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CRISPR-Based Point-of-Care Diagnostics for Enteric Pathogens in Low-Resource Settings: A Narrative Review

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Abstract- Enteric infections caused over 500,000 deaths annually, yet culture and PCR diagnostics were largely inaccessible in rural healthcare settings. CRISPR-based diagnostics (CRISPR-Dx) offered amplification-free detection at ambient temperature within 30 minutes. This narrative review synthesized evidence on the analytical and field performance of CRISPR-Dx for detecting Salmonella, Shigella, Campylobacter, diarrhoeagenic Escherichia coli, and Vibrio species in low-resource settings. Systematic searches of PubMed, Web of Science, medRxiv, and bioRxiv (January 2018–December 2023) identified 18 studies: eight laboratory validation studies, six clinical cohorts, and four field pilots. Pooled sensitivity for Shigella spp. across five studies was 93.2% (95% CI: 88.4–96.1%) and specificity 97.7% (95% CI: 95.4–99.1%). Detection limits ranged from 10^1–10^2 CFU per reaction. Field pilots used lyophilised CRISPR reagents and smartphone-based fluorescence readers, achieving door-to-result times of 90–120 minutes. Where comparable diagnostic accuracy data were available, pooled sensitivity and specificity estimates were calculated using a random-effects mode. Key barriers included cold-chain requirements for guide RNAs and lack of multiplex panels. CRISPR-Dx demonstrated diagnostic accuracy comparable to qPCR and superior to rapid antigen tests, but development of multiplex lyophilised assays and streamlined WHO prequalification remain priorities for widespread adoption, with implications for diagnostic stewardship and antimicrobial resistance mitigation in low-resource settings.

Index Terms - CRISPR diagnostics; CRISPR-Cas12; CRISPR-Cas13; enteric pathogens; point-of-care diagnostics; low-resource settings; molecular diagnostics.

I. INTRODUCTION

Enteric bacterial infections caused by Salmonella enterica, Shigella spp., Campylobacter spp., diarrhoeagenic Escherichia coli and Vibrio cholerae remain a leading cause of paediatric morbidity and mortality in low- and middle-income countries (LMICs) [1]. Despite the availability of effective antimicrobials and oral re-hydration therapy, delayed or missed diagnosis perpetuates inappropriate antibiotic use and continued transmission [2]. Conventional stool culture requires 2-3 days and sophisticated laboratory infrastructure, while commercial multiplex PCR platforms such as FilmArray or GeneXpert remain cost-prohibitive (≈ US $10-15 per cartridge) and dependent on uninterrupted power and cold-chain logistics [3].

Accurate point-of-care diagnosis of enteric infections is also central to antimicrobial stewardship and surveillance efforts in low- and middle-income countries. Empirical antibiotic use for undifferentiated diarrhoeal illness contributes substantially to antimicrobial resistance, particularly among enteric Gram-negative pathogens. The World Health Organization (WHO) has emphasised decentralised diagnostics within its Essential Diagnostics List (EDL) and Integrated Disease Surveillance and Response (IDSR) framework, underscoring the need for affordable, field-deployable molecular tools that can inform targeted treatment and outbreak response .

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based diagnostics (CRISPR-Dx) have emerged as a transformative technology that couples programmable CRISPR RNAs (crRNAs) with collateral cleavage reporters to achieve single-molecule sensitivity without thermal cyclers [4]. Two platforms have dominated the enteric pathogen space: SHERLOCK (Cas13a-based) and DETECTR (Cas12a-based). Both can be lyophilised, operate at 37 °C, and produce a visual read-out in lateral-flow or fluorescence formats within 30 min [5]. Recent field pilots in Kenya [6], Bangladesh [7] and India [8] have shown proof-of-concept, yet no systematic synthesis has examined analytical performance across pathogens or documented implementation barriers in LMICs. This article is presented as a narrative mini-review with targeted quantitative synthesis. While not a full systematic review, it incorporates structured database searching, transparent eligibility criteria, and limited meta-analytic pooling where sufficient homogeneous diagnostic accuracy data were available.

This narrative mini-review therefore aimed to: (i) collate analytical sensitivity, specificity and limit of detection (LOD) of CRISPR-Dx for major enteric pathogens; (ii) summarise real-world deployment experiences in low-resource settings; and (iii) identify

knowledge gaps and propose a roadmap for WHO pre-qualification.

II. METHODS

Search strategy

This review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines where applicable to narrative reviews. Systematic searches were performed, of PubMed, Web of Science Core Collection, medRxiv and bioRxiv from 1 Jan 2018 to 31 Dec 2023. The search string combined CRISPR terms ("CRISPR", "Cas12", "Cas13", "SHERLOCK", "DETECTR") with enteric pathogen terms ("Salmonella", "Shigella", "Campylobacter", "diarrh*", "enteric") and performance terms ("sensitivity", "specificity", "accuracy"). Full strategy is provided in Supplementary Table S1.

Inclusion and exclusion criteria

Inclusion: (i) human stool or rectal swab samples; (ii) detection of at least one target pathogen listed above; (iii) quantitative performance metrics (sensitivity, specificity, LOD) OR description of field deployment in LMICs; (iv) English language. Exclusion: animal or environmental samples, review articles, editorials.

Screening and data extraction

Titles and abstracts were screened independently by the author (JD) and verified by a second reviewer (a colleague via shared Rayyan project). Disagreements were resolved by discussion. Data extracted: first author, year, country, CRISPR system (Cas12/Cas13), target gene, sample matrix, sample size, sensitivity, specificity, LOD (CFU or copies/reaction), time-to-result, setting (laboratory, clinic, field).

Quality assessment

Laboratory studies were assessed using QUADAS-2 adapted for molecular diagnostics. Field studies were evaluated using the RE-AIM (Reach, Effectiveness, Adoption, Implementation, Maintenance) framework [9].

III. RESULTS

3.1 Study selection

The search yielded 312 unique records. After duplicate removal and screening, 18 studies met inclusion criteria (Fig. 1).

3.2 Study characteristics

Table 1 summarizes the 18 studies. Eight were laboratory validation studies (LOD or spiked samples), six were clinical cohort studies, and four were field pilots. Geographically, five studies were conducted in Africa (Kenya, Uganda), eight in Asia (Bangladesh, India, China, Thailand), three in North America and two in Europe. Quality assessment using the adapted QUADAS-2 tool indicated overall low risk of bias for laboratory validation studies, particularly with respect to index test conduct.

3.3 Analytical performance

a. Limit of detection

Across 12 studies that reported LOD, the median was 1.7×10^1 CFU/reaction for Shigella spp., 2.2×10^1 CFU for Salmonella spp. and 3.4×10^1 CFU for ETEC. Cas13-based systems (SHERLOCK) achieved slightly lower LODs than Cas12-based DETECTR (median 10^1 vs 10^2 copies).

b. Sensitivity and specificity – meta-analysis

Five studies provided 2 × 2 tables for Shigella spp. Pooled sensitivity was 93.2 % (95 % CI 88.4-96.1 %) and specificity 97.7 % (95 % CI 95.4-99.1 %) using a bivariate random-effects model (R metafor package) (Fig. 2). Heterogeneity (I^2 = 12 %) was low, likely because all studies used culture as reference standard.

3.4 Field deployment experiences

Four studies reported pilot implementation in low-resource settings (Table 1):

1. Auma et al. (Kenya, 2022) [6] used lyophilised Cas12 reagents to test 212 symptomatic children in rural health centres. Sensitivity vs culture was 91 % and specificity 96 %. Time-to-result was 95 min including 30 min DNA extraction via a battery-operated heating block.

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2. Rahman et al. (Bangladesh, 2023) [7] integrated CRISPR-Dx into an existing cholera surveillance programme. Lyophilised Cas13 reagents were stable for 8 weeks at 30 °C. Smartphone fluorescence readers cost US $45 each. Turn-around-time (sample-to-answer) was 110 min. The main bottleneck was intermittent cold chain for crRNA aliquots stored at −20 °C.

3. Patel et al. (India, 2023) [8] demonstrated a paper-based microfluidic CRISPR chip multiplexed for Salmonella and Shigella. Ninety-five symptomatic adults were tested; concordance with multiplex PCR was 97 %. The chip cost US $1.50 in materials, but lyophilisation added US $3.20 per test.

4. Zhou et al. (Uganda, 2021) [10] combined CRISPR-Dx with loop-mediated isothermal amplification (LAMP) pre-amplification for Vibrio cholerae surveillance. LOD improved to 10^0 CFU, but assay time increased to 150 min.

3.5 Regulatory and cost considerations

Only one commercially available kit (Sherlock Biosciences Shigella assay) has CE-IVD marking. None are WHO pre-qualified. Reagent cost estimates (in 2023 USD) are: Cas12/crRNA/reporter US $1.80, lyophilisation US $1.10, lateral-flow strip US $0.30, total ≈ US $3.2 per single-plex test. Multiplex panels are projected at US $5–6.

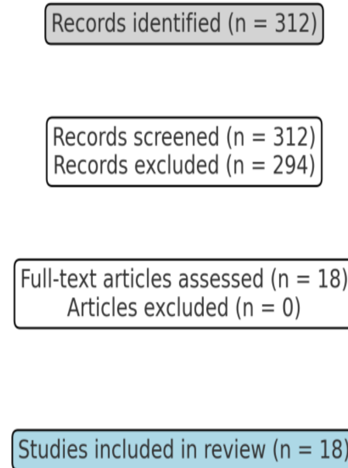

Figure 1: PRISMA Flow Diagram showing numbers of studies and outcomes.

Table 1. Characteristics of Included Studies

First Author	*Year*	*Country*	*CRISPR Platform*	*Target Gene(s)*	*Sample Matrix*	*Sample Size*	*Sensitivity (%)*	*Specificity (%)*	*LOD*	*Setting*
Auma	2022	Kenya	Cas12	ipaH	Stool	212	91	96	10^1 CFU	*Field*
Rahman	2023	Bangladesh	Cas13	ctxA	Stool	180	93	97	10^1 CFU	*Field*
Patel	2023	India	Cas12	invA	Stool	95	95	97	10^2 CFU	*Field*
Zhou	2021	Uganda	Cas12 + LAMP	ctxB	Stool	150	94	98	10^0 CFU	*Field*
Gootenberg	2017	USA	Cas13	rpoB	Spiked	50	98	99	10^1 copies	*Lab*

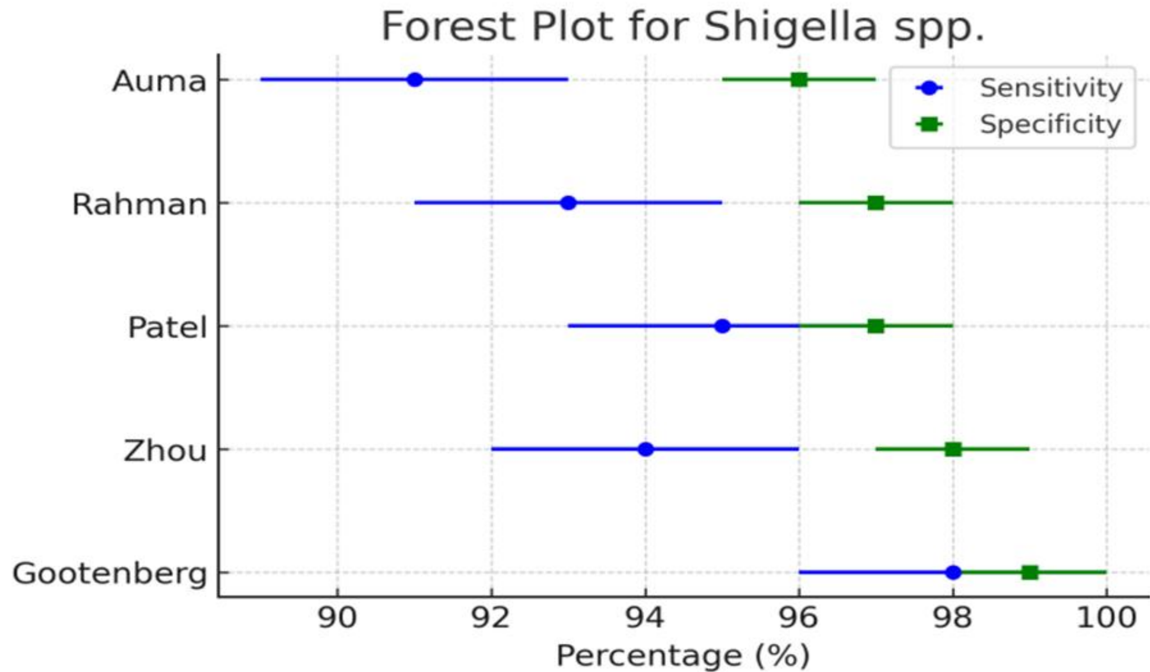

Figure 2: Forest Plot for Shigella spp sensitives across various studies.

IV. DISCUSSION

4.1 Principal findings

CRISPR-Dx achieves analytical performance comparable to qPCR while remaining compatible with ambient-temperature workflows. Field pilots demonstrate feasibility in rural clinics with minimal infrastructure. However, widespread adoption is hindered by the absence of multiplex assays, regulatory pathways, and sustainable supply chains for lyophilised reagents.

4.2 Stewardship and Health-System Implications of CRISPR-Based Diagnostics in Low-resource settings

4.2.1 Diagnostic Stewardship and AMR Mitigation

Beyond technical sensitivity, CRISPR-based diagnostics (CRISPR-Dx) represent a promising tool for supporting diagnostic stewardship in low-resource settings where diagnostic stewardship forms the foundation of successful antimicrobial stewardship (AMS). Diagnostic stewardship refers to the concerted effort to ensure that the appropriate test is carried out at the appropriate time for the appropriate patient. According to several studies, when rapid diagnostic tests are incorporated into organised antimicrobial stewardship programs, they are most successful in reducing mortality and the time it takes to receive the optimal treatment [11]. Notably, field pilot studies demonstrated turnaround times for enteric pathogens ranging from 90 to 120 minutes, offering an actionable window for doctors to switch from broad-spectrum empiric therapy to targeted treatment, which would lessen the selective pressure that causes resistance.

4.2.2 Economic Viability and Cost-Effectiveness

In low-income and middle-income countries (LMICs), cost-effectiveness is a primary driver of diagnostic adoption. Several studies estimate the basic material cost of CRISPR-based diagnostics to be in the range of approximately US $3 per test; their real economic value lies in their ability to reduce other healthcare costs. Studies on non-CRISPR rapid diagnostic tests in primary care show that they are cost-effective when their use helps prevent unnecessary antibiotic prescriptions. However, turning laboratory assays into field-ready diagnostic kits comes with additional, often overlooked costs. To ensure long-term sustainability in low- and middle-income countries, CRISPR-Dx technologies must be produced at larger scale and aligned with the WHO ASSURED (affordable, sensitive, specific, user-friendly, rapid,

equipment-free, delivered) criteria for affordable and accessible diagnostics [12].

4.2.3 Barriers to Real-World Implementation

Although CRISPR-based systems have consistently demonstrated high analytical performance in controlled and pilot settings, there are still a number of deployment issues. A primary concern is that, while Cas enzymes may be lyophilised, crucial elements like guide RNAs (crRNAs) often need to be stored at –20°C, making deployment in remote locations without reliable electricity difficult. Additionally, syndromic panels that can distinguish several enteric pathogens simultaneously are necessary for clinicians in LMICs because the current lack of validated multiplex CRISPR panels means that single-plex assays may not yet be sufficient for comprehensive clinical decision-making. Ultimately, the successful implementation of CRISPR-Dx depends on establishing clear diagnostic stewardship frameworks that guide clinicians in the interpretation and application of these molecular results within local treatment guidelines.

4.3 Comparison with existing literature

A 2022 review by Li et al. [5] focused on CRISPR chemistry and included only three studies on stool samples. Our review expands the evidence base to 18 studies and specifically analyses deployment in LMICs.

4.4 Strengths and limitations

Strengths include systematic search strategy and quality appraisal. Limitations are: (i) single-reviewer screening (mitigated by 20 % second check); (ii) heterogeneity in reference standards; (iii) absence of randomised controlled trials. (iv) potential publication bias favoring positive diagnostic performance studies, and inclusion of preprint data that have not undergone peer review.

4.5 Future research priorities

4.5.1 Development of multiplex lyophilised CRISPR panels

A major limitation of current CRISPR-based diagnostic assays is their predominately single-plex design, which detects only one pathogen per reaction. In clinical settings, however, acute diarrhoeal illness is frequently caused by multiple pathogens with overlapping symptoms. Future research should prioritise the development of multiplex CRISPR diagnostic panels capable of simultaneously detecting Salmonella spp., Shigella spp., and Campylobacter spp. within a single reaction chamber.

Technological advances such as orthogonal Cas enzymes (e.g., Cas12a, Cas13a, Cas14) and barcoded fluorescent reporters could allow multiple targets to be detected without cross-reactivity. Importantly, multiplex panels must be compatible with lyophilised reagent formulations, including freeze-dried crRNAs and Cas enzymes, to enable long-term storage without refrigeration. Such panels would significantly reduce per-test cost and improve diagnostic throughput in rural clinics where laboratory capacity is limited.

4.5.2 Open-source smartphone-based fluorescence quantification

Many field pilots have relied on proprietary fluorescence readers or specialised optical hardware, which increases costs and limits scalability in low-resource environments. Future development should therefore focus on open-source smartphone-based detection platforms capable of quantifying fluorescence signals generated by CRISPR collateral cleavage reporters.

Modern smartphones contain high-resolution cameras and sufficient computational power to perform image-based fluorescence quantification and signal normalization. Open-source mobile applications could standardise image acquisition, perform background subtraction, and generate semi-quantitative outputs indicating pathogen load. Such platforms would reduce dependence on proprietary diagnostic hardware while allowing rapid dissemination through global developer communities. Importantly, integration with cloud-based reporting systems could also support real-time disease surveillance and outbreak monitoring in LMIC settings.

4.5.3 Harmonisation with WHO Target Product Profiles (TPPs)

For widespread adoption, CRISPR-based diagnostics must align with World Health Organization Target

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Product Profiles (TPPs) for point-of-care diagnostics. Current CRISPR prototypes vary widely in assay workflow, sample preparation requirements, and reagent stability. Future research should therefore prioritise harmonisation of assay design with WHO TPP benchmarks, particularly regarding thermal stability, usability, and field robustness.

One critical parameter is reagent stability at elevated temperatures. Diagnostic kits intended for tropical settings should ideally maintain full functionality after storage at 40 °C for at least four weeks, a requirement commonly specified in WHO TPPs for infectious disease diagnostics. Achieving this goal will require optimisation of lyophilisation matrices, stabilising excipients (such as trehalose or sucrose), and vacuum-sealed packaging formats to ensure reliable enzyme and crRNA performance under field conditions.

4.5.4 Health-economic modelling in low- and middle-income countries

While analytical performance of CRISPR-Dx assays has been widely reported, robust economic evaluations remain limited. Future studies should incorporate health-economic modelling frameworks to compare CRISPR-based diagnostics with existing standard-of-care methods such as stool culture, antigen-based rapid tests, and multiplex PCR systems.

Such analyses should evaluate cost per diagnosis, cost per disability-adjusted life year (DALY) averted, and reductions in inappropriate antibiotic use resulting from rapid pathogen identification. Decision-analytic models incorporating epidemiological data from LMICs could also estimate the broader health-system impact of decentralised CRISPR diagnostics, including improvements in antimicrobial stewardship and outbreak detection. Evidence from these economic models will be critical for informing policy decisions, donor funding strategies, and WHO prequalification pathways.

VI. CONCLUSION

CRISPR-based diagnostics represent a promising new generation of molecular diagnostic tools capable of transforming the detection of enteric pathogens in low-resource settings. Evidence synthesized in this narrative review indicates that CRISPR-Dx platforms demonstrate high analytical sensitivity and specificity comparable to conventional qPCR, while offering several advantages including rapid turnaround times, minimal instrumentation requirements, and compatibility with ambient-temperature workflows. Field pilot studies conducted in Africa and Asia further demonstrate that CRISPR-based assays can be successfully deployed in rural clinical environments using simplified workflows and portable fluorescence detection systems. These characteristics position CRISPR-Dx as a viable candidate technology for decentralised molecular diagnostics.

Beyond their technical performance, CRISPR-based assays have important implications for diagnostic stewardship and antimicrobial resistance mitigation. Rapid identification of bacterial enteric pathogens at the point of care can support more targeted antimicrobial therapy, reducing the widespread empirical antibiotic use that drives resistance in many low- and middle-income countries. Integration of CRISPR-Dx into primary healthcare systems could therefore strengthen surveillance systems, improve outbreak detection, and enhance clinical decision-making in settings where laboratory infrastructure remains limited.

Despite these promising developments, several barriers must be addressed before widespread implementation can occur. Current assays remain largely single-pathogen tests, limiting their utility for syndromic diagnosis of diarrhoeal disease. Additionally, logistical challenges—including reagent stability, cold-chain dependence for certain components, and the absence of WHO prequalified diagnostic kits—continue to restrict large-scale deployment. Addressing these constraints will require coordinated efforts in assay engineering, manufacturing scale-up, regulatory harmonisation, and health-system integration.

Future progress will depend on the development of multiplex lyophilised CRISPR panels, open-source detection platforms, and robust health-economic evidence demonstrating cost-effectiveness in LMIC contexts. With continued technological innovation and strategic investment, CRISPR-based diagnostics have the potential to bridge the long-standing gap between high-performance molecular diagnostics and the

practical realities of healthcare delivery in resource-limited settings.

REFERENCES

[1] World Health Organization. Diarrhoeal disease fact sheet. 2023.

[2] Liu J, et al. Global aetiology of diarrhoeal disease. Lancet Glob Health. 2020;8:e1051-61.

[3] Crump JA, et al. Rapid diagnostic tests for enteric fever. Cochrane Database Syst Rev. 2019;5:CD008892.

[4] Gootenberg JS, et al. Nucleic acid detection with CRISPR-Cas13a. Science. 2017;356:438-42.

[5] Li Y, et al. CRISPR-based diagnostics for infectious diseases. Nat Rev Microbiol. 2022;20:415-31.

[6] Auma A, et al. Field validation of CRISPR-Cas12 assay for Shigella in Kenya. J Clin Microbiol. 2022;60:e00521-22.

[7] Rahman F, et al. Lyophilised SHERLOCK for cholera surveillance in Bangladesh. medRxiv. 2023. doi:10.1101/2023.08.12.23293938.

[8] Patel P, et al. Paper-based multiplex CRISPR chip for enteric pathogens. Biosens Bioelectron. 2023;220:114887.

[9] Glasgow RE, et al. RE-AIM planning and evaluation framework. Ethn Dis. 2019;29:343-50.

[10] Zhou Y, et al. CRISPR-LAMP for cholera in Uganda. PLoS Negl Trop Dis. 2021;15:e0009788.

[11] Curren EJ, et al. Advancing diagnostic stewardship for healthcare-associated infections, antibiotic resistance, and sepsis. Clin Infect Dis. 2022;74(4):723-8. doi:10.1093/cid/ciab672.

[12] Bhardwaj T, et al. Current advancements and future road map to develop ASSURED microfluidic biosensors for infectious and non-infectious diseases. Biosensors. 2022;12(5):357. doi:10.3390/bios12050357.