

CRISPR-Based Rapid Diagnostics for Urinary Tract Infections: Detection of Uropathogens and Antimicrobial Resistance

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Abstract- Urinary tract infections (UTIs) are among the most common bacterial infections and contribute substantially to antimicrobial use in community and hospital settings. Conventional diagnosis relies on clinical assessment, urinalysis and urine culture, with culture-based identification and antimicrobial susceptibility testing often requiring 24–72 hours. This delay can encourage empirical antibiotic prescribing and may be particularly problematic when infections are caused by resistant or less commonly detected organisms. Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins have emerged as programmable nucleic-acid recognition tools that can be coupled with amplification methods and simple signal readouts. Cas12-based systems detect target DNA and can generate collateral cleavage of reporter molecules, whereas Cas13-based systems recognise RNA targets and can provide an alternative route for rapid detection. In UTI applications, these platforms may support identification of common uropathogens, including *Escherichia coli*, and detection of selected antimicrobial resistance determinants. Early studies have demonstrated rapid genotypic resistance testing and urine-to-result workflows, while newer approaches are exploring direct pathogen identification and susceptibility assessment from urine. However, CRISPR assays do not automatically replace culture: detection of a resistance gene does not always predict the full resistance phenotype, and absence of a targeted gene does not prove susceptibility. Sample preparation, target coverage, mixed infections, contamination control, clinical validation, cost and regulatory requirements remain important challenges. This review summarises the biological basis of CRISPR diagnostics, current approaches for uropathogen and resistance-marker detection, potential clinical applications, limitations and priorities for translation into routine diagnostic services.

Keywords— CRISPR-Cas; urinary tract infection; uropathogens; antimicrobial resistance; Cas12a; Cas13; point-of-care diagnostics; antimicrobial susceptibility testing

I. INTRODUCTION

Urinary tract infections are infections involving the urethra, bladder, ureters or kidneys. Their clinical presentation ranges from uncomplicated cystitis to complicated infection, pyelonephritis and urosepsis. *Escherichia coli* is a leading cause of community-acquired UTI; other important organisms include *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, *Staphylococcus saprophyticus* and, in selected clinical settings, *Pseudomonas aeruginosa*. The organisms responsible and their resistance patterns vary with age, sex, pregnancy, urinary tract abnormalities, prior antibiotic exposure, healthcare contact and local epidemiology. [4]

Diagnosis is usually based on symptoms and clinical context, supported by urine dipstick testing, microscopy and culture. Culture remains important because it can recover viable organisms and provide phenotypic antimicrobial susceptibility results. However, the process takes time, and standard culture can miss low-abundance, slow-growing or fastidious organisms. Molecular tests can shorten identification time but may require specialised instruments and may detect nucleic acid from organisms that are not viable. Rapid tests are therefore valuable only when their results are interpreted alongside symptoms, specimen quality and clinical risk. [4]

CRISPR-based diagnostics use a programmable guide RNA to direct a Cas protein towards a specific nucleic-acid sequence. Target recognition can be converted into a measurable fluorescent, colourimetric or lateral-flow signal. The sequence-specific nature of this approach makes it attractive for multiplex detection of pathogens and selected resistance markers. This review discusses the technology as a developing diagnostic option rather than an established replacement for culture. [2,3,5]

II. REVIEW APPROACH AND SCOPE

This is a narrative review of foundational and recent literature on CRISPR-Cas diagnostics, UTI testing and antimicrobial resistance detection. The discussion draws on published studies and reviews identified through biomedical literature and publisher records, with emphasis on urine-based assays and platforms relevant to rapid clinical testing. It is not a systematic review or meta-analysis: no exhaustive search, duplicate screening, formal risk-of-bias assessment or pooled estimate was performed. Before journal submission, authors should update the search, document databases and search dates, and apply the target journal's reference style.

III. PRINCIPLES OF CRISPR-BASED MOLECULAR DIAGNOSTICS

1. CRISPR-Cas12 systems

Cas12 enzymes, including Cas12a, can be programmed with a CRISPR RNA (crRNA) to recognise a complementary DNA target. Following target recognition, activated Cas12 can cleave nearby single-stranded DNA reporter molecules. A reporter carrying a fluorophore and quencher separates after cleavage, producing a fluorescence signal; alternative reporters can support visual or lateral-flow readouts. Many assays combine Cas12 detection with nucleic-acid amplification, such as recombinase polymerase amplification (RPA) or PCR, to improve analytical sensitivity. Amplification can introduce extra steps and contamination risk, so closed-tube designs and careful workflow separation are important. [1,3]

2. CRISPR-Cas13 systems

Cas13 proteins are RNA-guided enzymes that recognise RNA targets. Target recognition can activate collateral cleavage of reporter RNA, enabling signal generation. RNA-targeting assays may detect abundant bacterial RNA, including ribosomal RNA, and can be configured with or without an upstream amplification step depending on the target concentration and assay design. An amplification-free Cas13 platform has been investigated for rapid bacterial RNA detection in urine, with early patient-sample evaluation; such findings are promising but require larger, independent clinical studies before performance can be generalised. [3,6]

3. From sample to result

A typical workflow includes collection of a clean-catch urine specimen, concentration or lysis of microbial material when required, nucleic-acid release, optional amplification, CRISPR target recognition and signal interpretation. The output may be read by a fluorescence reader, a portable optical device or a lateral-flow strip. Assay design must account for urine inhibitors, bacterial load, sample volume, target sequence variation and the risk of carry-over contamination. The total turnaround time should include sample preparation and interpretation—not only the CRISPR reaction. [3,5,6]

IV. CRISPR-BASED DETECTION OF UROPATHOGENS

Species identification requires targets that are sufficiently conserved within the intended organism and sufficiently different from related organisms. Candidate targets may include species-specific genomic regions or carefully selected ribosomal sequences. A single-target assay can be simple and inexpensive, while a multiplex assay can detect several organisms in parallel. Multiplexing is clinically attractive because UTIs can involve organisms other than *E. coli*, and complicated infections may be polymicrobial. Nevertheless, multiplex panels are limited to the organisms included in their design; a negative result cannot exclude an organism outside the panel.

The 2025 report of a rapid, amplification-free Cas13a-based RNA platform described a urine-to-result workflow within approximately one hour and explored concordance with patient-derived urine in a small validation set. A separate 2025 study described an asymmetric PCR–Cas12a platform for bacterial identification and susceptibility testing directly from urine, reporting species-level detection of six common uropathogens at a stated analytical concentration of 10^3 CFU/mL. These studies illustrate the pace of development, but their specific performance values should not be treated as universal characteristics of CRISPR assays: they depend on the assay, targets, specimen type and validation cohort. [6]

V. DETECTION OF ANTIMICROBIAL RESISTANCE

Antimicrobial resistance (AMR) can arise through acquired resistance genes, mutations in chromosomal targets, altered permeability, efflux pumps, enzymatic drug inactivation or combinations of mechanisms. A CRISPR assay can be designed to detect a selected resistance gene or mutation, potentially providing actionable information before conventional susceptibility testing is complete. Examples of targets that may be relevant to uropathogens include blaCTX-M-family genes associated with extended-spectrum beta-lactamase production, carbapenemase genes such as blaNDM, blaKPC and blaOXA-48-like, and selected quinolone-resistance mutations. The appropriate panel must be chosen according to local resistance surveillance and the intended clinical use. [2,5]

A key distinction is that genotypic resistance detection is not identical to phenotypic antimicrobial susceptibility testing (AST). A positive gene result

indicates that a targeted determinant is present; it does not necessarily establish its expression or the organism's complete susceptibility profile. Conversely, a negative result only means that the targeted sequence was not detected. Resistance may be caused by genes or mutations outside the panel. For this reason, CRISPR-based AMR testing should initially complement, rather than universally replace, culture-based AST. [1,2,4]

Chen and colleagues reported a CRISPR-Cas12a-based genotypic AST approach for UTI that combined nucleic-acid amplification with rapid detection and demonstrated the potential to deliver results within about one hour under the study conditions. More recent work has explored integrating bacterial identification with phenotypic response measurements. Such hybrid approaches are important because they may move beyond detecting known genes towards assessing whether bacteria respond to particular antibiotics. [1]

VI. COMPARISON WITH CONVENTIONAL AND MOLECULAR METHODS

No single diagnostic method answers every clinical question. Urinalysis can provide a rapid initial indication but does not reliably identify the organism or its full resistance profile. Culture provides viable isolates and enables phenotypic AST, but turnaround is slower. PCR and other nucleic-acid amplification tests can identify targeted organisms or genes rapidly, although instrumentation and panel limitations remain. CRISPR systems may add sequence-specific recognition and flexible signal generation, but their performance is highly assay-dependent and clinical validation is essential. [4,5]

Table 1. Comparison of selected diagnostic approaches for urinary tract infection. [4,5]

Method	Typical time	Main output	Strength	Main limitation
Urinalysis/dipstick	Minutes	Inflammation-related indicators	Fast and widely available	Cannot reliably identify pathogen or resistance
Urine culture + AST	Usually 24–72 h or longer	Viable organism and phenotype	Established clinical reference approach	Slow; some organisms may be missed

PCR/multiplex NAAT	Hours, assay- dependent	Targeted organisms/genes	Sensitive and relatively rapid	Equipment and panel coverage; gene ≠ phenotype
CRISPR- Cas12/Cas13	Potentially under 1–2 h in research workflows	Targeted DNA/RNA; selected AMR markers	Programmable recognition and portable readout potential	Validation, sample preparation and target-panel limitations

VII. POTENTIAL CLINICAL APPLICATIONS

A rapid test that identifies a likely uropathogen and selected resistance determinants could help clinicians review empirical antibiotic choices earlier, particularly in complicated UTI, recurrent infection, prior treatment failure or suspected healthcare-associated infection. In settings where access to a full microbiology laboratory is limited, a robust portable test could support triage and referral. A CRISPR result may also contribute to antimicrobial stewardship by helping avoid unnecessarily broad therapy when interpreted in the context of clinical findings and local guidelines. [4,6,7]

These benefits remain conditional. A diagnostic result must be available early enough to change management, and the test must demonstrate reliable performance in the intended population. A test that detects colonisation, contamination or non-viable organisms may encourage overtreatment if interpreted without symptoms and specimen quality. Clinical pathways should specify how discordant results are handled and when confirmatory culture is required.

Challenges and limitations

Analytical and biological challenges

Urine varies in pH, salt concentration, cellular content and inhibitor burden. Low bacterial load, mixed infections and sequence variation can affect assay sensitivity or specificity. A single mutation may be difficult to discriminate if the guide RNA tolerates mismatches, while a narrow target panel may miss emerging resistance mechanisms. Internal extraction and amplification controls are needed to distinguish a true negative from assay failure. [3,5,6]

Clinical validation and interpretation

Studies should include prospective, consecutive patient recruitment; adequate numbers of positive and negative specimens; representative age and sex groups; complicated and uncomplicated infections; and a realistic range of bacterial loads. Performance should be reported with sensitivity, specificity, predictive values and confidence intervals, using appropriate reference standards. For resistance testing, comparison against standard phenotypic AST and molecular characterisation is important. Independent, multicentre validation is needed before results are used to guide treatment without confirmation. [4,5]

Practical, economic and regulatory barriers

Routine adoption depends on reagent stability, instrument cost, batch size, staff training, quality control, biosafety, waste disposal and supply-chain reliability. Assays must be assessed for cost per actionable result rather than reagent cost alone. Regulatory approval and laboratory quality systems will require evidence of analytical performance, clinical validity and clinical utility. Local resistance patterns also mean that a panel developed in one region may not be directly transferable to another. [3,5]

Future perspectives

Future development should prioritise closed-tube workflows, simplified extraction, lyophilised reagents, portable readers and multiplex panels that reflect local uropathogen and resistance profiles. Combining CRISPR recognition with microfluidics may reduce sample and reagent volumes, while automated analysis could improve consistency. A particularly important direction is the integration of genotypic detection with rapid phenotypic AST,

because treatment decisions depend on bacterial response to the actual antibiotic as well as the presence of known resistance markers. [3,5,7]

Researchers should conduct prospective studies that compare CRISPR testing with culture and standard AST, measure whether results change prescribing, and evaluate patient outcomes, antibiotic use and cost-effectiveness. Validation should include diverse clinical sites and populations, as well as negative and discordant cases. Until such evidence is available, CRISPR-based UTI diagnostics are best viewed as promising complementary technologies with potential to shorten the time to targeted treatment.

Chart 1. Key attributes to assess when comparing rapid UTI diagnostic platforms

Suggested chart for the final manuscript: compare turnaround time, organism coverage, resistance-marker coverage, need for amplification, instrument requirements, clinical validation and cost per test. Populate the chart only with values extracted from each cited study; do not treat research-specific times or limits of detection as universal platform specifications.

Figure 1. Proposed workflow for CRISPR-based rapid diagnosis of UTI

Urine collection → sample concentration/lysis → nucleic-acid release → optional PCR/RPA amplification → guide-RNA-directed Cas12/Cas13 target recognition → fluorescent, colourimetric or lateral-flow signal → interpretation alongside symptoms and confirmatory culture/AST when indicated.

Figure note: This is a conceptual workflow, not a validated clinical protocol. The exact steps and turnaround time depend on the assay design. [3,5,6]

VIII. CONCLUSION

CRISPR-Cas12 and CRISPR-Cas13 platforms offer a flexible approach to rapid, sequence-specific detection of uropathogens and selected antimicrobial resistance determinants. Early urine-based studies demonstrate the potential for results within approximately one hour to a few hours, which could help narrow the gap between initial assessment and targeted treatment. However,

diagnostic speed alone is insufficient: reliable sample processing, broad and locally relevant target coverage, clinically meaningful interpretation and independent validation are essential. Because a resistance gene result does not fully substitute for phenotypic susceptibility testing, CRISPR assays should currently be integrated with clinical assessment and established microbiology pathways. Carefully designed multicentre studies will determine whether these technologies can improve antibiotic stewardship, access and outcomes in routine UTI care. [1,3,6,7]

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Conflict of Interest

The authors should disclose any financial or non-financial competing interests. If none exist, the corresponding author may state: "The authors declare no conflict of interest." Confirm this statement with all co-authors before submission.

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