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Review Article

CRISPR-Based Point-of-Care Diagnostics for Antimicrobial Resistance: From Molecular Precision to Clinically Deployable Bioengineering Systems

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Abstract

The issue of antimicrobial resistance (AMR) has become a defining feature of global health, driven not only by microbial evolution but also by persistent delays in delivering actionable diagnostic information to clinicians. Although molecular technologies have significantly enhanced pathogen characterization, their clinical utility remains constrained by centralized infrastructure, lengthy turnaround times, and limited accessibility in resource-restricted settings. Based on programmable guide RNAs and the collateral cleavage activity of Cas enzymes, CRISPR-based nucleic-acid diagnostics have rapidly evolved into point-of-care (POC) testing applications. However, analytical sensitivity is no longer the primary barrier to clinical translation; instead, challenges related to engineering robustness in complex biological matrices, multiplex resistance detection, workflow automation, reagent stability, and clinical performance have become dominant. This Perspective re-evaluates CRISPR-based AMR testing as an integrated bioengineering system rather than a single molecular assay. We trace key translational bottlenecks and propose system-level benchmarks required to transition CRISPR diagnostics from experimental platforms into clinically deployable tools supporting antimicrobial stewardship and surveillance programs.

Antimicrobial resistance as a diagnostic system's failure

Antimicrobial resistance is frequently described as a microbiological crisis; however, its clinical consequences are largely driven by delayed access to diagnostic information. Traditional culture-based antimicrobial susceptibility testing typically requires 48–96 hours, during which empirical broad-spectrum antibiotics are commonly administered. However, although molecular diagnostics can shorten this timeline, most platforms remain centralized, technically complex, and poorly integrated into frontline clinical workflows.

Consequently, AMR has increasingly become a time-to-information problem rather than a lack-of-information problem. The need for rapid, decentralized resistance detection has therefore stimulated interest in point-of-care diagnostic systems capable of delivering actionable results within the first clinical decision window. CRISPR-based diagnostics have emerged as strong contenders in this space, with early studies demonstrating robust molecular specificity and analytical feasibility in infectious disease applications [1,2].

CRISPR diagnostics: From gene editing to molecular sensing

The discovery of collateral cleavage activity in Cas12a and Cas13 enzymes transformed CRISPR systems from genome-editing tools into highly programmable molecular sensors. Precise recognition of resistance determinants is enabled

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by guide RNAs, while collateral reporter cleavage generates detectable optical or colorimetric signals under isothermal conditions.

In contrast to probe-based PCR assays, CRISPR diagnostics combine molecular flexibility, portability, and reduced instrumentation requirements. These characteristics make them particularly attractive for decentralized AMR detection. However, laboratory feasibility alone does not equate to clinical deployability. Translation into practice requires the coordinated integration of molecular chemistry, device engineering, workflow design, and result interpretation frameworks.

Early clinical demonstrations of CRISPR-based AMR detection

Multiple studies have demonstrated CRISPR-based detection of clinically significant resistance determinants. Cas13-based assays have shown high analytical specificity for carbapenemase genes, including OXA-48 and GES, with reported sensitivities exceeding 95% and limits of detection in the 10–100 copies per reaction range [3]. Using Cas12a systems combined with isothermal amplification, *bla*kpc and *bla*ndm genes have been visually detected in urine and bacterial samples within 30–60 minutes, with performance comparable to established molecular diagnostics [4,5].

These studies confirm that CRISPR assays can achieve clinically relevant sensitivity and specificity. However, they also highlight variability in workflow complexity, sample preparation requirements, and operator dependence, factors that ultimately determine feasibility at the point of care.

Sample matrix complexity and inhibitor tolerance

One of the most significant translational barriers lies in biological specimen complexity. Blood, urine, sputum, and swab samples contain enzymatic inhibitors, variable nucleic-acid concentrations, and heterogeneous microbial loads. Assays optimized in buffered laboratory conditions often perform inconsistently when applied to clinical matrices.

Recent advances in Cas12a signal-amplification chemistries have demonstrated improved tolerance to inhibitory environments [6]. Nevertheless, sample preparation remains a leading cause of diagnostic failure. Diagnostic reliability is therefore less dependent on maximal enzymatic sensitivity and more on workflow resilience in the face of biological variability, a challenge that is fundamentally bioengineering-driven rather than molecular.

Multiplex detection as a core engineering requirement

AMR is inherently multigenic. Resistance may arise from *bla*kpc, *bla*ndm, *bla*oxa-48-like, or *bla*ges genes, which often coexist within a single isolate. Single-target diagnostics therefore risk incomplete resistance characterization, potentially leading to suboptimal antimicrobial selection.

Recent developments in split-crRNA architectures have enabled multiplex CRISPR detection using programmable molecular logic while preserving specificity [7]. Complementary approaches employing spatially separated CRISPR microarrays further enhance multiplex scalability and enable smartphone-based signal acquisition [8]. These advances reposition CRISPR diagnostics as information-processing systems rather than isolated detection reactions, aligning them with the computational orientation of modern bioengineering.

Amplification-free and one-pot detection strategies

Workflow simplification is central to successful point-of-care implementation. Amplification-free CRISPR strategies aim to eliminate pre-amplification steps, thereby reducing contamination risk and manual handling. Under optimized conditions, DSAC-Cas12a and localized reaction architectures have demonstrated rapid nucleic-acid detection without amplification [9].

More recently, one-step detection systems incorporating OR-gated molecular logic circuits have been described [10]. Combined one-pot strategies emphasize reaction compatibility, enzyme kinetics, and buffer harmonization to enable deployable formats [11]. However, amplification-free approaches must be clinically contextualized, as low pathogen burdens may still necessitate amplification to maintain diagnostic sensitivity.

Reagent stability and manufacturability

Cold-chain dependence and batch variability remain significant barriers to widespread adoption. Freeze-dried CRISPR reagents capable of long-term storage at ambient temperatures represent a critical advance for deployment in low-resource settings [12]. Beyond stability, manufacturability requires standardized enzyme production, quality-control frameworks, and reproducible operating conditions. Without these elements, CRISPR diagnostics risk remaining research tools rather than regulated clinical devices.

Clinical utility and stewardship integration

Most CRISPR-based AMR studies emphasize analytical performance metrics. However, clinical value is ultimately defined by impact on patient management. Key outcomes include reduced time to effective therapy, decreased inappropriate antibiotic exposure, and faster infection-control responses.

Future validation studies should therefore move beyond contrived samples toward pragmatic evaluations embedded within real clinical workflows. Long-term adoption will depend on integration with antimicrobial stewardship programs and resistance surveillance networks.

Perspective and outlook

CRISPR-based AMR diagnostics have reached a technological inflexion point. Molecular detection of resistance genes is well established; the remaining challenge lies in

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translating biological precision into reproducible, interpretable, and scalable diagnostic systems. Progress will depend on standardized workflows, multiplex intelligence, stable reagent formulations, and clinically meaningful performance benchmarks. When approached as bioengineering systems, CRISPR diagnostics have the potential to form a cornerstone of the global response to antimicrobial resistance.

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