrite and leukocyte esterase dipsticks could be adequate for community clinics. Rapid assays that provide AST without pathogen identification such as the Bacterioscan or UroQuick might be most useful for characterization of rectal flora before TRUS-guided prostate biopsy to provide targeted prophylaxis. Biosensor or microfluidic systems capable of integrated pathogen identification and AST might provide the greatest clinical benefit for complicated UTI.

Conclusions

The need for improved and efficient diagnosis of UTI is considerable. The ability to initiate evidence-based treatment guided by rapid profiling of bacterial pathogens and antimicrobial susceptibility can improve patient care and help stem the rise of multidrug-resistant pathogens. The technologies described in this Review illustrate a number of advances that are currently approved for other applications and can be adapted for direct urine testing or are in development to achieve the goals of improved screening for bacteriuria, decreasing the time to result for microbial identification and AST, or point-of-care testing.

MALDI–TOF, FISH, and multiplex PCR are capable of expediting the identification of uropathogens, but currently remain dependent on initial isolation of bacterial colonies from urine, delaying bacterial identification by at least 12 hours. Adaptation of these technologies for direct-from-urine testing is the best route to expedite uropathogen identification for the future application of these technologies. However, although considerable information can be gleaned from identifying the pathogen responsible for UTI, the rapid determination of antimicrobial susceptibility is perhaps even more important to achieving the goal of appropriate antibiotic therapy. Phenotypic ASTs are best suited for UTI diagnostics, owing to the wide variety of uropathogens and antibiotic-resistance mechanisms. Direct-from-urine analysis can be challenging with many rapid AST technologies, owing to the complexity of the sample and variable bacterial concentration. Biosensors and microfluidics provide great promise for development of new diagnostic tools. Clinical-laboratory-based biosensor systems are likely to be the first step in the next generation of molecular diagnostic technology capable of direct testing from clinical samples. Integration of advanced microfluidic handling systems for various sample preparation steps of molecular diagnostics including pipetting, mixing, and concentrating are key to facilitate direct urine testing for both pathogen identification and AST in a point-of-care device.

To expand these advanced technologies for UTI diagnostics, one must also consider the balance between the time and cost of the diagnostic relative to what information is essential for improved treatment. In the point-of-care setting, a reliable screening method to eliminate negative urine samples coupled with a rapid molecular method to determine if the infection is caused by a member of the *Enterobacteriaceae* family and a limited AST of the most common oral antimicrobials is probably sufficient to direct appropriate therapy. If point-of-care testing is inconclusive, additional in-depth analysis can then be undertaken in a clinical laboratory (FIG. 1).

Substantial improvements in sensitivity and specificity have been achieved, but the commercialization of biosensors for infectious diseases is still in its infancy. One challenging bottleneck for point-of-care device development is translating sample preparation techniques. Most biosensors perform excellently with pristine samples, such as pure bacterial cultures, viral cultures, or purified biomolecules from clinical samples. Unprocessed clinical samples, especially urine, can be quite variable. Differences in salt concentration, pH, and viscosity can interfere with analyte detection. Moreover, the most critical challenge to enable the technology to transfer from laboratories into the clinic remains system integration; hurdles need to be overcome in the integration of detection

mechanisms, microfluidics-based sample preparation strategies, and detection mechanisms into a fully automated, stand-alone platform that is easily operated by the end user, although these modules have been successful in isolation. The likelihood of translating research-grade biosensors from the research laboratories into the clinic will be considerably increased once these issues are addressed.

The greatest effect on public health in implementing these new assays in the clinic will be to reduce the societal burden of multidrug-resistant infection. This goal cannot be achieved by adoption of these assays in isolated clinics, but rather requires systematic change and wide adoption of the new technologies combined with antibiotic stewardship. In order to achieve this goal, the devices brought to market must maintain cost-effectiveness and ease of use. Initially, these advanced diagnostic tools might be targeted for use in patients with complicated UTI, as this population would probably receive the greatest benefit from rapid diagnosis. As new technologies gain acceptance for complicated UTI, the benefits to a wider patient population and public health can be determined. To fully realize the benefits, the many stakeholders in the health-care system including physicians, hospitals, clinical microbiology laboratories, insurance companies, and biotechnology companies must coordinate to facilitate implementation of rapid UTI diagnosis.

The implementation of any rapid diagnostic test should be used as a complement to thorough clinical evaluation. Clinical assessment will enable the care provider to select the appropriate test for the patient. For most patients with symptoms of UTI, a test for bacterial infection is appropriate; however, some populations are susceptible to urinary tract pathogens that are not typically detected by standard culture. For example, a diagnostic assay for catheterized patients might need to include identification of fungi, or, in regions where urinary *Schistosomiasis* is endemic, the diagnostic assay might need to be adapted for the detection of parasitic organisms. Finally, clinical judgment must be used to determine the appropriate course of treatment, especially to avoid overtreatment in the scenario of asymptomatic bacteriuria.

Development and adoption of rapid, simple, accurate tests for UTI would enable treatment based on objective microbiological analysis. As scientific knowledge of host–pathogen interactions increases, the next generation of point-of-care diagnostics could be refined to analyse the host response biomarkers to further assess the appropriate course of treatment. Providing an early, refined course of treatment might not only benefit the patient but result in improved antibiotic stewardship. Advances in UTI diagnostics have the potential to provide precision medicine for this common bacterial infection, giving the right drug, at the right dose, to the right patients, at the right time.

Acknowledgments

Glossary

Antimicrobial susceptibility

Antimicrobial susceptibility refers to phenotypic response of the bacteria in the presence of antimicrobial agents.

Multidrug-resistant pathogens

Bacterial pathogens that have developed resistance to multiple antimicrobials. Common multidrug resistant uropathogens include *Enterobacteriaceae* that produce AmpC β-lactamase, extended-spectrum β-lactamase and carbapenamase.

Antimicrobial resistance

Antimicrobial resistance refers to the inherent or acquired genetic mechanisms by which bacteria withstand antimicrobial agents.

Antimicrobial stewardship

Coordinated interventions to improve the appropriate use of antimicrobials by reducing the administration of unnecessary antimicrobials and promoting the selection of the optimal antimicrobial drug, dose, duration of therapy, and route of administration when needed. The major goals of antimicrobial stewardship include achieving optimal clinical outcomes at the same time minimizing toxicity and adverse events, limiting the selection pressure on bacterial populations that drives the emergence of antimicrobial-resistant strains, and reducing excessive costs related to suboptimal antimicrobial use.

Sample preparation

Multistep assay preparation that includes pipetting (such as reagent transfer and mixing), centrifugation (separation and concentration), and washing.

Lateral flow assays

A single-use, point-of-care diagnostic tool based on liquid transport driven by capillary action without the requirement of external support. The major advantages of these test strips include simplicity, portability, and cost-effectiveness. Examples include urinalysis test strips.

Mass spectrometry

A technique in which charged molecules are created by ionization and their identity determined based on the mass:charge ratio. Matrix-assisted laser desorption ionization–time of flight (MALDI–TOF) mass spectrometry can be used for the identification of large biological molecules enabling its use in pathogen identification. In the current clinical application of MALD–TOF mass spectrometry for pathogen identification, the sample (such as urine) is first cultured to isolate the bacteria and a colony from the culture plate is analysed by MALDI–TOF mass spectrometry.

System integration

Integration of the functional building blocks of microfluidic components including pumps, mixers, concentrators, and valves to create an automated system capable of 'sample-in, answer-out' for the end users. System integration is a major hurdle in translating

microfluidic devices into practical applications. Key factors include throughput, cost, multiplexity, diversity of components, accuracy, and programmability.

Fluorescence *in situ* hybridization (FISH)

A cytogenetic technique that uses fluorescent probes that bind to complementary sequences in target cells (such as bacterial pathogens).

Matrix

Components present in biological samples can affect the detection of the analyte of interest. Urinary constituents that can cause matrix effects in diagnostics include somatic cells, electrolytes, organic molecules, proteins, and crystals. Matrix effects can affect assay sensitivity and reproducibility.

Minimum inhibitory concentration (MIC)

The lowest dose of antimicrobial to which a bacterial strain is sensitive.

Biosensors

A molecular sensing device composed of a recognition element that binds specifically to a target analyte and generates a measurable signal via a transducer. For quantitative detection, the magnitude of the signal is proportional to the analyte concentration.

Microfluidics

A multidisciplinary field based on the manipulation of small amounts of fluids at the micron scale. Microfluidics-based platforms commonly integrate reagent transfer, target isolation, and sample-mixing steps in a multilayered cartridge containing channels, valves, and reagent reservoirs. Such 'lab-on-a-chip' platforms offer the potential advantages of microfluidics including low fluid volumes (reduced reagent use and cost), short assay time, low power consumption, rapid generation of small liquid compartments, and a high degree of parallelization.

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Biographies

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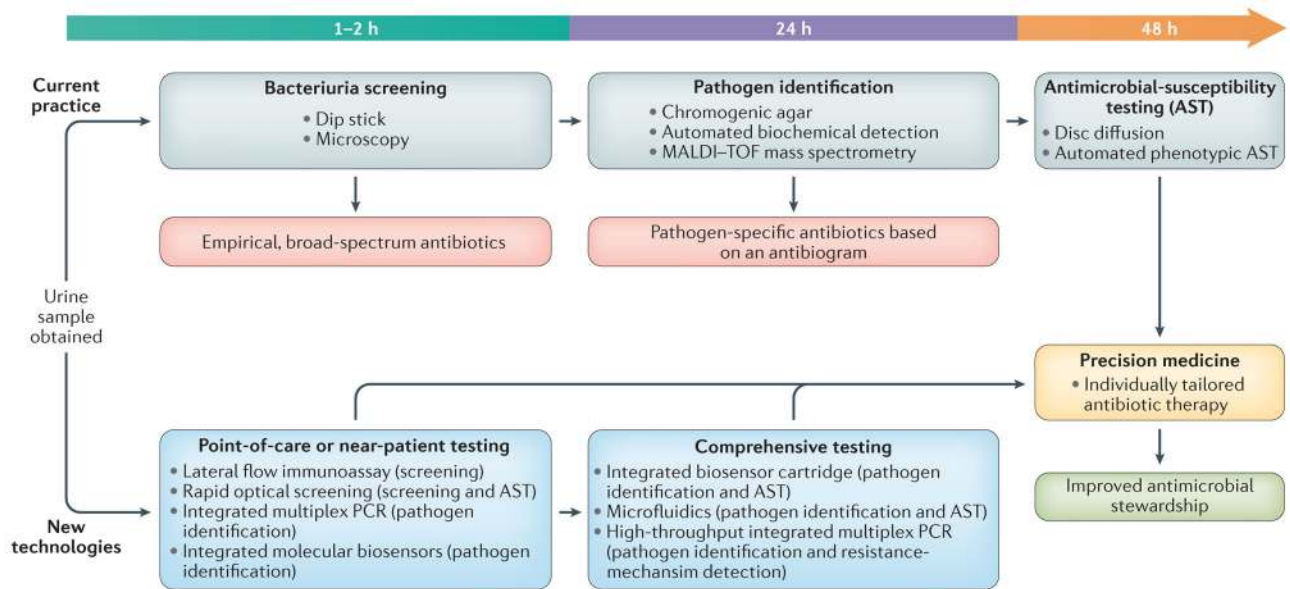
**Figure 1. Overview of the clinical workflow of existing and future diagnostic technologies for UTI**

In current practice (illustrated in the grey boxes) once a urine sample is collected it is transferred to a clinical microbiology laboratory. In the laboratory, sample processing is initiated with a screening assay to assess for the presence of bacteria followed by pathogen identification, and, if positive, antimicrobial-susceptibility testing (AST). Information from each successive assay enables providers to prescribe specific antibiotic therapy. However, truly infection-specific antibiotic treatment cannot be prescribed until results from AST are available — at least 48 hours after sample submission. The new technologies in development have the potential to expedite this process and transform the clinical microbiology workflow (depicted in blue boxes). Urine samples collected in clinic can be analysed at the point of care. In this setting, integrated platforms can determine both pathogen identity and AST enabling precise, infection-specific treatment in a matter of hours from presentation. For complex samples or those collected from clinics without access to point-of-care testing, integrated platforms can provide similarly robust and efficient information in a clinical laboratory. MALDI-TOF, matrix-assisted laser desorption ionization–time of flight.
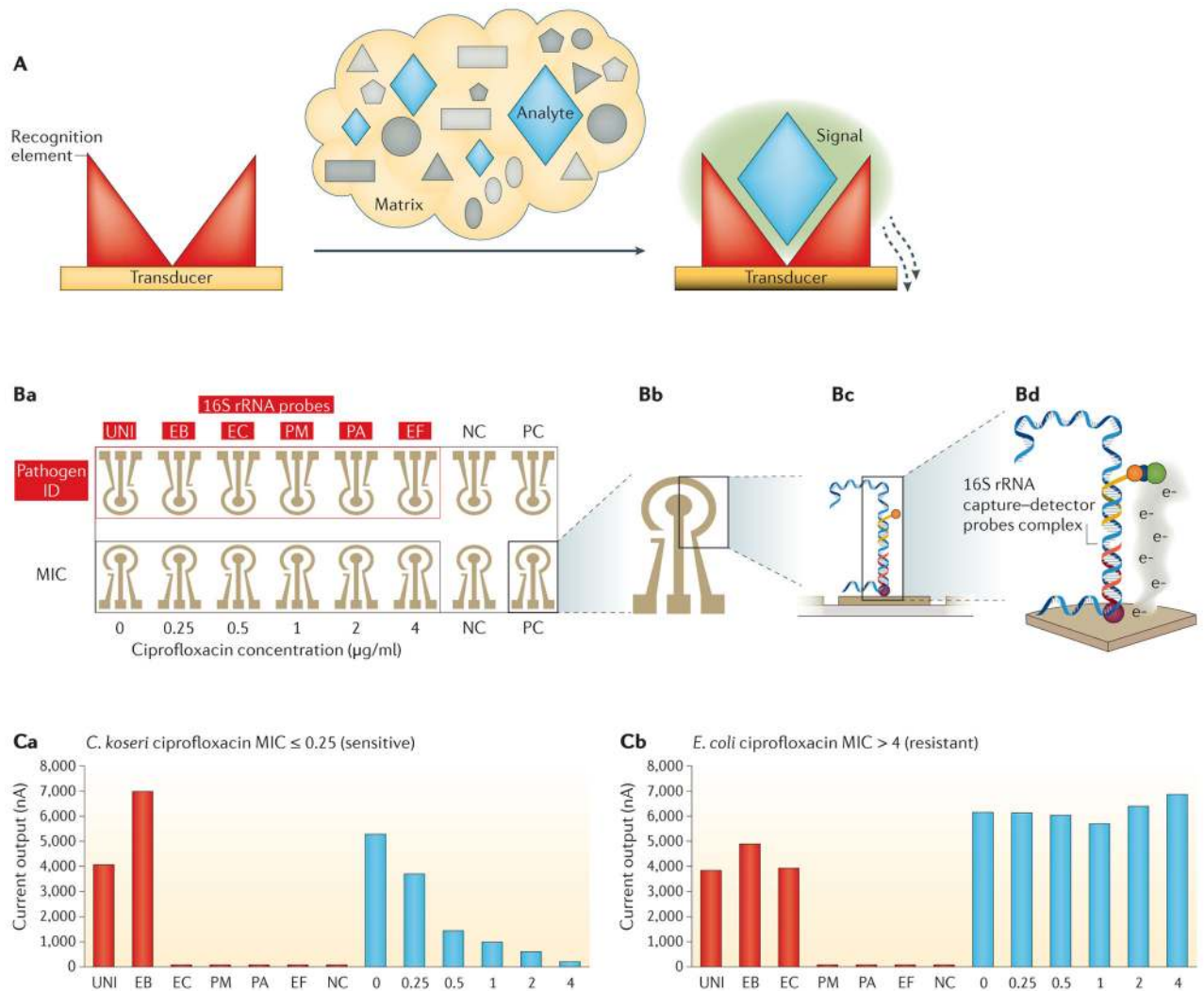
Figure 2. Biosensor-based diagnosis of UTI

A | A biosensor is a molecular sensing device composed of a recognition element and a transducer. Specific binding of the target analyte to the recognition element generates a measurable signal that is detectable via the transducer. The matrix is the biological medium (for example urine or blood) with varying biochemical parameters and nonspecific cells and molecules that could influence the performance of the biosensor. **B** | Biosensor-based molecular diagnosis of UTI with pathogen identification (ID) and antimicrobial-susceptibility testing (AST). **Ba** |The biosensor array consists of 16 sensors functionalized with DNA probes for pathogen ID (top row). Sensors are functionalized with a universal bacterial probe (UNI), an *Enterobacteriaceae* (EB) probe, and probes for *Escherichia coli* (EC), *Proteus mirabilis* (PM), *P. aeruginosa* (PA), and *Enterococcus faecalis* (EF). To determine the phenotypic AST (ciprofloxacin, minimum inhibitory concentration (MIC), the bottom row of sensors were functionalized with an EB probe to measure 16S ribosomal (r)RNA levels after culture in the presence of increasing ciprofloxacin concentrations. **Bb** | Each sensor is composed of a central working electrode and peripheral reference and auxiliary electrodes. **Bc** | Sandwich hybridization between capture and detector probes with

target rRNA binding is facilitated by electrokinetic heating and mixing to improve hybridization stringency. **Bd** | An electrochemical signal is generated and measured. **C** | Representative results for integrated biosensor pathogen identification (red bars) and ciprofloxacin MIC (blue bars) in clinical urine samples. **Ca** | The sample was positive for *Citrobacter koseri*, an *Enterobacteriacea*. Consistent with clinical microbiology results, the biosensor revealed a ciprofloxacin MIC of 0.5 mg/ml, whereby the signal decreased with increasing ciprofloxacin concentration. **Cb** | The sample was positive for *E. coli* and demonstrated resistance to ciprofloxacin, with no reduced signals measured by the MIC sensors, consistent with clinical microbiology results. NC, negative control; PC, positive control. Permission for part **A** obtained from Elsevier © Mach, K. E. *et al. Trends Pharmacol. Sci.* **32**, 330–336 (2016). Permission for parts **B** and **C** obtained from Elsevier © Altobelli, E. *et al. Eur. Urol. Focus* (2016).
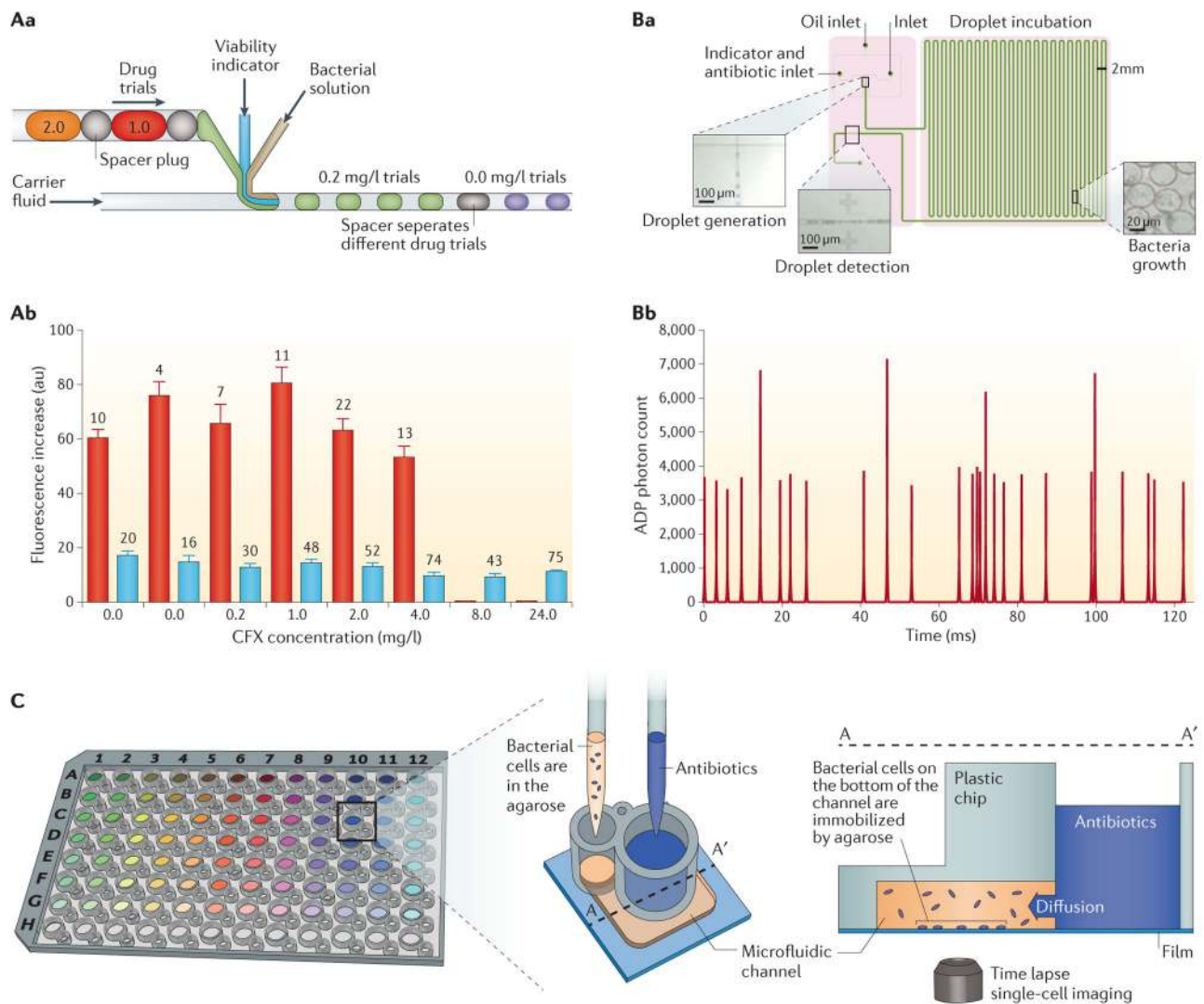
Figure 3. Single-cell analysis of antimicrobial susceptibility

A | A microfluidic plug-based, single-cell antimicrobial-susceptibility test (AST).The top panel shows a flow-focusing design that is used for the formation of 50 nl-sized plugs of bacteria, a viability indicator, and an antibiotic at varying concentrations. The bottom graph shows the average change in fluorescence intensity of threefold greater than (solid) or less than (striped) the base line. A MIC of cefoxitin (CFX) to methicillin-sensitive *Staphylococcus aureus* (MSSA) of 8.0 mg/l was determined. *n* indicates the number of plugs for each condition. **B** | A microfluidic chip used for single-cell AST. **Ba** | A microfluidic device that comprises a flow-focusing design for generating 5pl-sized droplets of bacteria, a viability indicator, and an antibiotic, an elongated serpentine channel for incubation and a restricted channel region for in-line fluorescent detection. **Bb** | High-throughput, in-line detection of droplets. The fluorescence intensity of each droplet is quantified to determine the cellular vitality. **C** | The microfluidic agarose channel (MAC) chip integrated with a 96-well-plate platform for high-throughput analysis. The MAC chip is composed of microfluidic channels containing bacteria in agarose, and a well to supply antibiotics and

nutrients. The imaging region was the interface between the liquid medium and the microfluid. ADP; avalanche photo diode. Permission for part **A** obtained from Royal Society of Chemistry © Boedicker, J. Q. *et al. Lab Chip* **8**, 1265–1272 (2008). Part **B** reproduced with permission from Chemical and Biological Microsystems Society, Kaushik A. *et al.* 19th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2015) (2015). Permission for part **C** obtained from The American Association for the Advancement of Science © Choi, J. *et al. Sci. Transl Med.* **6**, 267ra174 (2014).

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Table 1

Approved technologies for pathogen detection

Technology	Commercial assay	AST	Advantages	Disadvantages	Refs
Nitrite and leukocyte esterase	Dipstick (lateral flow assay)	No	Point of care	Poor specificity	29
Conventional culture	• VITEK • MicroScan	Yes	Standard of care, sensitive, and inexpensive	Time consuming, not translatable to point-of-care applications	25,26
Urinalysis and microscopy	• sediMAx • CLINITEK Atlas • Sysmex UF-1000i • Iris iQ200	No	Fast, detects the presence of bacteria	No pathogen identification	32–34
MALDI-TOF mass spectrometry	• VITEK MS • Bruker MALDI-TOF	Under development	Fast, sensitive, specific, potential for simultaneous AST detection	Expensive for initial equipment	49–54
Fluorescent *in situ* hybridization (FISH)	AdvanDx *QuickFISH*	Under development	Rapid detection, high sensitivity and specificity	Requires multiple probes for all possible urinary pathogens	61–63
Microfluidics	UTI Biosensor Assay (not FDA approved)	Under development	Integrated platform, rapid detection direct-from-patient samples, small footprint	System is not fully automated, poor data from low concentration of bacteria	82,83, 94,95
PCR (clinical isolates)	• GeneXpert • SeptiFast • FilmArray	Resistance-gene probes available	Specific, sensitive, and rapid	Requires multiple probes for all possible urinary pathogens and extensive initial processing	68–73
Immunological-based assays	RapidBac	No	Rapid and inexpensive	Poor specificity and sensitivity	31
Forward light scattering	• Uro-Quick • BacterioScan	Under development	Inexpensive potential for AST	No species indentification	40,41

AST, antimicrobial susceptibility testing; MALDI-TOF, matrix-assisted laser desorption ionization–time of flight; MS, mass spectrometry.