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Loop-Mediated Isothermal Amplification (LAMP) Assay for Rapid Detection of *Salmonella enterica* in Low-Resource Settings

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Abstract

Typhoid fever remains a significant global health burden, particularly in regions with inadequate sanitation and limited access to safe water supplies. Traditional diagnostic methods, such as blood culture and biochemical assays, although considered the best standards, are labor-intensive and time-consuming, thereby limiting their practical utility in resource-constrained settings. This study aimed to develop a loop-mediated isothermal amplification (LAMP) assay for the rapid detection of S. enterica using conserved genomic regions (367 bp) as targets. Unlike conventional PCR, which requires expensive thermal cyclers, our LAMP protocol utilizes a water bath to maintain a constant reaction temperature of 63°C for 90 minutes, significantly reducing equipment costs. Amplified products were rapidly detected by the addition of SYBR Green dye, which produced a distinct green fluorescence under blue light, enabling easy visual interpretation. Comparative analysis revealed that the LAMP assay demonstrated higher sensitivity—detecting Salmonella in 90% of isolates—compared to 85% for PCR, with both methods showing high specificity and no cross-reactivity with non-Salmonella species. These findings suggest that the LAMP assay provides a rapid, cost-effective, and sensitive alternative for typhoid diagnosis, offering promise for enhanced disease surveillance and management in low-resource settings.

Keywords: Typhoid fever, Salmonella enterica, loop-mediated isothermal amplification (LAMP),, rapid detection.

INTRODUCTION

Typhoid fever is a systemic infection caused by the human-restricted pathogen *Salmonella enterica*serovar Typhi (Manesh *et al.*, 2021) It is primarily transmitted via the faecal-oral route and results in acute systemic infections with potentially life-threatening complications such as intestinal perforations (Sukri *et al.*, 2024) Patients recovering from typhoid fever may also develop a chronic carrier state, contributing to the pathogen's persistence in human populations (Manesh *et al.*, 2021) The disease remains a major global health concern, particularly in low- and middle-income countries where access to clean water and sanitation is inadequate (Debellut *et al.*, 2024) According to the World Health Organization (Murthy *et al.*, 2025), an estimated 9–14 million cases of typhoid fever occur annually worldwide, leading to approximately 110,000–161,000 deaths each year. Children and young adults are the most vulnerable due to weaker immune defenses and increased exposure to contaminated food and water sources. Furthermore, the disease has a high prevalence and transmission rate in Sub-Saharan Africa (SSA), where over 7.2 million cases are reported annually, with an incidence rate of 762 per 100,000 people per year (Mahmoud *et al.*, 2023) This highlights the urgent need for targeted intervention strategies to control the spread of typhoid fever.

The persistence of typhoid fever is strongly linked to inadequate water, sanitation, and hygiene (WASH) infrastructure, particularly in urban slums and rural areas with poor drainage systems and open defecation practices (Debellut *et al.*, 2024) The WHO (2024) emphasizes that improving WASH services is crucial for reducing transmission rates. However, implementing sustainable sanitation infrastructure requires a financial and political commitment, which remains a challenge in many endemic regions. In addition to improving WASH services, vaccination programs, such as the Typhoid Conjugate Vaccine (TCV), have demonstrated significant effectiveness in reducing incidence rates (Murthy *et al.*, 2025). However, access to

these vaccines is still limited in some areas due to financial and logistical barriers (Kishore *et al.*, 2024). Despite the availability of antibiotics for treating typhoid fever, the increasing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Salmonella enterica* serovarTyphi has complicated treatment strategies (Murthy *et al.*, 2025) Therefore, a combination of preventive measures, early detection, and improved antibiotic stewardship is necessary to mitigate the global impact of typhoid fever (Debellut *et al.*, 2024) The disease continues to pose a significant public health challenge in Nigeria, where poor sanitation, antibiotic misuse, and limited healthcare access exacerbate the situation (Akinyemi *et al.*, 2018).

Accurate estimation of the actual burden of typhoid fever and other *Salmonella*-associated diseases is challenging due to the lack of comprehensive epidemiological surveillance systems, particularly in developing countries (Debellut *et al.*, 2024) In high-income regions such as the United States and Europe, typhoid fever is predominantly diagnosed in travelers returning from endemic areas (Teh *et al.*, 2014) However, in developing nations like Nigeria, typhoid fever remains endemic, particularly in densely populated urban centers with inadequate sanitation facilities and widespread misuse of antibiotics, which accelerate the development of antibiotic-resistant strains. A retrospective study conducted in Lagos, Nigeria, documented over 80,000 cases of Salmonella infections and 800 associated deaths over ten years (Akinyemi *et al.*, 2018). This alarming statistic highlights the urgent need for effective diagnostic and therapeutic strategies to control the spread of the disease. The persistent prevalence of typhoid fever in Nigeria highlights the need for improved diagnostic tools and enhanced public health interventions (Akinyemi *et al.*, 2018; Murthy *et al.*, 2025).

Current conventional methods for identifying *S. enterica* serovars rely on cell culture, colony counting, and biochemical tests (Cheesbrough, 2006) While these methods are effective, they are labor-intensive, time-consuming, and may take up to a week to yield definitive results (Sayad *et al.*, 2016) These limitations hinder timely diagnosis and management of the disease, particularly in resource-limited settings where laboratory infrastructure is inadequate (Debellut *et al.*, 2024) To address these setbacks, various molecular and immunological methods have been developed, including enzyme-linked immunosorbent assay (ELISA) (Kuhn *et al.*, 2015), microarray immunoassays (Yamasaki *et al.*, 2021), pulsed-field gel electrophoresis (PFGE) (Goay *et al.*, 2016), plasmid analysis (Wang *et al.*, 2018), ribotyping (Shi *et al.*, 2015), polymerase chain reaction (PCR) (Anejo-Okopi *et al.*, 2016), and genome sequencing (Rao & Sun, 2015). For a definitive diagnosis of typhoid fever, WHO recommends bacterial isolation from blood or bone marrow samples. Bone marrow culture, obtained through aspiration of the iliac crest or sternum, is considered the gold standard, with a suggested sensitivity of 90% after four days of culture (Murthy *et al.*, 2025) However, due to the invasive nature of bone marrow biopsies, the diagnosis typically depends on blood culture or the Widal test (Mahmoud *et al.*, 2023) The Widal test, although widely used in developing countries, has limitations in specificity and sensitivity, leading to frequent misdiagnosis and unnecessary antibiotic use (Tegene & Eshetie, 2025).

While PCR has revolutionized pathogen detection with high sensitivity and specificity, its widespread application in low-resource settings is limited by the need for expensive equipment, trained personnel, and sophisticated infrastructure (Neupane *et al.*, 2021). To address these limitations, isothermal nucleic acid amplification techniques such as loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and helicase-dependent amplification (HDA) have emerged as practical alternatives (Neupane *et al.*, 2021; Sohrab *et al.*, 2022).

LAMP stands out among these methods for its speed, sensitivity, and simplicity. It amplifies DNA at a constant temperature (60–65 °C) using 4–6 primers that target 6–8 regions of the gene and a strand-displacing Bst polymerase, producing up to 10^9 copies in under an hour (Notomi *et al.*, 2015; Oliveira *et al.*, 2021) LAMP requires no thermal cycling or initial denaturation and is less affected by inhibitors, which enhances its robustness in unpurified samples (Oliveira *et al.*, 2021) Furthermore, its results can be visualized through turbidity or simple colorimetric changes, enabling naked-eye detection without sophisticated instrumentation—ideal for field diagnostics (Sohrab *et al.*, 2022).

Compared to LAMP, RPA functions at a lower temperature (37–42 °C) using only two primers and achieves amplification in 20–40 minutes. It

is known for its rapid turnaround and tolerance to inhibitors (Sohrab *et al.*, 2022; Zou *et al.*, 2020). However, RPA requires proprietary enzymes and is prone to nonspecific amplification if primers are not highly optimized. HDA, on the other hand, uses helicase and accessory proteins to unwind and amplify DNA but tends to be slower and less sensitive, making it less suitable for rapid diagnostics (Sohrab *et al.*, 2022).

LAMP was selected for this study because it combines high specificity, rapid amplification, and low equipment requirements, making it particularly suitable for detecting Salmonella *enterica* in resource-limited laboratory environments. Unlike RPA or HDA, LAMP provides a balance of robust amplification, visual result interpretation, and field adaptability (Oliveira *et al.*, 2021; Sohrab *et al.*, 2022; Zou *et al.*, 2020). Its demonstrated superior sensitivity—10 to 100 times that of PCR—further justifies its use in this context (Oliveira *et al.*, 2021).

In this research, we aim to develop a simple yet efficient method for the rapid detection of *S. enterica* using the LAMP platform. The objectives of this study are therefore (1). To isolate and identify *Salmonella enterica* using conventional methods. (2) To develop a rapid and efficient LAMP method for detecting *S. enterica* based on conserved genomic sequences.

METHODOLOGY

Twenty clinical isolates of *S. enterica* were collected from different hospitals, including Rasheed Shekoni Teaching Hospital (8), General Hospital Dutse (7) and Shirbaline Clinic (5) all in Dutse, Jigawa State, Nigeria. The isolates were first inoculated into Selenite F broth for selection and enrichment, after 24h, each samples was plated onto *Salmonella Shigella* Agar (SSA), isolates that produced opaque colonies with black centres after 24h were then subcultured onto Xylose Lysine Deoxycholate Agar. After 24h colonies that appeared red in colour with black centres on the XLD were subcultured again onto Nutrient Agar plates to obtain pure cultures. Each culture was subjected to a series of biochemical tests to confirm its identity as *S. enterica* before proceeding to molecular methods. The biochemical tests included triple sugar iron (TSI) agar for carbohydrate fermentation and hydrogen sulfide (H_2S) production, citrate utilization test (CT), urease test (UT), lysine decarboxylase test (LT), indole test (IT), Methyl Red Test (MR) Vogues Proskeur Test (VP) and motility test (MT). These tests represent the current gold standards for confirming the presence of *S. enterica* (Dhayananth, 2024).

Following biochemical confirmation, DNA extraction was performed using a heat treatment method (Lee *et al.*, (2020). A loopful of bacterial colonies was suspended in 100 µL of double-distilled water, heated at 99 °C for 5 minutes, and immediately chilled on ice for 10 minutes. The crude cell lysate was centrifuged at 13,400 rpm for 3 minutes, and the supernatant containing the extracted DNA was used as the template for PCR and LAMP assays. The purity of the extracted DNA was evaluated using spectrophotometric analysis, as indicated by the A260/A280 ratio, where a value between 1.8 and 2.0 indicates pure DNA (Lee *et al.*, 2020).

For the PCR amplification, *Salmonella*-specific primers, targeting the flagella antigen gene *fliC*((Mina *et al.*, 2024) as shown in Table 1 were used. The PCR reaction was carried out in a 25 µL volume containing 1X PCR buffer, 1 mM MgCl2, 0.02 mM of each deoxynucleoside triphosphate (dNTP), 0.4 µM of each primer, 1 U of Taq DNA polymerase, and 5 µL of DNA template (~50 ng/µL). The thermal cycling conditions included an initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of 95 °C for 50 seconds, 55 °C for 1.5 minutes, and 72 °C for 2 minutes, with a final extension at 72 °C for 7 minutes. The PCR products were analyzed on a 1.5% agarose gel and visualized using a gel documentation system (Mina *et al.*, 2024).

Table 1: Primers for PCR Amplification of *fliC* Gene from *Salmonella* Isolates

Gene	Primers (5'-3')	Amplicon Size (bp)	Reference
fliC	F: ACTGCTAAAACCACTACT R: TGGAGACTTCGGTCGCGTAG	367	(Mina *et al.*, 2024)

Primers for the loop-mediated isothermal amplification (LAMP) assay targeting *Salmonella enterica were adopted from* Yang *et al.* (2018). These primers were validated through BLAST

analysis on the NCBI server to ensure specificity to conserved regions of the invA gene. The invA gene was selected due to its critical role as a marker for *Salmonella spp*. detection in over 74% of published assays. This gene is conserved across multiple *Salmonella* serovars and offers robust diagnostic reliability (Nagamine *et al.*, 2002).

Table 2: LAMP Assay Primers

Primer	Sequence (5′-3′)	Notes	Reference
F3	GAACGTGTCGCGGAAGTC	Forward outer primer	(Yang *et al.*, 2018)
B3	CGCAATAGCGTCACCTT	Reverse outer primer	(Yang *et al.*, 2018)
FIP	GCCGCGCATCCGCATCAATA-TCTGGATGGTATGCCCGG	Forward inner primer	(Yang *et al.*, 2018)
BIP	GCGAACGCGGAAGCGTACTG-TCGCACCGTCAAAGGAAC	Backward-inner Primer	(Yang *et al.*, 2018)
LF	TCAAATCGGGCATCAATACTCATG	Loop forward primer	(Yang *et al.*, 2018)
LB	AAAGGGAAGCGCAGCTTTACG	Loop-backward primer	(Yang *et al.*, 2018)

Loop-mediated isothermal amplification (LAMP) was performed using a Loopamp DNA amplification kit (Eiken Chemical Co., Ltd., Japan). The reaction mixture, with a final volume of 25 µL, consisted of 12.5 µL of 2X reaction mix, 40 pmol of each primer (FIP, BIP, F3, B3, LF, LB), 8 U of Bst DNA polymerase, and 2.5 µL of DNA template. The reaction was incubated at 63°C for 90 minutes in a Scantrik Medical Equipment Laboratory Water Bath model HH-4, followed by enzyme inactivation at 80°C for 2 minutes using a separate Scantrik Medical Equipment Laboratory Water Bath model HH-4. For visualization of the LAMP products, 1 µL of 10X SYBR Green I dye (Guangzhou Dongsheng Biotech Co., Ltd.) was added to each reaction tube after the amplification was completed. The tubes were then gently mixed by pipetting. The presence of amplified DNA was determined by visual inspection under blue light illumination (470 nm) using a VisionMed TI470 Transilluminator. A distinct green fluorescence indicated a positive reaction, while a negative reaction showed no visible fluorescence. The sensitivity of the LAMP assay, along with a comparative PCR assay, was evaluated using known cultures of bacterial isolates. Distilled water was used as a negative control to confirm the absence of contamination during the amplification process. The design and optimization of the LAMP primers followed the principles described by Notomi *et al.* (2015). The sensitivity of both PCR and LAMP assays was assessed by subjecting known cultures of other bacterial isolates to amplification, with distilled water serving as a negative control (Notomi *et al.*, 2015).

RESULTS

Twenty clinical isolates of *Salmonella* enterica, obtained from clinical samples collected at Rasheed Shekoni Teaching Hospital, General Hospital Dutse, and Shirbaline Clinic, were subjected to confirmatory identification using a combination of cultural, morphological, and biochemical techniques. The phenotypic evaluation involved both colonial observations on selective media and standardized biochemical assays commonly used in clinical microbiology for identifying enteric pathogens.

On Salmonella-Shigella Agar (SSA), all isolates produced round, colourless colonies that were flat in elevation and dry in texture. The colonies exhibited a non-mucoid appearance, a typical trait of *Salmonella* species. Gram staining of the isolates revealed they were Gram-negative rods, consistent with the known morphology of *S.* enterica. On Xylose Lysine Deoxycholate (XLD) agar, the colonies appeared red with characteristic black centres, indicating hydrogen sulfide production.

In the biochemical tests, all isolates produced a triple sugar iron (TSI) reaction characterized by a red slant with a yellow butt and a black precipitate, indicating glucose fermentation with hydrogen sulfide (H_2S) production, but no lactose or sucrose fermentation. Urease activity was negative in all cases, further supporting the identity as *Salmonella*, which typically lacks urease activity. The Methyl Red (MR) test yielded positive results, indicating the production of stable acids through glucose

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fermentation. All isolates tested negative for the Voges-Proskauer (VP) test and the Indole test, consistent with the expected biochemical profile of *S.* enterica. Citrate utilization was positive, indicating the organism's ability to utilize citrate as a sole carbon source. Lysine decarboxylase activity was also positive in all isolates, and motility was confirmed via positive results in the motility test medium, further supporting the identification as motile *Salmonella* species. Collectively, these cultural and biochemical characteristics matched the classical description of *Salmonella* enterica, confirming the phenotypic identity of all 20 isolates (Dhayananth, 2024)

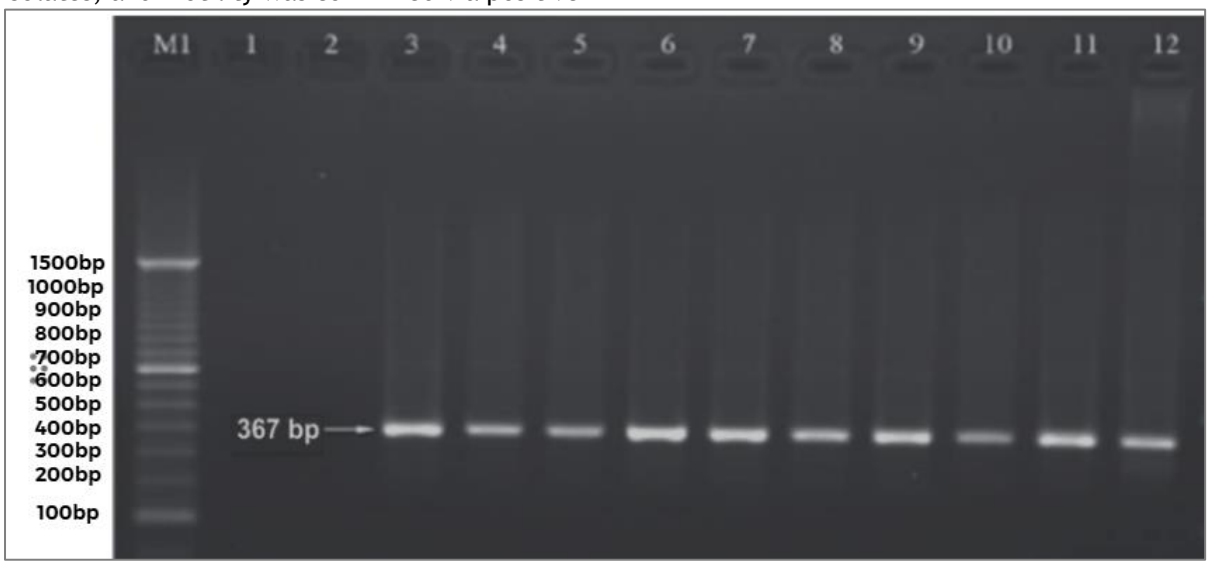

Figure 1: Gel Documentation of PCR Products of Salmonella Isolates
Key: Lane M1: DNA Ladder, Lanes 3-12: Clinical Isolates

Subsequent DNA extraction was performed using a simple heat lysis method. The quality and purity of the extracted DNA were assessed by spectrophotometry, and the A260/A280 ratio ranged from 1.8 to 2.0, indicating the presence of good-quality nucleic acids free from protein contamination. This confirmed that the extraction method was suitable for downstream molecular applications without the need for further purification. PCR amplification was then carried out targeting the *fliC* gene, which encodes the phase 1 flagellin protein and serves as a reliable molecular marker for *S.* enterica. Specific primers designed for the *fliC* gene yielded the expected 367 bp amplicon in 17 of the 20 isolates tested. The PCR products were visualized through agarose gel electrophoresis and are documented in Figure 1. The absence of amplification in two isolates could be attributed to a low template concentration, degraded DNA, or a possible absence/mutation in the target gene region.

The LAMP assay was designed to target the invA gene, a well-established diagnostic marker for *Salmonella enterica*. Positive results were observed in 18 of the 20 isolates, as confirmed through SYBR Green I dye visualization. Representative results are presented in Figure 2.

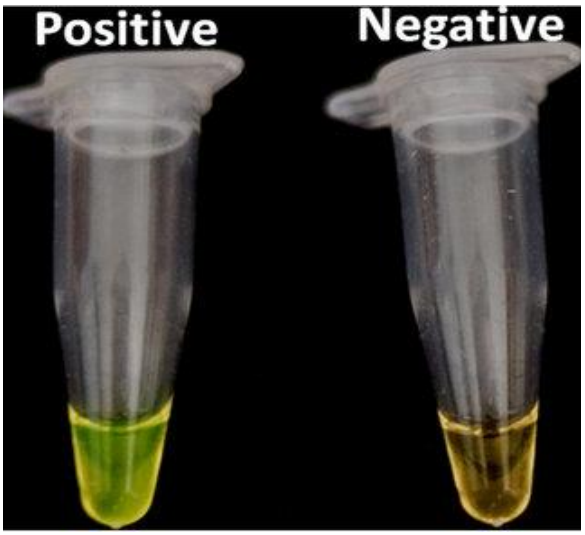

Figure 2: SYBR Green I dye visualization

The specificity of both PCR and LAMP assays was evaluated using negative controls and non-*Salmonella* isolates. Both assays displayed specificity for amplification of *S. enterica*. The results showed that the Lamp assay had more sensitivity than the PCR assay, as 18 (90%) of the isolates showed a change in color to green within 1 hour, while only 17 (85%) of the PCR samples produced amplicons within the expected range.

Table 3: Specificity Testing of PCR and LAMP Assays

Bacterial Species	PCR Result	LAMP Result
Salmonella enterica	Positive	Positive
Escherichia coli	Negative	Negative
Klebsiella pneumoniae	Negative	Negative
Staphylococcus aureus	Negative	Negative

Two (2) of the isolates in this study were subjected to molecular analysis, and the 16S rRNA sequence signified the isolates to have 99.83% and 99.81% similarity with *Salmonella enterica* subsp enteric serovar Typhi BTSB492 and *Salmonella enterica* AT MJH isolates in the gene bank with accession numbers PQ628081.1 and LC773422.1, respectively. The accession numbers of the isolates are PV715987 and PV734124, respectively.

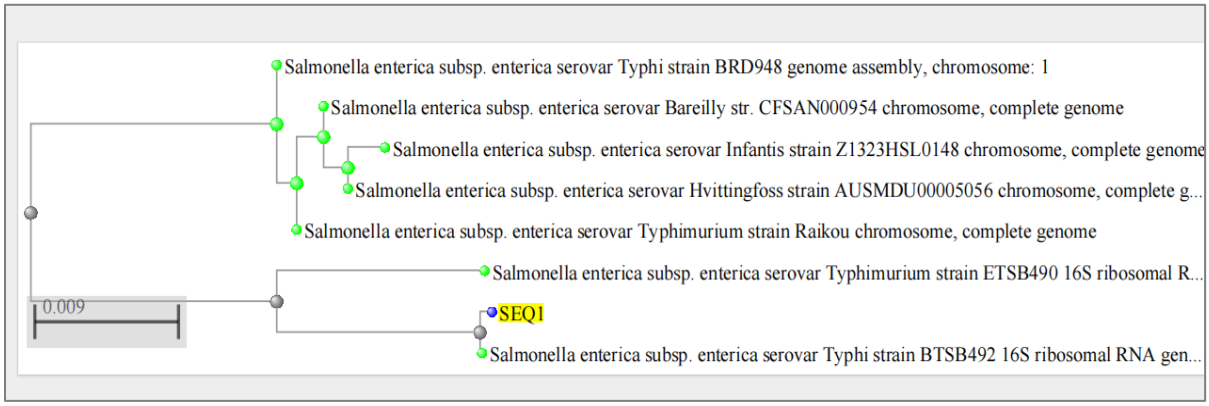

Figure 3: Phylogenetic Tree of *Salmonella enterica* Isolates.

DISCUSSION

This study aimed to develop a rapid, cost-effective loop-mediated isothermal amplification (LAMP) assay for detecting *Salmonella enterica* using a water bath instead of a thermal cycler, addressing the need for accessible diagnostic tools in low-resource settings. While conventional microbiological techniques remain the gold standard for *Salmonella* identification (Bell *et al.*, 2016) Polymerase Chain Reaction (PCR), though highly specific and widely regarded as a superior molecular tool, demands expensive equipment, technical expertise, and may have limitations in detecting low pathogen loads (Sunar *et al.*, 2016) Given these constraints, LAMP has emerged as a viable alternative due to its rapidity, cost-effectiveness, and minimal equipment requirements.

The Polymerase Chain Reaction produced a 367 bp amplicon in 85% of isolates, demonstrating high specificity, as no cross-reactivity was observed with non-*Salmonella* species. However, its sensitivity was slightly lower than that of the LAMP assay, suggesting that PCR may not always be the most efficient tool for low-burden infections. The LAMP assay detected *Salmonella* in 90% of isolates, confirming its superior sensitivity compared to PCR. The use of SYBR Green I dye enabled rapid and clear visual differentiation between positive and negative reactions, making it a practical and accessible method for quick diagnosis. The innovative use of a water bath for temperature maintenance reduced costs and improved practicality in resource-limited settings. This finding aligns with those of Ou *et al.* (2021) and Wang *et al.* (2018), who reported that LAMP assays offer greater sensitivity and faster results than PCR.

Furthermore, Ou *et al.* (2021 reported a 97.4% sensitivity for LAMP using real-time fluorescence and 89.5% using visual observation. As such, the 90% sensitivity observed in this research closely aligns with their findings, reinforcing the reliability of our methodology. Similarly, our specificity results align with those of Fan et al. (2015), who demonstrated that LAMP successfully amplified target genes in S. enterica while avoiding nonspecific amplification in non-Salmonella strains, thereby supporting its utility as a diagnostic tool.

However, our LAMP sensitivity was slightly lower than the 97.7% reported by Edel et al. (2023). This variation may be attributed to differences in primer design, target genes, or sample size. Additionally, while our LAMP assay detected *Salmonella* in 90% of isolates, studies by (Hara-Kudo *et al.*, 2005) demonstrated that LAMP assays could detect *Salmonella* at levels as low as 10^2 CFU/mL, significantly lower than the detection limits of conventional PCR (Berenger *et al.*, 2022) This suggests that further optimization of the LAMP assay, including refining primer sequences and enhancing reaction conditions, could improve its sensitivity and broaden its application in clinical diagnostics.

LAMP may be more specific than PCR due to its use of multiple primers targeting six to eight distinct regions of the target gene, compared to PCR's reliance on two primers (Soroka *et al.*, 2021) This multi-primer approach enhances specificity by reducing the likelihood of nonspecific amplification (Soroka *et al.*, 2021) Additionally, LAMP's strand-displacement polymerase provides continuous amplification without requiring thermal cycling, reducing the risk of amplification artifacts commonly observed in PCR (Garg *et al.*, 2022) Furthermore, LAMP does not require stringent reaction conditions, making it less susceptible to variations in sample purity and inhibitors, which often affect PCR efficiency (Soroka *et al.*, 2021) These factors collectively contribute to LAMP's superior specificity and reliability, particularly in complex sample matrices where PCR may fail to amplify due to inhibitory substances (Garg *et al.*, 2022).

Despite LAMP's advantages, one challenge observed in this study is the need for rigorous primer optimization to ensure maximum specificity. No false positives were recorded, as all positive LAMP reactions corresponded with confirmed *Salmonella* isolates, indicating high assay accuracy. However, previous studies, such as those by Edel et al. (2023), have reported rare instances of cross-reactions with non-target species in LAMP assays, highlighting the necessity for careful primer selection and extensive validation. Implementing confirmatory techniques, such as sequencing or additional molecular assays, could further enhance diagnostic precision and ensure consistency across diverse sample sources.

Another potential limitation of the LAMP assay is the reliance on subjective visual interpretation of color changes when using SYBR Green I dye. While this method is convenient and cost-effective, real-time fluorescence detection could further enhance accuracy and reduce the likelihood of ambiguous results. Future studies could explore real-time fluorescence detection and integrate digital image analysis to improve sensitivity and specificity.

The confirmation of *Salmonella enterica* isolates (accesion numbers PV715987 and PV734124) underscores the reliability of the identification methods used in this study. This high degree of similarity validates the accuracy of the isolates' classification and highlights the importance of sequencing in bacterial diagnostics and epidemiological surveillance. The findings also reinforce the effectiveness of the loop-mediated isothermal amplification (LAMP) assay used for rapid *Salmonella* detection. LAMP demonstrated higher sensitivity than conventional PCR, which aligns with previous studies reporting that LAMP is an efficient and cost-effective diagnostic method for *S. enterica*in clinical and foodborne infections (Wang *et al.*, 2018) The strong agreement between LAMP and sequencing results suggests that LAMP can be a viable alternative to PCR for rapid screening, particularly in low-resource settings where access to thermal cyclers and sequencing facilities is limited. Combining LAMP for initial detection with 16S rRNA sequencing for confirmation provides a practical two-tiered diagnostic approach, where LAMP serves as a rapid, point-of-care tool and sequencing acts as a definitive method in specialized laboratories.

The findings of this study support the integration of molecular diagnostics into routine clinical practice to improve the detection and characterization of *Salmonella* infections. The combination of phenotypic screening, LAMP, and sequencing provides a more accurate and efficient diagnostic workflow, which is particularly beneficial for regions with limited access to advanced microbiological facilities. The confirmation of *S. enterica* isolates through sequencing strengthens the case for expanding the use of molecular tools in public health surveillance and outbreak response. Further research should focus on optimizing LAMP for broader applications, refining sequencing methodologies for real-time epidemiological monitoring, and exploring the role of whole-genome approaches in understanding the genetic evolution of *Salmonella* strains.

CONCLUSION

This study successfully established a loop-mediated isothermal amplification (LAMP) assay as a rapid, sensitive, and specific method for detecting Salmonella *enterica* from clinical isolates. While conventional phenotypic methods confirmed the identity of 20 isolates, the LAMP assay demonstrated superior sensitivity (90%) compared to PCR (85%) and enabled faster detection without the need for advanced equipment. Using a simple water bath for isothermal amplification and SYBR Green dye for visual detection, the assay proved highly effective for low-resource settings. The absence of cross-reactivity with non-Salmonella species further affirmed its diagnostic accuracy. These findings support the potential of LAMP as a cost-effective alternative to traditional methods, making it suitable for both clinical and field-based applications where the timely detection of typhoid pathogens is critical.

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