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Single-Gene Target PCR Assays for Identification of *Salmonella typhi*

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Abstract

Objectives: The present study focused on the molecular identification and evaluation of specific gene targets for the rapid and accurate detection of *Salmonella typhi* from blood samples of patients diagnosed with typhoid fever. **Methodology**: Three representative isolates were subjected to 16S rRNA gene sequencing, and their identity as *S. typhi* was confirmed via BLASTn analysis with 100% sequence identity and query coverage. To develop a DNA-based diagnostic tool, single gene-target PCR assays were optimized using six primer sets targeting *STY2020*, *STY2021*, *STY0201*, *STY0307*, *STY0322*, and *STY0326*. **Results**: Five of these assays successfully identified all *S. typhi* isolates with 100% sensitivity and specificity, while the *STY0322* assay failed to amplify any strains. Detection limit analysis using serially diluted genomic DNA revealed that *STY0307* was the most sensitive target (5.0 pg), followed by *STY0201* and *STY2021* (10 pg). Lower sensitivity was observed with *STY2020* and *STY0326* (50 pg), with only faint gel electrophoresis bands. These results indicate that *STY0307*, *STY0201*, and *STY2021* are highly reliable markers for *S. typhi* detection. Furthermore, low expression levels of *STY2020* and *STY0326* during infection underscore the need for further population-based studies. **Conclusion:** *STY0307* and *STY2021* emerged as the most promising gene targets for diagnostic purposes and could be leveraged for the development of low-cost, rapid, and accurate point-of-care diagnostics suitable for use in resource-limited settings.

Keywords: Diagnostic tool, Gene-specific markers, PCR assay, *Salmonella typhi*, Typhoid fever

1 Introduction

Salmonella enterica serovar typhi (*S. typhi*) is the Gram-negative bacterium leading cause of community-acquired bloodstream infections in many low- and middle-income countries[1, 2] and responsible for typhoid fever, a systemic infection characterized by prolonged fever, headache, and abdominal symptoms[2].

Salmonella enterica serovars *typhi*, *paratyphi* A, *paratyphi* B, and *paratyphi* C are collectively known as typhoidal *Salmonella*, which are human host-restricted bacteria responsible for typhoid and paratyphoid (enteric) fever. All other serovars are grouped as nontyphoidal *Salmonella* (NTS), which may infect a wide range of animals or be host-adapted to specific nonhuman species[3].

Accurate and rapid diagnosis of typhoid fever is crucial for effective treatment and to prevent the spread of the disease, especially in endemic regions. While traditional methods like blood culture remain the gold standard, they are often time-consuming and can have limited sensitivity, particularly if antibiotics have been initiated.

Molecular identification techniques, especially those based on the Polymerase Chain Reaction (PCR), have revolutionized *S.*

typhi detection by offering superior speed, sensitivity, and specificity. PCR-based methods amplify specific DNA sequences unique to the pathogen, allowing for its direct detection in clinical samples[4].

The success of PCR for *S. typhi* identification hinges on the careful selection of target genes that are both highly conserved within *S. typhi* and absent or significantly different in other *Salmonella* serovars and common commensal bacteria. Several genes have been explored as PCR targets, each with its own advantages and limitations.

The *tviA* gene (or *tviB*) within the *viaB* operon is frequently targeted due to its strong association with *S. typhi* and its essential role in Vi antigen production. PCR assays targeting *tviA* have shown high specificity for *S. typhi*[5, 6]. However, it's important to note that *S. paratyphi C* and some *S. dublin* strains also possess the Vi capsule, which can lead to false positives for *S. typhi* if not combined with other targets in a multiplex PCR. While *fliC-d* is characteristic of *S. typhi*, it is also found in some other *Salmonella* serovars (e.g., *S. muenchen*). Therefore, using *fliC-d* alone for *S. typhi* identification lacks absolute specificity. Similarly, the *tyv* gene is specific for *S. typhi*, while *prt* is found in *S. paratyphi A*. These can be useful for differentiating between typhoidal serovars[7]. However, like *fliC-d*, they may not be exclusively specific to *S. typhi* when considered in isolation from all other *Salmonella* serovars.

With the availability of whole-genome sequences, bioinformatic approaches have identified chromosomal genes that are highly conserved in *S. typhi* but absent or significantly divergent in other *Salmonella* serovars and non-Salmonella bacteria[8, 9]. These genes represent potentially more specific targets. Studies have reported several such genes (e.g., *STY0307, STY0322, STY0326, STY2020, STY2021, STY0201, stoD*) demonstrated 100% sensitivity and specificity when tested against panels of *S. typhi*, non-typhi *Salmonella*, and other bacterial isolates. These targets aim to overcome the limitations of previously used genes that might share homology with other serovars[10]. These target genes are reported in detecting *S. typhi* but their specificity needs to be evaluated with more clinical strains.

These newer, highly specific targets are increasingly being incorporated into single-gene PCR or multiplex PCR assays for robust and definitive *S. typhi* identification. In the present investigation, we aimed to analysed single-gene target PCR assays (*STY0201, STY0307, STY0322, STY0326, STY2020, STY2021*) for the reliable molecular identification of *Salmonella typhi* strains recovered from blood cultures of patients presenting with typhoid fever.

2 Materials and Methods

Screening and identification of *Salmonella typhi*

Salmonella typhi, the test organism, was isolated from blood samples obtained from patients clinically diagnosed with typhoid fever at Adesh Hospital, Bathinda. Isolation was conducted using Xylose Lysine Deoxycholate (XLD, Hi-media) selective agar. Briefly, 100 µl of blood was aseptically inoculated into 10 ml of nutrient broth and incubated at 37°C for 24 hours for enrichment. Subsequently, 100 µl of the enriched broth culture was spread onto XLD selective agar plates, which were incubated at 37°C for 24 hours or until bacterial colonies developed. All isolates used in this study were confirmed as S. typhi through conventional culture techniques, biochemical characterization, and 16S rRNA gene analysis.

Optimization of blood culture PCR assay

To increase PCR sensitivity, it's crucial to first remove inhibitory human DNA from the sample. Additionally, a short pre-incubation of blood with culture broth boosts PCR amplification by increasing target DNA concentration. The 2 ml blood was added to 20 ml of culture media [3% (w/v) ox bile/tryptone soya broth] in 50 ml falcon tubes (Tarson) and incubated for 5 hrs at 37°C. Bacteria were concentrated by centrifugation at 8000 rpm for 10 min and the supernatant was removed. The human DNA removal by mixing thoroughly with an equal volume of mammalian cell lysis buffer (2 M Na_2CO_2 pH 9.8, 1% Triton-X100) for three minutes at ambient temperature to allow for complete fragmentation of human chromatin into DNA fragments. After the incubation step an equal volume of neutralization buffer (1.0 M Tris–HCl, pH 4.5) was added in order to prevent further cell lysis. The samples were then centrifuged at 5000 rpm for 10 min. The supernatants were discarded and bacterial pellets were resuscitated in 200 µl of 1x phosphate buffered saline (PBS) and used for isolation of bacterial DNA by a conventional method using the NaOH/SDS approach[11]. Similarly isolated colonies on XLD media also explored for the DNA isolation by NaOH/SDS approach and DNA processed for PCR amplification.

DNA Purity & integrity

The purity and concentration of the extracted DNA were assessed using a Cary UV-Visible Spectrophotometer (Agilent Technologies). DNA concentration was determined by measuring absorbance at 260 nm, while the A260/280 ratio was used to evaluate DNA purity. The extracted DNA was diluted with MilliQ water to a final concentration of 50 ng/µL and stored at −20 °C for subsequent analyses. Genomic DNA integrity was evaluated by electrophoresis on a 0.8% agarose gel at 80 V (BIO-RAD), followed by staining with ethidium bromide and visualization using a Gel Doc EZ imager (BIO-RAD).

Optimization of PCR

After *S. typhi* DNA extraction from the different patients and the amplification were done on BIO-RAD T100 Thermocycler with a set of six primers selected from previous study[10].

Table 1: Primer sequences used for PCR amplification

Target genes	Primer labels	Primer sequences (5'-3')	Target lengths (bp)
STY0201	0201F	ATGCTTTTAAAAAACACAACATGG	1176
	0201R	TTACGGATAGGTGATTGAAAATTG	
STY0307	0307F	ATGAAACCTTTATTCTCAGTGC	495
	0307R	TTAGCGTAATTCCCAGAACC	
STY0322	0322F	ATGAAATATAAAAAAATAAGAG	678
	0322R	CTATGGATTCATTTCCATTTC	
STY0326	0326F	ATGAATACGAATAATTCACC	261
	0326R	TTACCCTCCCCATGTCAC	
STY2020	2020F	ATGCCTGTTATGCATAATTG	429
	2020R	TTATGCTGTTAACGAGTCGTC	
STY2021	2021F	ATGAGTTTAGCGCAGCCTAAATCC	732
	2021R	TTAGAAGTCTCCTGCCTGGAAAC	
16S rRNA	16SF	CAGGCCTAACACATGCAAGTC	1362
	16SR	GGGCGGTGTGTACAAGGC	

Each PCR assay was optimized to assess the effects and interactions of two main variables: S. typhi-specific gene primer concentrations and annealing temperatures, each tested at three levels (primer concentrations: 1.00, 1.50, and 2.00 µM; annealing temperatures: 50, 55, and 60°C). PCR amplification was performed in a total reaction volume of 20 µl, comprising 10 µl of 2× PCR master mix (SRL), 5 µl of DNA template, 2–4 µl of forward and reverse primers (combined), and MilliQ water to a final volume of 20 µl. The thermal cycling conditions were as follows: initial denaturation at 94°C for 2 minutes; 34 cycles of denaturation at 94°C for 45 seconds, annealing at 50–60°C for 45 seconds, and extension at 72°C for 45 seconds; with a final extension at 74°C for 5 minutes. PCR products were analyzed alongside a 3 kb molecular weight marker (SRL) on a 1.2% (w/v) agarose gel containing ethidium bromide and visualized using a Gel Doc EZ imager (BIO-RAD).

Sequencing

The purified PCR amplicons were submitted to Central University, Bathinda, for definitive identification through 16S rRNA region sequencing (Table. 1). The resulting nucleic acid sequences, derived from both forward and reverse cycle sequencing reactions, underwent bioinformatic analysis using DNA Baser software. This analysis involved sequence trimming and subsequent alignment with previously curated sequence data available in the GenBank database via the NCBI Basic Local Alignment Search Tool (BLAST).

3 Results and Discussion

This study aimed at the molecular identification of *Salmonella typhi* isolates obtained from the blood samples of patients diagnosed with typhoid fever. Three representative isolates were selected for *16S* rRNA gene sequencing. The resulting sequences, submitted to GenBank with accession numbers MT065760, MT065761, and MT065762, were analysed using the NCBI BLASTn algorithm. The analysis confirmed their identity as *S. typhi*, showing 100% query coverage and sequence identity, with an E-value of 0.

Fig. 1: Agarose gel electrophoresis (1.2 %) of PCR amplified product of *Salmonella typhi* genes with different primers

Table 2: PCR amplified product of different primers

Lane No.	Primers	Amplified Product
1 & 20	DNA Ladder	100 bp-1500 bp
2, 3, 4, 5, 6, 7, 8,9	STY2020, STY2021, STY0201, STY0307, STY0322, STY2020, STY2021, STY0201	Non-salmonella strains
10.	STY2020	425 bp
11.	STY2021	730 bp
12.	STY0201	1170 bp
13.	STY0307	495 bp
14.	STY0322	Nil
15.	STY0326	261 bp
16,17,18, 19	STY0307, STY0322, STY2020, STY0201	Non-salmonella strains
20.	DNA Ladder	100 bp-1500 bp

To develop a rapid diagnostic tool, single gene-target PCR assays were optimized using six different primer sets, with an annealing temperature of 55°C. These assays targeted the

STY2020, *STY2021*, *STY0201*, *STY0307*, *STY0322*, and *STY0326* genes, as previously described by Goay *et al.*[10] Clinical evaluation of the assays demonstrated successful detection of *S. typhi* by five targets: *STY0307* (495 ± 5.0 bp), *STY0326* (261 ± 1.0 bp), *STY2020* (429 ± 4 bp), *STY2021* (732 ± 2.0 bp), and *STY0201* (1176 ± 6 bp). These assays accurately identified all *S. typhi* isolates without cross-reactivity or false positives among non-*Salmonella* isolates (Fig. 1; Table. 2), resulting in 100% sensitivity and 100% specificity. In contrast, the assay targeting *STY0322* failed to detect any *S. typhi* strains.

Serial dilutions of *S. typhi* genomic DNA were performed to determine the detection limits of the optimized PCR assays. The assay targeting the *STY0307* gene exhibited the highest sensitivity, with a detection limit of 5.0 pg. This was followed by the *STY0201* and *STY2021* assays, each with a detection limit of 10 pg. In contrast, the *STY2020* and *STY0326* assays demonstrated lower sensitivity, with detection limits of 50 pg and produced only faint bands during gel electrophoresis. These findings suggest that *STY0307*, *STY2021*, and *STY0201* are more reliable and sensitive molecular markers for the detection of *S. typhi* compared to *STY2020* and *STY0326*.

Notably, the primer targeting the *STY0322* gene failed to produce any visible amplification bands during gel electrophoresis for all patient-derived *S. typhi* isolates. This contrasts with the findings of Goay *et al.*[10], who reported that *STY0322*, along with four other genes, was highly conserved among *S. typhi* strains.

Ngan *et al.*[12] and Pratap *et al.*[13] demonstrated that the *STY0201* gene serves as an effective PCR target, with assays based on this gene exhibiting 100% sensitivity and specificity. The results of the present study are consistent with these findings. However, Goay *et al.*[10] reported that the *STY0201* gene showed only 97.2% specificity, noting cross-reactivity with *S. oslo* and *S. kissi*. Additionally, Tracz *et al.*[14] found that the *STY4220* locus was present in both *Salmonella* serovars Typhi and Paratyphi A.

Goay *et al.*[10] reported that the genes *STY0307*, *STY0322*, and *STY0326* encode hypothetical proteins, while *STY2020* and STY2021 encode putative bacteriophage proteins. Notably, *STY0307*, *STY0322*, and *STY0326* are located within Salmonella Pathogenicity Island 6 (SPI-6), although their roles in bacterial virulence and pathogenicity remain uncharacterized. In the present study, the *STY0322* gene was not detected among isolates from the local ethnic population, in contrast to its presence in Malaysian isolates as reported by Goay *et al.*[10], suggesting possible regional variation. Further studies are needed to substantiate this observation.

Additionally, the expression of *STY2020* and *STY0326* was found to be markedly low during typhoid infection in this study, compared to the findings of Goay *et al.*[10]. This highlights the need for further investigation into the expression profiles of these genes across different populations.

Overall, *STY0307* and *STY2021* emerged as the most specific and sensitive markers for the detection of *S. typhi*. These genes offer promising prospects for the development of novel point-of-care diagnostic assays that are cost-effective, straightforward, rapid, and accurate—particularly beneficial in resource-constrained environments.

4 Conclusion

In conclusion, clinical testing in the present study revealed that three *S. typhi*-specific genes—*STY0307*, *STY0201*, and *STY2021*—demonstrated high sensitivity and specificity, outperforming *STY2020* and *STY0326*. These genes represent promising diagnostic targets and could be effectively utilized in the development of DNA-based diagnostic tools for the accurate and sensitive clinical detection of typhoid fever.

References

1. Reddy EA, Shaw AV, Crump JA. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *The Lancet Infectious Diseases*. 2010;10(6):417-432. Available from: 10.1016/s1473-3099(10)70072-4
2. Deen J, von Seidlein L, Andersen F, Elle N, White NJ, Lubell Y. Community-acquired bacterial bloodstream infections in developing countries in south and southeast Asia: a systematic review. *The Lancet Infectious Diseases*. 2012;12(6):480-487. Available from: 10.1016/s1473-3099(12)70028-2
3. Feasey NA, Dougan G, Kingsley RA, Heyderman RS, Gordon MA. Invasive non-typhoidal *Salmonella* disease: an emerging and neglected tropical disease in Africa. *The Lancet*. 2012;379(9835):2489-2499. Available from: 10.1016/s0140-6736(11)61752-2
4. Panwar S, Duggirala KS, Yadav P, Debnath N, Yadav AK, Kumar A. Advanced diagnostic methods for identification of bacterial foodborne pathogens: contemporary and upcoming challenges. *Critical Reviews in Biotechnology*. 2023;43(7):982-1000. Available from: 10.1080/07388551.2022.2095253
5. Prabagaran SR, Kalaiselvi V, Chandramouleeswaran N, Deepthi KNG, Brahmadathan KN, Mani M. Molecular diagnosis of *Salmonella typhi* and its virulence in suspected typhoid blood samples through nested multiplex PCR. *Journal of Microbiological Methods*. 2017;139:150-154. Available from: 10.1016/j.mimet.2017.05.013
6. Mustafa AH, Raad AZ. Molecular identification of CdtB and TviA virulence genes of *Salmonella typhi* isolated from cholelithiasis patients in Erbil City, Iraq. *Journal of Applied and Natural Science*. 2024;16(4):1476-1484. Available from: 10.31018/jans.v16i4.5922
7. Faik AJ, Al-wardy AH, Mohammad RE. Multiplex PCR for identification of *Salmonella enterica* serovars Typhi and Paratyphi A by selective amplification of tyv, prt, viaB, fliC-d and fliC-a genes. *Journal of Biotechnology Research Center*. 2014;8(2):60-65. Available from: 10.24126/jobrc.2014.8.2.333
8. Wan Makhtar WR, Bharudin I, Samsulrizal NH, Yusof NY. Whole genome sequencing analysis of *Salmonella enterica* serovar Typhi: history and current approaches. *Microorganisms*. 2021;9(10):2155. Available from: 10.3390/microorganisms9102155
9. Maes M, Sikorski MJ, Carey ME, Higginson EE, Dyson ZA, Fernandez A, *et al.* Whole genome sequence analysis of *Salmonella typhi* provides evidence of phylogenetic linkage between cases of typhoid fever in Santiago, Chile in the 1980s and 2010–2016. *PLoS Neglected Tropical Diseases*. 2022;16(6):e0010178. Available from: 10.1101/2022.01.19.22269577
10. Goay YX, Chin KL, Tan CL, Yeoh CY, Ja'afar JA, Zaidah AR, *et al.* Identification of Five Novel *Salmonella typhi* -Specific Genes as Markers for Diagnosis of Typhoid Fever Using Single-Gene Target PCR Assays. *BioMed Research International*. 2016;2016:1-9. Available from: 10.1155/2016/8905675

11. Avneet Kaur Heyar, Kamaldeep Kaur, Amarjit Kaur Gill, Prabhjot Kaur Gill. Induction of clindamycin resistance in clinical isolates of *Staphylococcus aureus* from a tertiary care hospital. *International Journal of Medical and Biomedical Studies*. 2020;4(12):52-57. Available from: 10.32553/ijmbs.v4i12.1566
12. Ngan GJ, Ng LM, Lin RT, Teo JW. Development of a novel multiplex PCR for the detection and differentiation of *Salmonella enterica* serovars Typhi and Paratyphi A. *Research in Microbiology*. 2010;161(4):243-248. Available from: 10.1016/j.resmic.2010.03.005
13. Pratap CB, Kumar G, Patel SK, Verma AK, Shukla VK, Kumar K, *et al*. Targeting of putative fimbrial gene for detection of *S. typhi* in typhoid fever and chronic typhoid carriers by nested PCR. *The Journal of Infection in Developing Countries*. 2013;7(07):520-527. Available from: 10.3855/jidc.2561
14. Tracz DM, Tabor H, Jerome M, Ng LK, Gilmour MW. Genetic determinants and polymorphisms specific for human-adapted serovars of *Salmonella enterica* that cause enteric fever. *Journal of Clinical Microbiology*. 2006;44(6):2007-2018. Available from: 10.1128/jcm.02630-05