Emerging CRISPR-Cas Platforms for Rapid and Field-Deployable Detection of Multidrug-Resistant Microbes

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***Abstract*—The emergence and global spread of multidrug-resistant (MDR) microbes present a critical challenge to infectious disease management, necessitating rapid, accurate, and decentralized diagnostic solutions. Traditional microbiological methods remain constrained by prolonged turnaround times, infrastructure dependence, and suboptimal sensitivity. CRISPR-Cas systems, originally discovered as bacterial immune mechanisms, have since been repurposed into next-generation diagnostic platforms with remarkable specificity and versatility. This review explores the diagnostic capabilities of emerging CRISPR-based technologies such as SHERLOCK, DETECTR, and Cas12/13/14 systems, with a focus on their application in MDR pathogen detection. Particular attention is given to the ability of these platforms to target resistance genes in clinical and environmental samples, often without nucleic acid amplification. The integration of CRISPR diagnostics with isothermal amplification, microfluidic chips, and smartphone-compatible formats is also examined for field-deployable use. Current challenges including off-target effects, assay standardization, and regulatory hurdles are discussed alongside prospective solutions for broad clinical implementation against the rising AMR threat.**

***Index Terms*—CRISPR-Cas systems, multidrug-resistant microbes, antimicrobial resistance, molecular diagnostics.**

I. INTRODUCTION

The alarming rise in multidrug-resistant (MDR) microbial infections has become a major global health concern, threatening the effectiveness of current antimicrobial therapies and increasing the burden on healthcare systems worldwide. According to the World Health Organization (WHO), antimicrobial resistance (AMR) is one of the top ten global public health threats facing humanity, with projections estimating up to 10 million deaths annually by 2050 if urgent action is not taken [1]. The ability of bacteria and other microbes to acquire and disseminate resistance genes—either through chromosomal mutations or horizontal gene transfer—has led to the emergence of strains that are resistant to multiple classes of antibiotics, including last-resort drugs such as carbapenems and colistin [2].

Early and accurate detection of MDR pathogens is critical for initiating appropriate treatment, preventing the spread of resistance, and guiding antimicrobial stewardship. Conventional diagnostic methods, including culture-based techniques and polymerase chain reaction (PCR), though effective, are time-consuming, often requiring centralized laboratory infrastructure, trained personnel, and complex sample processing [3]. These limitations are particularly challenging in low-resource and outbreak-prone settings, where delays in diagnosis can lead to poor clinical outcomes and uncontrolled transmission of resistant strains.

In recent years, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and their associated Cas proteins have emerged as powerful tools beyond genome editing, offering new opportunities in nucleic acid-based diagnostics. Unlike traditional molecular tools, CRISPR-Cas systems possess inherent specificity due to their programmable RNA-guided cleavage activity, allowing them to detect DNA or RNA sequences with high precision. The development of CRISPR-based diagnostic platforms such as SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing), DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter), and others has demonstrated rapid, portable, and sensitive detection of pathogens and resistance genes, often without the need for extensive amplification or sophisticated equipment [4,5].

These platforms utilize various Cas effectors—such as Cas12, Cas13, and Cas14—which, upon binding to their target sequences, exhibit collateral cleavage activity that can be harnessed for signal generation. When integrated with isothermal amplification techniques (e.g., RPA or LAMP), paper-based lateral flow devices, and smartphone-based readouts, CRISPR-Cas diagnostics can be deployed in field settings, enabling real-time, on-site surveillance of AMR hotspots [6]. Moreover, their modularity and programmability make them easily adaptable for multiplexed detection, a feature particularly valuable for identifying co-infections or multiple resistance determinants in a single assay. Despite their promise, challenges such as sample preparation, off-target activity, and regulatory validation remain to be addressed before widespread clinical implementation can occur. Nonetheless, CRISPR-based diagnostics represent a paradigm shift in the fight against MDR infections, offering speed, precision, and accessibility that could significantly transform current diagnostic landscapes.

This review explores the state-of-the-art developments in CRISPR-Cas-based diagnostic technologies for MDR microbes, emphasizing their mechanisms, applications, advantages, limitations, and future prospects in clinical and point-of-care settings.

II. OVERVIEW OF MULTIDRUG-RESISTANT MICROBES

Multidrug-resistant (MDR) microorganisms pose a serious challenge to public health due to their ability to withstand treatment with multiple classes of antibiotics. The development of drug resistance in microbes is a multifactorial process involving both genetic and biochemical mechanisms. One of the primary mechanisms is the overexpression of efflux pumps, which actively transport antibiotics out of the bacterial cell, thereby reducing intracellular drug concentrations. These pumps, particularly the AcrAB-TolC system in *Escherichia coli* and the AdeABC system in *Acinetobacterbaumannii*, can confer resistance to a broad range of antibiotics including fluoroquinolones, β-lactams, and tetracyclines [7,8]. Another significant mechanism involves enzymatic inactivation of antibiotics. For instance, extended-spectrum β-lactamases (ESBLs) and carbapenemases such as NDM-1, KPC, and OXA-type enzymes hydrolyze β-lactam antibiotics, rendering them ineffective against pathogens like *Klebsiellapneumoniae* and *Enterobacter spp.* [9,10]. Additionally, target site modification through mutation or methylation impairs antibiotic binding, as observed in fluoroquinolone resistance due to mutations in gyrA and parC genes, or in macrolide resistance via 23S rRNA methylation [11].

Porin loss or modification also contributes to resistance by decreasing drug permeability. This is particularly relevant in Gram-negative organisms such as *Pseudomonas aeruginosa*, where downregulation of OprDporin reduces susceptibility to carbapenems [12]. Furthermore, horizontal gene transfer via plasmids, transposons, and integrons plays a pivotal role in the rapid dissemination of resistance genes among bacterial populations, especially in hospital environments where antibiotic pressure is high [13]. Clinically significant MDR pathogens collectively known as ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiellapneumoniae, Acinetobacterbaumannii, Pseudomonas aeruginosa,* and *Enterobacter spp.*) are notorious for causing hospital-acquired infections that are difficult to treat with conventional therapies [14].

Given the complexity and diversity of resistance mechanisms, rapid and precise diagnostic tools are essential to guide effective treatment decisions and contain the spread of MDR infections. Traditional culture-based techniques are time-consuming, often delaying critical therapeutic interventions. Hence, the integration of novel molecular approaches—such as CRISPR-based diagnostics—is urgently needed to enable point-of-care identification of resistance genes and accelerate clinical decision-making [15].

III. MOLECULAR MECHANISM OF CRISPR-CASON MDR BACTERIA

CRISPR-Cas systems, originally characterized as an adaptive immune defense in bacteria and archaea, have evolved into powerful molecular tools for nucleic acid detection due to their high specificity and programmability. The core mechanism involves CRISPR-derived RNA (crRNA) guiding Cas effector proteins to complementary nucleic acid targets, enabling sequence-specific cleavage. In the context of

diagnostic applications, Cas enzymes such as Cas9, Cas12, Cas13, and Cas14 have been repurposed to detect genetic signatures of multidrug resistance (MDR) in pathogens. For instance, Cas12 and Cas13 exhibit a unique collateral cleavage activity—upon recognition of their specific DNA or RNA targets, respectively, they indiscriminately cleave nearby single-stranded DNA (ssDNA) or RNA reporters, generating a detectable signal such as fluorescence or lateral flow readouts [16,17]. This property is harnessed in systems like DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter) and SHERLOCK (Specific High-Sensitivity Enzymatic Reporter unLOCKing), where resistance genes (e.g., blaNDM, mecA, mcr-1) are targeted with high specificity and sensitivity [18,19].

The process generally begins with amplification of the target sequence using isothermal techniques such as recombinase polymerase amplification (RPA) or loop-mediated isothermal amplification (LAMP), which obviate the need for thermocyclers and enable point-of-care use. The amplified product is then introduced to the CRISPR-Cas system programmed with specific crRNAs. Upon target recognition, the Cas enzyme activates its trans-cleavage function, cleaving synthetic reporter molecules (e.g., fluorophore-quencher probes), leading to a quantifiable signal. In the case of Cas12, DNA targets (such as plasmid-borne resistance genes) can be detected directly, while Cas13 enables RNA-level detection, facilitating expression profiling of resistance transcripts [20]. Furthermore, Cas14, a recently identified miniature effector, holds potential for ultra-sensitive single-stranded DNA detection due to its compact size and minimal sequence constraints [21].

The integration of CRISPR diagnostics with microfluidics, paper-based assays, and smartphone-based readouts enhances the portability and scalability of these platforms, particularly in low-resource or outbreak-prone settings. These CRISPR-based technologies are highly adaptable, allowing multiplexed detection of multiple resistance genes in a single assay, which is critical in diagnosing co-infections or identifying complex resistance profiles in clinical samples. Thus, the molecular mechanism of CRISPR-Cas systems provides the foundation for next-generation diagnostics capable of rapidly and accurately identifying MDR microbes with minimal equipment and high translational potential in public health and clinical microbiology [22].

IV. CRISPR-CAS SYSTEMS: MECHANISMS AND DIAGNOSTIC POTENTIAL

The CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated) system functions as an adaptive immune mechanism in bacteria and archaea, enabling them to recognize and defend against invading genetic elements such as bacteriophages and plasmids. The process begins with the acquisition phase, during which short sequences (spacers) from foreign DNA are integrated into the host genome within the CRISPR array. These arrays are transcribed and processed into small CRISPR RNAs (crRNAs) that guide Cas (CRISPR-associated) endonucleases to complementary sequences in future invaders during the interference phase, leading to targeted cleavage and inactivation of the foreign genome [23,24].

While CRISPR-Cas9 is widely known for genome editing, its diagnostic applications are rooted in the system's ability to detect specific nucleic acid sequences with high precision. Cas9, guided by crRNA and a trans-activating crRNA (tracrRNA), binds to double-stranded DNA targets and cleaves them at precise locations, requiring a protospacer adjacent motif (PAM) for recognition [25]. However, for diagnostic applications, Cas enzymes that possess collateral cleavage activitysuch as Cas12, Cas13, and Cas14—have gained particular interest. These enzymes, once activated by binding their specific target nucleic acid sequence, exhibit nonspecific cleavage activity toward surrounding single-stranded nucleic acids (either DNA or RNA), a property not observed in Cas9 [26].

Cas12 targets double-stranded DNA, and upon activation, it cleaves nearby single-stranded DNA reporters, enabling signal generation in diagnostic platforms like DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter) [27]. Cas13, on the other hand, targets single-stranded RNA and, once bound to its target, nonspecifically degrades nearby RNA molecules, which is utilized in the SHERLOCK (Specific High Sensitivity Enzymatic Reporter unLOCKing) platform [28]. Cas14, a more recently characterized miniature nuclease, can identify single-stranded DNA targets without a strict

PAM requirement, expanding the potential for detecting diverse genetic elements with minimal sequence constraints [29].

These CRISPR-based diagnostics are typically integrated with isothermal amplification techniques such as recombinase polymerase amplification (RPA) or loop-mediated isothermal amplification (LAMP) to increase sensitivity without the need for thermal cycling. The cleavage of reporter molecules—often fluorescently labeled or conjugated to lateral flow detection systems—allows for real-time, visual, or instrument-based readouts. This collateral cleavage mechanism is central to transforming CRISPR systems into powerful biosensors capable of detecting pathogen-specific genes, antimicrobial resistance determinants, and even point mutations with remarkable speed and accuracy [30, 31].

Thus, the reengineering of CRISPR-Cas systems from a natural immune tool to a programmable molecular detector has opened new frontiers in microbiological diagnostics. Especially in the context of multidrug-resistant pathogens, CRISPR-based platforms offer a promising alternative to conventional diagnostic approaches by combining specificity, rapidity, and adaptability in portable formats suitable for point-of-care use.

V. CRISPR-BASED DIAGNOSTIC PLATFORMS

CRISPR-Cas-based diagnostic platforms have rapidly emerged as transformative tools in the detection of multidrug-resistant (MDR) microbes, owing to their high specificity, programmability, and adaptability for use in low-resource or point-of-care settings. Among the earliest and most extensively studied systems is SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing), which utilizes the RNA-targeting CRISPR-Cas13a enzyme. SHERLOCK works by employing a synthetic CRISPR RNA (crRNA) to guide Cas13a to its RNA target—such as mRNA or reverse-transcribed DNA of a resistance gene—where, upon binding, Cas13a is activated to cleave surrounding single-stranded RNA non-specifically. This collateral cleavage of a fluorescent or colorimetric reporter molecule generates a detectable signal, making SHERLOCK highly suitable for detecting RNA viruses, bacterial resistance transcripts, or plasmid-borne resistance genes such as *blaNDM*, *mcr-1*, and *vanA* [32,33]. The system is compatible with isothermal amplification techniques like recombinase polymerase amplification (RPA), enabling rapid and instrument-free diagnostics in under an hour.

In contrast, DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter) is built upon the DNA-targeting Cas12aenzyme, which functions similarly but targets double-stranded DNA. When guided by a specific crRNA, Cas12a recognizes and binds to its DNA target—such as resistance genes embedded in bacterial genomes or plasmids—and upon activation, indiscriminately cleaves single-stranded DNA reporters [34]. DETECTR is especially suited for DNA-based resistance diagnostics, such as detecting *mecA* in methicillin-resistant *Staphylococcus aureus* or *blaCTX-M* genes in extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae [35]. Like SHERLOCK, DETECTR is also used in combination with isothermal amplification methods (e.g., LAMP or RPA), which boosts sensitivity and removes the need for thermal cycling equipment.

Several other CRISPR-based platforms have also emerged, each contributing to the growing toolkit for MDR diagnostics. HOLMES (One-HOur Low-cost Multipurpose Highly Efficient System) employs either Cas12a or Cas13a enzymes for DNA or RNA detection respectively, and has demonstrated femtomolar-level sensitivity for pathogen identification, including potential MDR markers [36]. FELUDA (FnCas9 Editor Linked Uniform Detection Assay), developed using the Cas9 enzyme from *Francisellanovicida*, differs mechanistically from SHERLOCK and DETECTR as it lacks collateral activity; instead, FELUDA relies on specific binding of FnCas9 to its DNA target and utilizes lateral flow-based readouts to detect hybridization events [37]. Although FELUDA lacks collateral cleavage, its precise targeting makes it suitable for SNP discrimination and resistance gene detection, with minimal cross-reactivity. miSHERLOCK, a portable, multiplexed version of SHERLOCK, integrates paper-based microfluidics and smartphone imaging for field-deployable detection of multiple pathogens or genes simultaneously, offering added value for real-time MDR surveillance [38].

These CRISPR-based diagnostic platforms vary in terms of sensitivity, cost, and operational complexity.

SHERLOCK and DETECTR typically reach attomolar to femtomolar sensitivity with amplification, and can deliver results within 30–60 minutes [37]. Compared to traditional PCR and culture methods, these platforms drastically reduce diagnostic time and infrastructure needs. Their modularity, speed, and programmability position them as powerful alternatives for MDR detection in clinical, environmental, and field settings.

VI. APPLICATIONS IN MDR PATHOGEN DETECTION

CRISPR-Cas systems have emerged as transformative tools in the field of molecular diagnostics for identifying multidrug-resistant (MDR) pathogens by enabling precise, rapid, and programmable detection of resistance genes at the nucleic acid level. Mechanistically, CRISPR-based diagnostic platforms function by utilizing guide RNAs (crRNAs) that are complementary to specific resistance-associated DNA or RNA sequences. Upon recognition of the target, Cas proteins such as Cas12 or Cas13 undergo conformational activation and exhibit collateral cleavage activity on nearby reporter molecules, typically fluorescent or colorimetric probes, thereby producing detectable signals without requiring complex instrumentation [39, 40]. This unique mechanism provides both high specificity due to sequence-directed targeting and sensitivity, especially when combined with isothermal amplification techniques such as loop-mediated isothermal amplification (LAMP) or recombinase polymerase amplification (RPA) [41].

One of the most impactful applications of CRISPR diagnostics has been the detection of clinically significant resistance genes, such as *blaNDM* (New Delhi metallo-β-lactamase), *mecA* (conferring methicillin resistance in *Staphylococcus aureus*), and *mcr-1* (mobilized colistin resistance gene), which are often associated with resistance to last-resort antibiotics. For instance, Li et al. (2019) developed a Cas12a-based assay capable of detecting *mcr-1* in bacterial genomic DNA with attomolar sensitivity, using a fluorescence readout format that bypassed the need for PCR [42]. Similarly, Wang et al. (2020) employed a Cas13a-based SHERLOCK platform for detecting *blaNDM-1* directly from patient-derived samples within 45 minutes, with a detection limit as low as 10 copies per microliter, highlighting its potential in urgent clinical settings [43].

Clinical validation studies have further demonstrated the applicability of these tools in real-world diagnostic environments. For example, Kaminski et al. (2021) conducted a field study using a CRISPR-Cas12a lateral flow assay to detect *mecA* genes in *S. aureus* isolates from wound swabs, achieving over 95% concordance with traditional PCR-based methods while reducing the time-to-result from hours to under 60 minutes [44]. Moreover, the robustness of CRISPR systems under varied environmental conditions makes them suitable for deployment in resource-limited settings where rapid and point-of-care diagnostics are critical for infection control and antimicrobial stewardship.

Importantly, CRISPR-based diagnostics are also amenable to multiplexed detection, enabling simultaneous identification of multiple resistance genes or co-infecting pathogens in a single assay. Multiplexing can be achieved by spatial separation of reaction wells, barcoded crRNAs, or orthogonal Cas enzymes with distinct cleavage preferences [45]. For instance, Gootenberg et al. (2018) demonstrated a Cas13-based multiplexed system capable of detecting multiple RNA targets including viral and antibiotic resistance transcripts using unique crRNAs coupled with fluorophore-quencher probes [46]. Such multiplexed platforms are particularly useful in hospital settings, where patients may harborpolymicrobial infections with varying resistance profiles.

Together, these advances in CRISPR-Cas-mediated detection hold tremendous potential for revolutionizing MDR pathogen surveillance and timely clinical decision-making by providing scalable, rapid, and accurate molecular tools adaptable for diverse diagnostic needs.

VII. INTEGRATION WITH PORTABLE AND FIELD-DEPLOYABLE TECHNOLOGIES FOR MDR MICROBES' DETECTION

To enable rapid and accessible detection of multidrug-resistant (MDR) microbes, the integration of CRISPR-Cas-based diagnostics with portable and field-deployable technologies has emerged as a transformative approach. Central to this integration are isothermal amplification techniques, such

asRecombinase Polymerase Amplification (RPA)andLoop-mediated Isothermal Amplification (LAMP)**.** These methods eliminate the need for thermocycling, thus reducing equipment complexity. RPA operates by using recombinase enzymes to pair primers with complementary DNA sequences at a constant low temperature (37–42 °C), while single-strand binding proteins stabilize the displaced strands, and a strand-displacing polymerase extends the primers, producing amplified DNA within 15–20 minutes [47]. In contrast, LAMP utilizes a set of 4–6 primers and Bst polymerase to amplify DNA at around 60–65 °C with high specificity, producing large quantities of amplicons and a visible turbidity due to magnesium pyrophosphate precipitation [48]. These isothermal platforms are commonly coupled with CRISPR effectors such as Cas12 or Cas13, whose target-specific collateral cleavage activity is triggered by the amplified product, enabling rapid signal generation.

The resulting CRISPR-Cas-mediated detection can be visualized using lateral flow assays (LFAs)**,** which provide a paper-based, user-friendly output suitable for low-resource settings. In a typical CRISPR-LFA, a reporter molecule labeled with biotin and fluorescein is cleaved by activated Cas enzymes upon detection of the target sequence. This cleaved or uncleaved reporter flows along a nitrocellulose strip and interacts with antibodies or streptavidin-gold nanoparticles at designated test and control lines, producing visible results within minutes [49]. The simplicity of LFAs, along with minimal sample preparation, makes them ideal for bedside or field use.

To enhance portability and accessibility, smartphone-based detection systemshave been developed as readout interfaces. These platforms exploit the high-resolution cameras and computing power of smartphones to detect fluorescence or colorimetric changes associated with CRISPR activity. For instance, miniaturized fluorescence readers or light-emitting diode (LED) attachments can detect CRISPR-mediated fluorescence, and dedicated mobile applications can quantify signal intensities and relay results to clinicians or databases in real time [50]. Such systems enable telemedicine applications and decentralized surveillance of MDR hotspots.

Further miniaturization and automation have been achieved through microfluidics and lab-on-a-chip (LOC) systems**,** which allow the integration of nucleic acid extraction, amplification, CRISPR-based detection, and signal readout within a single chip. These devices manipulate small volumes of fluids through microchannels, reducing reagent consumption and enabling high-throughput multiplexing. Passive or active fluid control methods (e.g., capillary flow, valves, centrifugal force) drive the sample through chambers containing lyophilized reagents, making the entire process instrument-free in some designs [51]. Microfluidic platforms integrated with CRISPR-Cas systems have demonstrated rapid detection of MDR genes such as *blaNDM* and *mecA* from clinical samples within 30–60 minutes [52]. Additionally, the closed-system design reduces contamination risk and improves biosafety, making such devices suitable for field deployment.

In summary, the convergence of CRISPR diagnostics with portable technologies such as isothermal amplification, lateral flow strips, smartphone interfaces, and lab-on-chip platforms significantly enhances the feasibility of point-of-care detection of MDR microbes. These advances bridge the diagnostic gap in resource-limited areas and represent a promising frontier in the global effort to control antimicrobial resistance

VIII. ADVANTAGES OF CRISPR-CAS-BASED DIAGNOSTICS: A MECHANISTIC PERSPECTIVE

CRISPR-Cas-based diagnostics offer a transformative approach to the detection of multidrug-resistant (MDR) microbes due to their inherent specificity, speed, adaptability, and simplicity of deployment. At the core of this precision lies the programmable nature of CRISPR-Cas systems, which utilize guide RNAs (gRNAs) to direct Cas effectors to complementary nucleic acid sequences. This programmable targeting mechanism ensures exceptional specificity, allowing discrimination between closely related microbial strains or even single nucleotide polymorphisms (SNPs) associated with resistance mutations, such as in the *blaNDM-1*, *mecA*, or *mcr-1* resistance genes [53,54]. Unlike traditional diagnostics that often rely on non-specific hybridization or probe binding, CRISPR-Cas systems

engage in sequence-directed recognition followed by enzymatic cleavage of the target, thereby minimizing false positives due to off-target hybridization.

A key mechanistic advantage contributing to the rapid turnaround of CRISPR-based assays is the use of collateral cleavage activity exhibited by specific Cas proteins, such as Cas12 and Cas13. Upon binding to the target sequence via complementary base pairing with the gRNA, these enzymes become catalytically activated and nonspecifically cleave nearby single-stranded DNA (Cas12) or RNA (Cas13) reporter molecules. This collateral cleavage results in a visible or fluorescent signal within minutes, eliminating the need for complex downstream processing [55,56]. This rapid signal generation capability enables most CRISPR-based assays to be completed in under an hour, with some platforms such as SHERLOCK and DETECTR demonstrating detection times as low as 30 minutes under optimized conditions [57].

Additionally, CRISPR diagnostics require minimal infrastructure**,** making them suitable for point-of-care and field-based settings. Traditional molecular diagnostics like PCR demand thermal cycling, precise temperature control, and extensive laboratory infrastructure. In contrast, CRISPR diagnostics are often paired with isothermal amplification techniques such as recombinase polymerase amplification (RPA) or loop-mediated isothermal amplification (LAMP), which operate at constant low temperatures (37–42°C). This compatibility with simple heat blocks or body heat makes CRISPR systems deployable even in resource-limited environments [58]. Furthermore, signal detection methods—ranging from lateral flow assays to smartphone-integrated fluorescence readers—remove the dependence on expensive or bulky instrumentation.

Another significant advantage is the customizability of CRISPR diagnostics to target newly emerging or mutating resistance determinants. Since only the gRNA needs to be redesigned to recognize a new sequence, the core diagnostic platform remains unchanged. This modularity permits rapid assay development against novel resistance genes or pandemic strains, making CRISPR an agile diagnostic tool in the face of evolving AMR threats [59]. For instance, gRNA libraries can be synthesized in bulk to allow multiplexed detection of several MDR genes in a single reaction, providing comprehensive resistance profiling in real-time [60]. The combination of specificity, speed, simplicity, and flexibility highlights the profound potential of CRISPR-based diagnostics to reshape current paradigms in AMR detection and surveillance.

IX. FUTURE PERSPECTIVES

The evolution of CRISPR-Cas diagnostics is being accelerated by innovations in synthetic biology, artificial intelligence, and global health informatics, promising a new generation of highly adaptable and field-deployable tools against multidrug-resistant (MDR) microbes. Synthetic biology plays a pivotal role in optimizing CRISPR-Cas systems by engineering Cas effectors with enhanced specificity and activity. For instance, engineered Cas12 and Cas13 variants have demonstrated reduced off-target effects and improved collateral cleavage kinetics, enabling more robust and consistent signal amplification in nucleic acid detection assays [61]. Furthermore, synthetic circuits integrating CRISPR modules with logic-gate functions are being explored to detect combinations of resistance genes, allowing a context-dependent diagnostic output tailored for complex clinical scenarios [62]. In parallel, artificial intelligence (AI) and machine learning models are being integrated into CRISPR guide RNA (gRNA) design platforms. These algorithms analyze large-scale genomic data to predict optimal gRNA sequences that avoid off-target binding and maximize on-target cleavage efficiency, especially for detecting resistance-conferring mutations in pathogens such as *Mycobacterium tuberculosis* or *Klebsiellapneumonia* [63, 64]. AI-based resistance prediction tools are also aiding in the dynamic updating of CRISPR diagnostics to match emerging resistance profiles, improving surveillance responsiveness.

Beyond the bench, efforts are underway to embed CRISPR-Cas diagnostics into global antimicrobial resistance (AMR) surveillance frameworks. Portable CRISPR devices, coupled with cloud-based data sharing, can enable real-time monitoring of resistance gene spread across regions, contributing to One Health-based surveillance strategies [65]. For instance, coupling diagnostics with GPS-tagged reporting systems can map resistance hotspots and inform rapid public health interventions. Additionally, innovations in wearable and home-

based biosensors are creating opportunities for decentralized diagnostics. CRISPR-Cas biosensors embedded in lateral-flow formats or smart textiles could facilitate non-invasive, point-of-care detection of MDR pathogens from saliva, sweat, or breath condensate [66]. This could revolutionize early detection in vulnerable populations or remote settings with minimal access to laboratories. However, realizing these advancements will require rigorous clinical validation, regulatory harmonization, and cost-effective manufacturing pipelines.

In conclusion, the integration of synthetic biology, AI-driven design, global health informatics, and wearable sensor platforms holds immense promise in advancing CRISPR-Cas diagnostics from the lab bench to real-world application in the fight against MDR microbes.

X. CONCLUSION

CRISPR-Cas-based diagnostic platforms represent a transformative leap in the detection of multidrug-resistant (MDR) pathogens, offering a rapid, specific, and field-deployable solution to combat the escalating antimicrobial resistance (AMR) crisis. The core mechanism relies on the RNA-guided recognition of target nucleic acid sequences by Cas enzymes, such as Cas12 and Cas13, followed by trans-cleavage of reporter molecules, enabling real-time detection without the need for complex equipment or lengthy amplification steps. These systems provide high sensitivity and specificity, often detecting femtomolar levels of resistance genes within an hour. Strategically, future research must focus on improving sample preparation methods, minimizing off-target activity, and enabling multiplexed detection of co-existing resistance determinants. Integration with isothermal amplification, microfluidics, and smartphone-based readouts will be critical for scalable deployment in resource-limited settings. Moreover, CRISPR diagnostics could become essential tools for AMR surveillance networks, enabling decentralized, rapid tracking of resistance gene dissemination in both clinical and environmental samples. By bridging molecular precision with portability, CRISPR-based diagnostics hold immense promise to reshape the diagnostic landscape and serve as a frontline tool in global AMR containment strategies.

FUNDING

This research did not receive any specific grant from funding agencies.

DATA AVAILABILITY

No datasets were generated or analysed during the current study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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