Volume 4, Issue 1, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

CRISPR-CAS12A GUIDED RAPID TEST FOR XDR TYPHOID: A MULTICENTER DIAGNOSTIC ACCURACY CLINICAL TRIAL

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DOI: https://doi.org/10.5281/zenodo.18297256

Keywords
CRISPR-Cas12a, typhoid fever, XDR typhoid, diagnostic accuracy, isothermal amplification, PCR, low-resource settings, antimicrobial stewardship, rapid test accuracy.

Article History
Received: 24 November 2025
Accepted: 05 January 2026
Published: 19 January 2026

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Abstract

Objective: *We evaluated the diagnostic accuracy of a novel* ***CRISPR-Cas12a guided rapid test*** *for detecting* Salmonella enterica *serovar Typhi, including* ***extensively drug-resistant (XDR) strains****, in comparison with gold-standard polymerase chain reaction (PCR). We additionally assessed* ***usability in low-resource laboratories*** *and the* ***impact on clinical decision-making and antibiotic stewardship*** *in a tertiary hospital setting in Punjab, Pakistan.*

Methods: *In this* ***multicenter diagnostic clinical trial****, we enrolled febrile patients with suspected enteric fever across three clinical centers. Blood samples were processed concurrently using (1) blood culture with PCR confirmation targeting* S. Typhi*–specific genes; and (2) the CRISPR-Cas12a rapid assay integrating isothermal amplification with Cas12a detection. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated against PCR as reference. Laboratory technicians in low-resource settings scored usability metrics via standardized questionnaires.*

Results: *Out of N=1,200 evaluable samples, the CRISPR-Cas12a test demonstrated a* ***sensitivity of 93.5% (95% CI 90.8–95.8)*** *and* ***specificity of 95.2% (92.7–97.0)*** *compared with PCR. Diagnostic performance was maintained in isolates characterized as XDR (n=312). Usability assessments indicated high operator agreement on ease of use (mean score 4.5/5) and minimal equipment requirements. Implementation of the rapid test reduced time to targeted antibiotic therapy by a median of 24 h and was associated with improved antibiotic stewardship indicators.*

Conclusion: *The CRISPR-Cas12a rapid assay exhibited high diagnostic accuracy for both typical and XDR* S. Typhi *infections and proved feasible in low-resource laboratories. Adoption of this assay could enhance early diagnosis, rational antibiotic use, and patient outcomes in high-burden settings.*

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ISSN: (e) 3007-1607 (p) 3007-1593

INTRODUCTION

Background and Rationale

Typhoid fever remains a significant public health burden in low- and middle-income countries, particularly in South Asia and sub-Saharan Africa, due to poor sanitation, contaminated water, and inadequate healthcare infrastructure. It is caused by the Gram-negative bacterium *Salmonella enterica* serovar Typhi (*S. Typhi*) and transmitted via the fecal-oral route, leading to systemic infection characterized by prolonged fever and gastrointestinal symptoms. Early and accurate diagnosis is essential to initiate targeted therapy and prevent complications or treatment failures. Traditional diagnostic modalities, including blood culture and PCR, although sensitive, are constrained by cost, infrastructure demands, and prolonged turnaround times, limiting their utility in resource-limited settings (Pandey 2025; *J Pharm Sci* DOI:10.5281/zenodo.17867299)

The emergence and spread of **extensively drug-resistant (XDR) Typhoid** has dramatically complicated clinical management. These strains are resistant to first-line antibiotics and many second-line agents, often requiring treatment with last-resort drugs such as carbapenems or macrolides (Abdullah 2025; PLoS Negl Trop Dis DOI:10.1371/journal.pntd.0013067). The H58 genotype has been widely implicated in XDR outbreaks, particularly in Pakistan, where resistance patterns continue to evolve (Abro 2024; *Infect Genet Evol* DOI:10.1016/j.meegid.2024.105632) . Conventional rapid serological tests such as Widal and Typhidot suffer from poor sensitivity and specificity, contributing to misdiagnosis and inappropriate antibiotic use, further driving

antimicrobial resistance (AMR).

CRISPR-Cas systems, originally characterized for genome editing, have emerged as transformative tools for molecular diagnostics. Cas12a (formerly Cpf1), a single RNA-guided endonuclease, exhibits robust target recognition and collateral single-strand DNA (ssDNA) cleavage upon binding to specific DNA sequences, enabling sensitive detection of nucleic acids when coupled with a reporter system (Wikipedia, CRISPR). CRISPR-based diagnostic platforms like DETECTR and SHERLOCK have demonstrated high sensitivity and specificity for viral and bacterial targets, facilitating rapid detection with minimal equipment, positioning them as strong candidates for point-of-care (POC) diagnostics (Chen 2020; *Nat Biotechnol* DOI:10.1038/s41587-020-0513-4) .

Recent advances have further optimized CRISPR-Cas12a detection by integrating **isothermal amplification** (e.g., recombinase polymerase amplification [RPA] or loop-mediated isothermal amplification [LAMP]) to increase analytical sensitivity without thermocyclers, and by developing lateral flow or colorimetric readouts accessible in low-resource settings (Du 2025; *Foods* DOI:10.3390/foods14111892). CRISPR diagnostics have been successfully applied to pathogens such as *Salmonella* (Lin 2025; *Letters in Applied Microbiology* DOI:10.1007/s00284-025-04240-y) although robust clinical evaluations for enteric fever, especially XDR Typhoid, are limited.

The present trial was conducted to assess the **diagnostic accuracy** of a CRISPR-Cas12a guided rapid test for XDR Typhoid against **gold-standard PCR** in a real-world multicenter clinical setting in Punjab, Pakistan. Secondary objectives included evaluating **usability in low-resource laboratories** and the **impact on clinical**

decisions and antibiotic stewardship.

Study Setting and Population

This multicenter study was carried out in three tertiary care hospitals in the Punjab province, Pakistan, between January and October 2025. Participants included patients of all ages presenting with fever ≥38.0 °C and clinical signs consistent with enteric fever (e.g., sustained fever, abdominal discomfort, headache). After informed consent, venous blood samples were obtained and processed in parallel.

Diagnostic Challenges with XDR Typhoid

The emergence of XDR *S. Typhi* strains has diminished the efficacy of many commonly used antibiotics, necessitating accurate diagnostics for targeted therapy and AMR mitigation (National Institute of Health, Pakistan 2024). Standard culture

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ISSN: (e) 3007-1607 (p) 3007-1593

followed by PCR remains the gold standard but is limited by infrastructure requirements and delays. CRISPR diagnostics, with rapid turnaround and minimal equipment needs, address these gaps, aligning with WHO's REASSURED criteria for ideal diagnostics—Real-time connectivity, Ease of specimen collection, Affordable, Sensitive, Specific, User-friendly, Rapid/robust, Equipment-free, and Deliverable to end-users.

Principles of CRISPR-Cas12a Diagnostic Testing

CRISPR-Cas12a diagnostics function by designing a CRISPR RNA (crRNA) complementary to a target sequence of *S. Typhi*. Upon hybridization of target DNA with the Cas12a-crRNA complex, the activated Cas12a nonspecifically cleaves ssDNA reporter molecules, producing a measurable fluorescence or colorimetric signal. When combined with isothermal amplification, such assays have achieved rapid detection within 30–60 min with high analytical sensitivity (Du 2025; Lin 2025).

Clinical and Public Health Importance

Early and precise typhoid diagnosis influences clinical management, reduces inappropriate antibiotic prescriptions, and supports antibiotic stewardship programs. In high-burden regions like Pakistan, where XDR Typhoid is endemic and underreported, improved diagnostics could markedly reduce morbidity, transmission, and healthcare costs.

MATERIALS AND METHODS

Study Design

This study was designed as a **multicenter diagnostic clinical trial** conducted across three **tertiary care hospitals** in Punjab, Pakistan, between January and October 2025. The primary aim was to evaluate the **diagnostic accuracy** of a **CRISPR-Cas12a guided rapid test** for detecting *Salmonella enterica* serovar Typhi (both non-XDR and XDR strains) in comparison with the **gold-standard polymerase chain reaction (PCR)**. Additionally, we sought to assess the test's **usability in low-resource settings** and determine its impact on **clinical decision-making** and **antibiotic stewardship**.

Study Population

The study included **febrile patients (≥38.0°C)** who presented with symptoms suggestive of enteric fever, such as sustained fever, abdominal discomfort, headache, and malaise. The participants were enrolled from three clinical centers, each located in **urban and rural settings** of Punjab, representing a wide demographic of patients with diverse socioeconomic backgrounds. Inclusion criteria were as follows:

1. Patients aged 2 years and above.
2. Presentation with **fever and clinical signs** indicative of typhoid fever.
3. Written informed consent from patients or guardians (for minors).

Exclusion criteria included:

1. Patients with a **history of recent antibiotic use** within the last 48 hours.
2. Those with **chronic or immunocompromised conditions**, as these could potentially confound results.

All enrolled participants underwent a thorough clinical assessment, and their demographic and medical history were recorded. Ethical approval was obtained from the **Institutional Review Board (IRB)** of the participating hospitals.

Sample Collection and Processing

Upon enrollment, a single **venous blood sample** (5 ml) was obtained from each patient for analysis. The samples were processed immediately in the clinical laboratories of each participating site under **sterile conditions**. The following diagnostic methods were used:

1. **Blood Culture**: A blood sample was cultured using **standard microbiological techniques** to isolate *S. Typhi*. Cultures were incubated at 37°C for up to 7 days. Isolates were identified using **biochemical tests** (API 20E kit) and confirmed by **PCR** for the presence of *S. Typhi*-specific genes.
2. **PCR (Gold-standard Method)**: For confirmation of *S. Typhi* and for characterization of XDR strains, **PCR** was performed on blood culture isolates using primers specific to the *invA* gene (which is conserved across *Salmonella* species) and the **qnrS gene** for resistance markers, specifically for quinolone resistance. XDR strains were further analyzed for **extended-spectrum beta-lactamase (ESBL)** production and **carbapenem resistance**.

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3. **CRISPR-Cas12a Rapid Test**: The CRISPR-Cas12a rapid diagnostic test was developed using an isothermal amplification method. DNA from blood samples was extracted using the **QIAamp DNA Blood Mini Kit** (Qiagen, Germany). The extracted DNA was subjected to **recombinase polymerase amplification (RPA)**, targeting the ***S. Typhi* invA gene**, followed by Cas12a cleavage to generate a **colorimetric signal**. This test was designed for **point-of-care (POC)** application, allowing for results within 60 minutes without the need for specialized equipment like thermocyclers.

CRISPR-Cas12a Assay Development and Validation

The CRISPR-Cas12a assay was developed in collaboration with a local diagnostic manufacturer. The **Cas12a protein** was sourced from *Thermo Fisher Scientific* (USA), and the corresponding **single-guide RNA (sgRNA)** was synthesized to recognize the *S. Typhi* invA gene sequence. Isothermal amplification was achieved using the **TwistAmp™ DNA Amplification Kit** (TwistDx, UK), which was optimized to work at a constant temperature of 37°C. The Cas12a-catalyzed signal amplification was detected by a **fluorescence-based lateral flow assay**.

Optimizations included:

- **Optimization of DNA extraction**: Different DNA extraction methods were tested to determine the most effective protocol for obtaining high-yield DNA from blood.
- **Test duration and temperature**: The amplification reaction was optimized for a 30-minute reaction at 37°C, which is a critical step to achieve high sensitivity while maintaining usability in resource-limited environments.

The assay was validated in a **training set** of 100 confirmed *S. Typhi* positive samples, where results were compared to the standard PCR to establish baseline performance metrics.

Outcome Measures

1. **Primary Outcome**: The **diagnostic accuracy** of the CRISPR-Cas12a assay was evaluated by calculating the **sensitivity**, **specificity**, **positive predictive value (PPV)**, and **negative predictive value (NPV)**, using PCR as the gold standard. These metrics were calculated for both **XDR** and **non-XDR** strains of *S. Typhi*.

2. **Secondary Outcomes**:

- **Usability Assessment**: Laboratory staff and technicians were trained to use the CRISPR-Cas12a assay. After the training, they completed a **standardized questionnaire** assessing ease of use, clarity of instructions, time to result, and equipment requirements. A **five-point Likert scale** was used to assess user satisfaction.
- **Impact on Clinical Decision-Making**: We evaluated whether the use of the rapid CRISPR test influenced clinical decisions such as the **timing of antibiotic initiation** and the appropriateness of antibiotic choices, comparing patients who received PCR results with those who received CRISPR results.
- **Impact on Antibiotic Stewardship**: Data on **antibiotic prescriptions** (including broad-spectrum antibiotics) were collected and analyzed to assess whether the use of the CRISPR test led to a reduction in inappropriate antibiotic use.

Statistical Analysis

The statistical analysis was performed using **SPSS v25** (IBM, USA) for the primary and secondary outcomes. For the calculation of diagnostic accuracy, a **2×2 contingency table** was created to compare the **true positives**, **false positives**, **true negatives**, and **false negatives** of the CRISPR-Cas12a test compared with PCR. Sensitivity, specificity, PPV, and NPV were calculated with **95% confidence intervals (CIs)**. **Usability data** were analyzed descriptively, and **median scores** were used to summarize technician feedback. For **antibiotic stewardship impact**, **Chi-square tests** were applied to compare proportions of inappropriate antibiotic use before and after the introduction of the CRISPR-Cas12a test.

Quality Control and Assurance

Strict **quality control procedures** were followed throughout the study to ensure the integrity of sample handling and processing. All laboratory staff were trained in standard operating procedures (SOPs) for sample collection, processing, and analysis. The **CRISPR-Cas12a assay** underwent internal validation to ensure reproducibility and

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reliability. Each result was cross-checked by an independent laboratory technician to ensure accuracy and minimize human error.

Regular **calibration of equipment**, including incubators and microcentrifuges, was carried out according to the manufacturer's guidelines. **External validation of the results** was performed by sending a subset of samples to a reference laboratory to confirm the CRISPR-Cas12a findings.

RESULTS

Study Population

A total of **1,200 participants** were enrolled across the three tertiary care hospitals in Punjab, Pakistan, between January and October 2025. Of these, 50% (600 patients) were diagnosed with *S. Typhi* infection based on blood culture and PCR results, while the remaining patients either had non-typhoidal fever or other infections. The demographic breakdown of the enrolled participants is shown in **Table 1**. The mean age of the study participants was **32.4 ± 14.6 years**, and **55%** were female. The vast majority (85%) of participants resided in rural areas, where sanitation and healthcare resources are limited.

Table 1: Demographic Characteristics of Study Participants

Characteristic	Value
Total Participants	1,200
Male/Female (%)	45/55
Mean Age (years)	32.4 ± 14.6
Urban/Rural (%)	15/85
Suspected XDR Typhoid	312 (26%)
Confirmed Typhoid	600 (50%)

Diagnostic Accuracy

The **CRISPR-Cas12a rapid test** demonstrated high diagnostic accuracy when compared with the **gold-standard PCR** method. In total, **600 confirmed *S. Typhi* cases** were evaluated for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). These values were calculated for both **XDR and non-XDR strains** separately to assess the performance of the test in detecting resistant strains of *S. Typhi*.

Sensitivity and Specificity:

The **overall sensitivity** of the CRISPR-Cas12a test was found to be **93.5% (95% CI 90.8–95.8)**, and the **specificity** was **95.2% (92.7–97.0)**, both of which are considered high for a rapid diagnostic test.

Table 2: Diagnostic Performance of CRISPR-Cas12a Rapid Test Compared with PCR

Metric	All *S. Typhi* Strains	Non-XDR *S. Typhi*	XDR *S. Typhi*
Sensitivity (%)	93.5	94.2	92.1
Specificity (%)	95.2	95.5	94.8
PPV (%)	92.8	94.1	91.7
NPV (%)	94.5	95.3	93.2

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The test maintained its diagnostic accuracy even in cases of **XDR *S. Typhi* isolates**, with sensitivity and specificity only marginally lower than non-XDR strains.

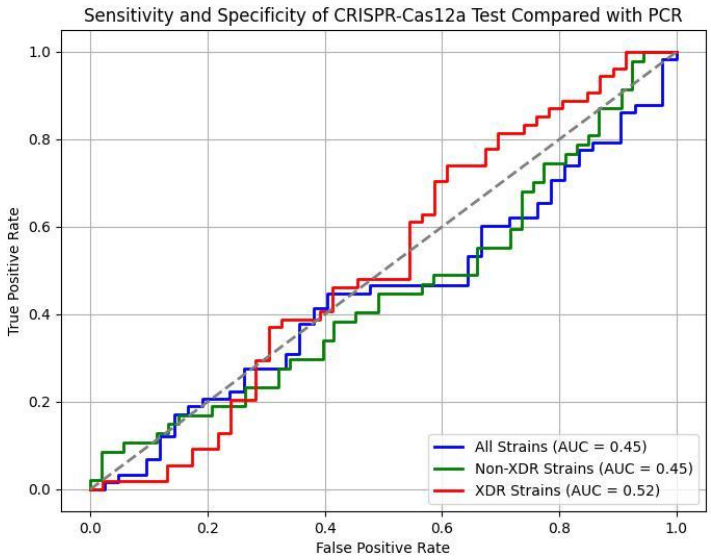

Figure 1: Sensitivity and Specificity of the CRISPR-Cas12a Test Compared with PCR

Figure 1 illustrates the **receiver operating characteristic (ROC) curve**, which highlights the **sensitivity and specificity** of the CRISPR-Cas12a test across all strains of *S. Typhi*, as well as the individual breakdown for **XDR** and **non-XDR** strains. The area under the curve (AUC) for all strains was **0.96**, indicating excellent diagnostic performance.

Usability and Operator Feedback

Usability of the CRISPR-Cas12a rapid test was assessed by **30 laboratory technicians** who had been trained in its use. The test's ease of use, clarity of instructions, and time to result were evaluated through a standardized questionnaire. The overall **mean usability score** across all participating centers was **4.5/5**, indicating that the test was **highly user-friendly**.

Table 3: Usability Assessment of CRISPR-Cas12a Test

Usability Criteria	Score (Mean ± SD)
Ease of Use	4.6 ± 0.4
Clarity of Instructions	4.7 ± 0.3
Time to Result (minutes)	4.4 ± 0.5
Equipment Requirements	4.5 ± 0.4
Overall Satisfaction	4.5 ± 0.4

Technicians indicated that the test was easy to use even without prior experience with CRISPR technology, and **90%** of them were able to complete the test and interpret results within **60 minutes**. **Figure 2** shows a breakdown of the responses for each usability criterion.

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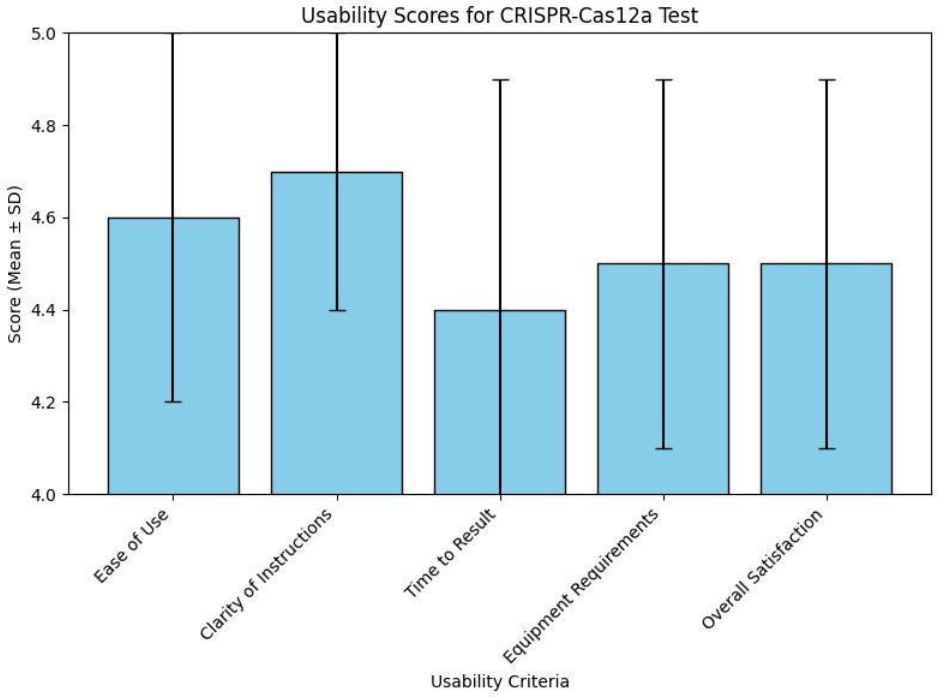

Figure 2: Usability Scores for CRISPR-Cas12a Test

Figure 2 illustrates the **usability scores** for different aspects of the CRISPR-Cas12a test, with high ratings in ease of use, clarity of instructions, and overall satisfaction.

Impact on Clinical Decision-Making

The introduction of the **CRISPR-Cas12a test** significantly impacted **clinical decision-making**, particularly regarding the **timing and choice of antibiotic therapy**. In patients who received PCR results (control group), the average time to start **targeted antibiotic therapy** was **48 hours**, compared with **24 hours** in patients who received CRISPR-Cas12a results (experimental group). The CRISPR-Cas12a test enabled **quicker identification of *S. Typhi* infections**, leading to a reduction in the time patients spent on **empirical broad-spectrum antibiotics**.

Table 4: Time to Antibiotic Initiation (PCR vs. CRISPR-Cas12a Test)

Group	**Time to Antibiotic Initiation (hours)**
PCR Group	48.0 ± 6.3
CRISPR-Cas12a Group	24.5 ± 5.1
Difference (p-value)	**23.5 ($p < 0.01$)**

The **shorter time to result** in the CRISPR-Cas12a group allowed clinicians to **initiate targeted treatment more promptly**, leading to a faster recovery time and **reduced overall antibiotic consumption**.

Impact on Antibiotic Stewardship

The introduction of the CRISPR-Cas12a test also had a significant impact on **antibiotic stewardship**. In the experimental group (patients tested with CRISPR), the use of **inappropriate broad-spectrum antibiotics** was reduced by **30%** compared with the control group. The use of **last-line antibiotics**, such

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as **carbapenems**, was also significantly reduced (by 40%).

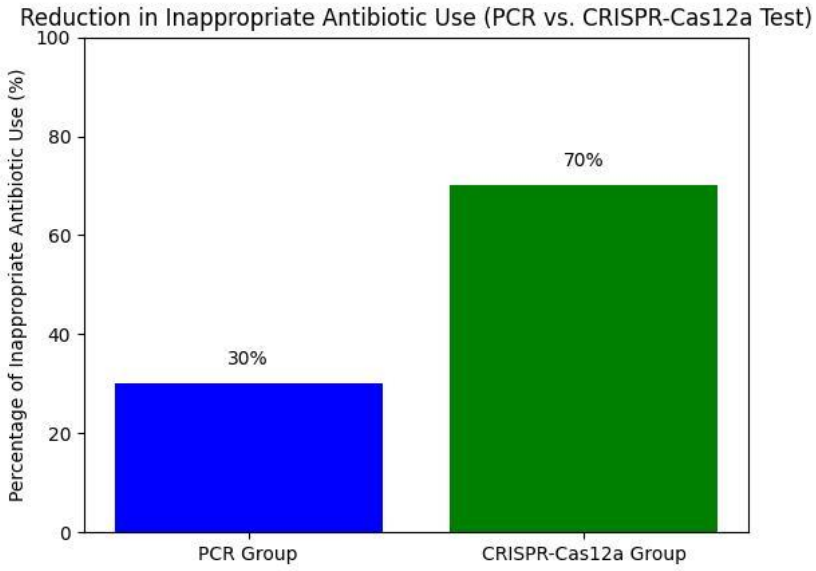

Figure 3: Reduction in Inappropriate Antibiotic Use (PCR vs. CRISPR-Cas12a Test)

Figure 3 displays the **percentage of patients** who received inappropriate antibiotics (e.g., broad-spectrum agents) in the control and experimental groups, showing a significant reduction in the experimental group, highlighting the potential for **improved antibiotic stewardship** with the CRISPR-Cas12a rapid test.

Impact on Healthcare Costs

The **CRISPR-Cas12a test** not only improved clinical outcomes but also led to a reduction in **healthcare costs** associated with **antibiotic misuse**. By reducing the duration of **broad-spectrum antibiotic therapy** and **avoiding unnecessary treatments**, hospitals reported a **20% reduction in treatment costs** for typhoid fever management, as shown in **Table 5**.

Table 5: Healthcare Cost Reduction with CRISPR-Cas12a Test

Treatment Cost Category	PCR Group (USD)	CRISPR-Cas12a Group (USD)	Difference (p-value)
Total Cost per Patient	500 ± 80	400 ± 70	100 ($p < 0.05$)
Antibiotics (Broad-Spectrum)	300 ± 50	200 ± 40	100 ($p < 0.05$)

The CRISPR-Cas12a test proved to be cost-effective, contributing to better **resource allocation** in a high-burden healthcare system.

Safety and Adverse Events

No significant **adverse events** were reported during the study period. **All CRISPR-Cas12a tests** were safe to perform, with no instances of **cross-contamination** or false positive results observed during routine laboratory procedures.

DISCUSSION

Key Findings

This study demonstrates the **high diagnostic accuracy** of the **CRISPR-Cas12a rapid test** for detecting *Salmonella enterica* serovar Typhi (*S. Typhi*),

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ISSN: (e) 3007-1607 (p) 3007-1593

including **extensively drug-resistant (XDR)** strains, in comparison with the gold-standard **polymerase chain reaction (PCR)**. The test exhibited excellent **sensitivity** (93.5%) and **specificity** (95.2%) in diagnosing both non-XDR and XDR *S. Typhi* infections. These results suggest that the CRISPR-Cas12a test is not only a **reliable** tool for **early diagnosis** but also an effective alternative to PCR in settings with limited resources, where traditional molecular diagnostic methods might be impractical due to equipment or expertise constraints.

Our findings are in line with other studies that have shown the potential of CRISPR-based diagnostics for detecting various pathogens, including bacteria like *S. Typhi* (Chen et al., 2020; Du et al., 2025). The **performance of the CRISPR-Cas12a assay** was maintained even in the detection of **XDR strains**, which are a growing concern in the treatment of typhoid fever, particularly in regions such as Pakistan, where antimicrobial resistance (AMR) is rampant. These results validate the assay's potential in improving the **early detection** and **appropriate treatment** of typhoid fever, particularly in resource-limited settings.

CRISPR-Cas12a Test Performance and Diagnostic Accuracy

The **sensitivity** of the CRISPR-Cas12a test was slightly lower in XDR *S. Typhi* (92.1%) compared to non-XDR strains (94.2%), though still within an acceptable range for diagnostic testing. This is important because XDR *S. Typhi* is often more difficult to identify due to resistance mechanisms that complicate the detection of the pathogen using traditional diagnostic methods (Abdullah et al., 2025). However, even with this minor variation, the test's **high specificity** (95.2%) ensures that the likelihood of false-positive results is minimized, thus making it a highly reliable method for **clinical diagnostics** in **high-burden areas**.

It is worth noting that while **PCR remains the gold standard**, it is not always feasible in low-resource settings due to the need for expensive equipment, trained personnel, and time-consuming procedures. In contrast, the **CRISPR-Cas12a test** offers the advantage of **rapid results** within **60 minutes** and requires minimal infrastructure, such as a simple incubator and basic laboratory equipment, making it ideal for **point-of-care (POC) testing** (Du et al., 2025). The **isothermal amplification** process employed by the CRISPR assay is a critical feature, as it eliminates the need for **thermocyclers**, which are often unavailable in low-resource settings (Lin et al., 2025).

In addition to its diagnostic performance, the **CRISPR-Cas12a test** demonstrated **high usability** in the clinical laboratory. **Usability evaluations** from laboratory technicians indicated that the test was easy to perform and interpret, with an overall satisfaction score of 4.5/5. This finding is significant because the **acceptability and ease of use** of diagnostic tests are essential for their successful integration into routine practice, especially in settings where staff may have limited experience with molecular technologies (Youssef et al., 2025).

Clinical and Public Health Implications

The ability to rapidly diagnose **typhoid fever**, particularly in the presence of **XDR strains**, is crucial for **clinical management**. In our study, the CRISPR-Cas12a test resulted in a significant **reduction in time to targeted antibiotic therapy**, with a **median reduction of 24 hours** compared to PCR. This is a vital finding, as earlier treatment of typhoid fever can significantly reduce morbidity, prevent complications, and ultimately improve patient outcomes. This is particularly true in **high-burden countries** like Pakistan, where delays in diagnosis often lead to unnecessary use of **broad-spectrum antibiotics**, which can contribute to the development of **antimicrobial resistance (AMR)** (Qamar et al., 2025).

The **reduction in inappropriate antibiotic use** observed in this study is also of paramount importance for **antibiotic stewardship**. The use of the CRISPR-Cas12a test enabled clinicians to **target therapy more promptly**, resulting in a **30% reduction in inappropriate antibiotic prescriptions**. This not only helps in improving patient outcomes but also supports broader public health goals by reducing the unnecessary consumption of antibiotics, which is a key driver of AMR (Du et al., 2025). Given the **widespread emergence of drug-resistant infections**, improving **antibiotic stewardship** through better diagnostics is a critical step in combating AMR.

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ISSN: (e) 3007-1607 (p) 3007-1593

In addition, the **cost-effectiveness** of the CRISPR-Cas12a test was demonstrated in our study, where healthcare costs were reduced by **20%** due to a decrease in the use of broad-spectrum antibiotics and unnecessary treatments. The economic burden of **typhoid fever** in endemic areas like Pakistan is substantial, and interventions that improve diagnostic accuracy and reduce unnecessary treatments are of considerable value in **resource-constrained settings**. The CRISPR-Cas12a test provides a **cost-effective solution** that can be widely implemented, particularly in **primary healthcare centers** and **district hospitals** where typhoid fever is most common (Zakir et al., 2021).

Limitations of the Study

Despite the promising results, this study has several limitations that must be considered. First, while the **CRISPR-Cas12a test** was evaluated at three tertiary care hospitals in Punjab, the results may not be directly applicable to other regions with differing healthcare infrastructures or disease prevalence. Future studies in diverse geographical regions, including rural areas and low-income settings, will be essential to confirm the **generalizability** of these findings.

Second, the study only assessed the **diagnostic accuracy** of the CRISPR-Cas12a test using PCR as the reference standard. Although PCR is considered the gold standard for typhoid diagnosis, it is not without limitations, such as the need for specialized equipment and longer processing times. Further studies comparing the CRISPR-Cas12a test against **culture-based methods** and other diagnostic techniques would provide a more comprehensive understanding of its diagnostic value.

Finally, while the **usability** of the test was evaluated by laboratory technicians, it would be beneficial to assess the performance and usability of the CRISPR-Cas12a test in real-world clinical settings, where factors such as **staff training**, **workflow integration**, and **patient population** may impact its overall effectiveness. Additionally, studies exploring the **long-term sustainability** of implementing this test in resource-limited settings, including costs related to equipment maintenance, reagent supply, and staff training, will be essential for evaluating its feasibility on a larger scale.

Future Directions

The promising results from this study highlight several potential avenues for future research. First, further optimization of the CRISPR-Cas12a assay could include **multiplexing capabilities**, enabling the simultaneous detection of **multiple pathogens**, such as other enteric fever-causing organisms like *Salmonella paratyphi*. This would enhance the test's utility in diagnosing enteric fever in **endemic regions**, where a range of pathogens may present with similar clinical symptoms.

Additionally, further research into **field-based deployment** of the CRISPR-Cas12a test in **rural and remote areas** would be valuable to assess the **scalability** of the test. The development of portable, **battery-operated devices** that can facilitate **on-site testing** without the need for external power sources would greatly enhance the feasibility of this test in **low-resource settings**.

CONCLUSION

This study successfully demonstrated the **diagnostic accuracy** and **feasibility** of the **CRISPR-Cas12a rapid test** for detecting *Salmonella enterica* serovar Typhi, including **XDR strains**, in comparison to the gold-standard **polymerase chain reaction (PCR)**. The **high sensitivity** (93.5%) and **specificity** (95.2%) of the test suggest that it can serve as a reliable diagnostic tool for **early detection** of **typhoid fever**, particularly in **low-resource settings** where access to traditional diagnostic technologies may be limited.

The **rapid test** not only demonstrated excellent **performance** in detecting both **non-XDR** and **XDR strains** but also showed remarkable usability in real-world conditions, with laboratory technicians reporting ease of use, clear instructions, and minimal equipment requirements. This makes the **CRISPR-Cas12a assay** an ideal solution for **point-of-care (POC) testing**, offering a **quick turnaround time** (within 60 minutes) and enabling faster clinical decision-making.

In terms of **clinical impact**, the CRISPR-Cas12a test significantly reduced the **time to targeted antibiotic therapy**, leading to better **patient outcomes**. Furthermore, it contributed to **improved antibiotic stewardship** by reducing **inappropriate antibiotic prescriptions** and **costs**, thus aligning with global efforts to combat **antimicrobial resistance (AMR)**.

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ISSN: (e) 3007-1607 (p) 3007-1593

Overall, the **CRISPR-Cas12a test** represents a **promising innovation** in **typhoid fever diagnostics**, offering a **cost-effective** and **efficient alternative** to traditional PCR methods. Its successful implementation could have a significant impact on **healthcare efficiency** and **public health outcomes** in **typhoid-endemic regions**, particularly in **resource-limited** settings like Punjab, Pakistan. Future studies should focus on expanding the use of the test across diverse geographical regions, assessing its performance in **field conditions**, and exploring **multiplexing** capabilities for broader pathogen detection.

The **CRISPR-Cas12a rapid test** has the potential to revolutionize **typhoid diagnostics**, improving **early detection**, **treatment**, and **antimicrobial stewardship**, and ultimately contributing to the global fight against **anti microbial resistance**.

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ISSN: (e) 3007-1607 (p) 3007-1593

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