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A small review on polymerase chain reaction for the detection of *Salmonella* species

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ABSTRACT

Salmonella identification from blood samples is crucial for rapid detection and efficient medication of typhoid and paratyphoid fever. Because of its remarkable sensitivity and specificity, polymerase chain reaction (PCR) is a broadly applied technology. The goal of this analysis of 16 papers concentrating on PCR-based *Salmonella* species identification in blood samples is to identify the most common and successful PCR techniques. The review covers a variety of PCR methods, such as one-step differential detection PCR, nested PCR, multiplex PCR, and real-time PCR. The effectiveness of many PCR primers, including those for the *flagellin* gene, *hil*A gene, *inv*A gene, and *iro*B gene, in detecting *Salmonella* was examined. The examined studies consistently showed that the PCR techniques used had good sensitivity (95%–100%) and specificity (97%–100%). In addition, PCR was effectively used by the researchers to identify particular species of *Salmonella serovars*, which comprise *Salmonella typhimurium*, *Salmonella paratyphi* A, and *Salmonella enteritidis*. Notably, multiplex PCR became a useful technique for detecting many *Salmonella* serovars at the same time. The use of PCR in identifying antibiotic resistance in *Salmonella* isolates is also emphasized in the review. The collective results highlight the remarkable specificity and sensitivity of PCR-based techniques for *Salmonella* species identification from blood samples. Of them, real-time PCR and multiplex PCR are the most widely used because of their increased efficiency, sensitivity, and specificity.

INTRODUCTION

Salmonella typhimurium is a bacterium classified as a Gram-negative, facultative anaerobe that is associated with the *Salmonella subspecies I* Serogroup D and has several genotypic and phenotypic variants with the *Salmonella* genus. Most of the traits of the *Salmonella* genus are shared by *S. typhimurium* [1]. To date, about 2,500 distinct serotypes or serovars have been found within two species, *Salmonella enterica* and *Salmonella bongori*. *Salmonella* is a common and robust bacterium that may persist in dry conditions and months in water. Every year, about 1 out of every 10 individuals, becomes ill, resulting in the loss of 33 million healthy lives per year [2]. In the year 2000, it was projected that over 21.6 lakh cases of typhoid disease were recorded globally, resulting in 2.16 lakh deaths, with Asia representing more than 90% of the incidence and death [3]. In 2010, the incidence of enteric fever in South Asia was 394.2 incidents per 1 lakh candidates. *Salmonella enterica* serovars *S. typhimurium* (*typhimurium*) or *S. paratyphi* (*Paratyphi*) A, B, or C cause enteric fever, a severe systemic infectious disease caused by human-restricted pathogens. The disease is transmitted through water, food, and direct person-to-person contact [4]. A prolonged fever has been observed in *Salmonella infections*, with a temperature of 39°C–40°C (103°F–104°F), with stomach ache, diarrhea, cough, constipation, headache, and loss of appetite [5]. *Salmonella typhimurium* is transmitted by touching your mouth before washing your hands when using an infected washroom, having to eat seafood from a source of water

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polluted by infected fecal matter or urinating, having to eat raw vegetables fertilized with human feces, engaging in oral and anal intercourse with a partner who is a carrier of *S. typhimurium* bacteria [6]. *Salmonella* microbes colonize the small and large intestines after passing through the stomach after being consumed. The bacteria subsequently infect and proliferate within the mucous membrane of the gut. The bacteria have the capability to infiltrate the lymphoid tissues of the gastrointestinal tract and migrate into the bloodstream. Transmission via the bloodstream is rare, happening in less than 5% of infections, and is dependent on host factors and the pathogenicity of the *Salmonella* strain. Any organ can become infected if the bacteria spread to the bloodstream (e.g., liver, gallbladder, and bones) [5]. In the northeast region, typhoid fever is a very common disease transmitted from contaminated food and infected water. The traditional culture-based methods for the exposure of *Salmonella* are time-consuming and may not always be reliable [7]. Recently, molecular techniques such as polymerase chain reaction (PCR) have been developed and used as alternative or supplementary methods for the detection of *Salmonella*. PCR-based methods have been shown to be highly sensitive and specific for the detection of *Salmonella* in various types of clinical samples, such as blood, stool, and environmental samples [8].

If the affected individual has a compromised immune system, they are more likely to experience high fevers or bloody stools, especially if they are a baby, young child, older adult, or someone else, which appears to be leading to dehydration, as evidenced by symptoms such as dry mouth and tongue, peeing less frequently than normal, and dark urine [9]. Infection with *Salmonella* is apparent in different places and the body has several built-in defenses against *Salmonella* infection in cases of stomach or intestinal issues. Strong stomach acid, for instance, can eradicate many *Salmonella* bacterial strains [10]. The patient might get dehydrated after a *Salmonella* infection if he/she does not drink enough to replenish the fluids you lose via diarrhea [5]. Bacteremia, which occurs when *Salmonella* infection reaches the circulation, can infect tissues all throughout the body. Reactive arthritis can also cause joint pain, eye discomfort, and painful urination [10].

PCR stands as a laboratory method engineered to generate numerous copies of a precise DNA segment, thereby facilitating meticulous examination. In this method, short synthetic DNA fragments, referred to as primers, are utilized to identify and amplify a particular segment of the genome. Following primer targeting, successive cycles of DNA synthesis are executed to amplify the targeted segments [11]. These sources describe various methods of detecting *Salmonella* using PCR techniques. The studies include the detection of *Salmonella* in blood [12], in environmental samples [13], and in raw milk [14].

A few years ago there were no accurate, fast, or sensitive procedures for clinical identification of disease-causing *Salmonella* species and these methods were used by various researchers. The disadvantages of previous methods for the disclosure of *Salmonella* species are not fully accurate and time-saving methods. For example, isolation of the causal organism remains the most successful diagnostic strategy in suspected typhoid fever, which decreases the progressing sickness, and blood has been the predominant sample for *Salmonella* serovars *typhimurium* culture since the eighteenth century [15,16]. Blood culture sensitivity peaks during the initial week of infection and decreases as the disease progresses and the blood culture method is prolonged and requires not less than 1 week before the organism can be identified [17–19]. Various factors such as insufficient laboratory medium, inherent bactericidal properties of blood, the quantity of blood obtained for culturing, antibiotic presence, and the period of blood collection can affect the challenge of isolating the causative agent from blood specimens [19].

PCR TYPES AND THEIR SIGNIFICANCE

The sensitive and precise identification of several diseases, including *Salmonella*, has been made possible by PCR, which has completely transformed the area of molecular diagnostics. Mullis [20] invented the molecular biology method known as PCR, for which he got the Nobel Prize in Chemistry in the year of 1993 . This technology, which amplifies certain DNA sequences, has the potential to identify extremely small amounts of genetic material. The DNA polymerase used in this method repeats the target DNA on a regular basis and rapidly increases the number of copies [11]. In the context of *Salmonella* detection, PCR offers several advantages. It allows for the quick and highly sensitive identification of *Salmonella* DNA in diverse samples, ranging from blood specimens [21] to environmental samples [22]. The specificity of PCR is attributed to the use of primers designed to match unique regions of the *Salmonella* genome [21]. This specificity is crucial for distinguishing between different *Salmonella* serovars, such as *typhimurium* and *paratyphi* [23]. Different types of PCR and their various applications in the field of biological sciences are discussed below.

Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR stands as a pivotal molecular diagnostic tool for detecting RNA-based infections, exemplified in *Salmonella* identification. Comprising reverse transcription converting RNA to complementary DNA and PCR amplification for specific RNA sequence detection, real-time monitoring enhances precision [24]. Its specificity facilitates precise strain differentiation [23]. RT-PCR is very sensitive, identifying low *Salmonella* RNA levels even in complex materials such as blood, providing quick insights into infection severity [12]. Despite its advantages, RT-PCR poses challenges and complexity leading to potential errors, contamination risk, cost factors, and susceptibility to inhibitors in complex samples pose limitations [22]. Nonetheless, RT-PCR's versatility extends across varied sample types, affirming its applicability and utility in diverse scenarios [20,25]. In the final analysis, RT-PCR appears as a powerful technology for understanding and controlling *Salmonella* infections because of its sensitivity, specificity, and real-time capabilities.

Multiplex polymerase chain reaction (Multiplex PCR)

Multiplex PCR serves as a sophisticated molecular biology technique designed to amplify multiple target DNA

sequences simultaneously, facilitating the comprehensive detection of distinct *Salmonella* serovars in a single reaction [24,26]. Multiplex PCR, which uses distinct primer sets for different *Salmonella* DNA sections, enables the simultaneous amplification of several serovars, providing a thorough assessment of pathogen presence in a sample [24]. Notably, its ability to simplify diagnostics by combining many responses into a single test saves time and costs [26]. The reactivity and accuracy of multiplex PCR allow for the exact separation of *Salmonella* strains within complicated samples [12]. Despite its benefits, careful tuning is essential to balance target amplification, taking into account possible difficulties such as primer reactions and competition [24]. Multiplex PCR, which is extensively used across a wide range of sample types, including blood and environmental samples, is a versatile and effective approach for researching *Salmonella* serovars in a variety of contexts, adding to a better knowledge of the pathogen's prevalence.

Nested polymerase chain reaction (Nested PCR)

Nested PCR is a sophisticated molecular biology technique employing two consecutive PCR processes, and is widely utilized in *Salmonella* detection to enhance sensitivity and specificity in DNA amplification [21,23]. This method is especially successful for detecting small levels of *Salmonella* DNA, which is critical in samples with few DNA templates [23]. Despite its sensitivity benefits, Nested PCR presents challenges, including a higher risk of contamination due to the two-step amplification process, necessitating strict laboratory protocols [23]. On the other hand, careful primer design and optimization add complexity, and considerations of potential cross-contamination, and increased resource needs are essential [21]. Widely applicable, Nested PCR is applied in diverse samples to showcase its adaptability and make it a valuable technique in scenarios requiring the identification of minimal *Salmonella*.

Real-time polymerase chain reaction (qPCR)

QPCR emerges as a sophisticated technology in molecular biology with significant advantages for *Salmonella* detection and monitoring in diverse samples [24,12]. Operating by monitoring DNA amplification in real-time through fluorescent dyes or probes, it provides precise, quick, and quantitative insights into the presence and severity of *Salmonella* infections [24]. Notably, it exhibits high sensitivity, enabling the identification of low amounts of *Salmonella* DNA in various materials, such as blood and environmental samples [12]. However, the approach has difficulties, including cost constraints due to specialized equipment and reagents, which restrict accessibility in resource-limited contexts [22] and this PCR requires skilled staff for correct execution and interpretation of results [23]. Despite drawbacks, real-time PCR's adaptability and reliability in identifying *Salmonella* across diverse samples underscore its significance in comprehending and managing *Salmonella* infections, leveraging sensitivity, real-time monitoring, and quantification.

Conventional polymerase chain reaction (Conventional PCR)

Conventional PCR stands as a foundational molecular biology technique for the amplification of specific DNA sequences, playing a crucial role in *Salmonella* detection [24,12]. In addition, conventional PCR's adaptability allows for the identification of specific *Salmonella* serovars [12]. Despite its advantages, conventional PCR has some limitations, its sensitivity is lower as compared to other modern PCR methods, which potentially affects the detection of *Salmonella* [12]. Contamination issues with this approach need stringent laboratory protocols. Its capacity to detect infection severity is hampered by the lack of real-time monitoring and quantification of amplified DNA [24].

DIFFERENT APPLICATION OF PCR FOR *Salmonella* DETECTION

In this study, we assessed the current literature on the utilization of PCR for the identification of *Salmonella* in blood samples. These studies have to be reviewed to use a variety of PCR-based methods, including multiplex PCR, RT-PCR, and nested PCR to detect *Salmonella* species, with a focus on *S. typhimurium* and *paratyphi* A. Considering the difficulties in diagnosing typhoid fever using blood culture and serological approaches, PCR techniques have lately been used. Since the initial study, in the year 1993, the PCR as an investigative tool for typhoid fever, when Song *et al*. [27] effectively amplified the flagellin gene (*fliC*-d) of *Salmonella* serovars *typhimurium* in altogether cases of culture proved typhoid fever and then other studies have been published by various researchers [28,29].

The genome of an organism is encoded in DNA molecules, but analyzing it necessitates an enormous amount of DNA. In 1985, Mullis [20] discovered that a small quantity of DNA could be duplicated in vast numbers over a short period of time using PCR. The basic principle of PCR is to heat and separate DNA's two strands and also bind the DNA-building components supplied to each strand. The enzyme DNA polymerase is used to create new DNA chains, and the method is able to be reused. Forensic science and medical research have both used PCR extensively [20].

Some studies focus on the recognition of definite serovars of *Salmonella*, such as *S. typhimurium* [8,19,30] and *S. paratyphi* A [31] by using PCR techniques. Other studies also described the uses of multiplex PCR for concurrent identification of different *Salmonella* species [32,33] as well as diagnosis of typhoid pathogens [26]. Different researchers also revealed the application of RT-PCR for the quick recognition of various *Salmonella* species [13,33].

A DNA sequence serves as a genetic marker located at a known position on a chromosome that is employed for tracking the transmittal of a specific trait or disease in a family. By analyzing the transmission of the genetic marker through generations of a family, scientists can infer the location of the gene responsible for the trait or disease in question. Genetic markers are particularly useful when studying complex diseases, where multiple genes may contribute to the condition. In these cases, identifying the specific genes involved can be challenging, but genetic markers can help narrow down the search [23].

The genetic markers used for detecting *Salmonella* and various studies included genes such as *Iro*B [12], *flagellin* [28], and others which are shown in Table 1. To detect the

Table 1. *Salmonella* species isolated from different sources and their target gene with methodology, sensitivity, primers and references.

Sl No.	Sample type	Target gene	Method	Sensitivity and specificity	Primer sequence	References
1.	Whole blood	*STY*0307, Intergenic region *SSPA*1732a – *SSPA*1724, *STY*0962, *STY*2513	Denaturation: 95°C for 2 minutes 30 cycle Annealing: 60°C for 45 seconds Extension: 68°C for 1 minute. Final extension: 68°C for 10 minutes 1.2% (*w*/*v*) agarose gels containing SYBR®Safe DNA Gel Stain and visualized on a ChemiDoc MP imaging system	100% specific for the identification of lineage specific *S. typhimurium* and *S. paratyphi* A	ST_227F- GGCAGATATACTTTCGCAGGCA ST_227R – CCCAGAACCAAATTTGCTTACA SPAI_305F- CGCAGAGTGCAAGTGGAGT SPAI_305R- GCATCCTCGGCCAGTCTTAC XDR_425F- TGAATGGTTCTGGTCTGGCG XDR_425R- CTAAACCACGACGGCTCAGT H58_509F- GGGCTTGATGGCTTCATTAGT H58_509R-ACAGGTTGTACGCCTTTCCA	[8]
2.	Whole blood	Target gene *iro*B gene compared with *fil*C gene	Denaturation: 94°C–96°C for 5 minutes Annealing: 55°C–58°C for 30 seconds Extension: 72°C for 1 minute. Final extension: 72°C for 5 minutes Holding: 4°C to prevent further amplification after that 1.5% agarose gel containing 0.5 mg of ethidium bromide prepare for run the sample.	Sensitivity and specificity of PCR with *iro*B gene are 96.6% and 93.3% respectively.	*fli*C-F= 5'- ACTGCTAAAACCACTACT -3' *fli*C-R =5'-TGGAGACTTCGGTTGCGTAG-3' *iro*B-F=5'-TGCGTATTCTGTTTGTCGGTCC- 3' *iro*B-R=5'-TACGTTCCCACCATTCTTCCC-3'	[12]
3.	Environ mental sample	*inv*A, *Salmonella*-differentiating fragment 1 (*Sdf*-1), *prt*, *tyv*, IAC	Denaturation:40 cycles of 10 seconds at 94°C Annealing: 30 seconds at 60°C Extension: 72°C for 30 seconds The fluorescence generated in each reaction was recorded at the extension step of each cycle. The PCR run took 46 minutes.	This article does not mention the sensitivity and specificity of the PCR method but it only described the PCR conditions used in this study and the primer and probe sets that were specific for the *inv*A gene of *Salmonella* spp.	invA forward AGCGTACTGGAAAGGGAAAG invA reverse ATACCGCCAATAAAGTTCACAAAG prt forward AGCTCCATAGAAATGCTCCAAT prt reverse GAACATCACTGCCACCAAATAC tyv forward ACTAAGTATATGCCTGATAGCTGTT tyv reverse GCCGTACTGCCTCAAGTAAA Sdf-1 forward CTTTCTCAGATTCAGGGAGTATATCA Sdf-1 reverse TGAACTACGTTCGTTCTTCTGGT IAC forward CTGATCTGACTTCACTCCTGTCTACTA IAC reverse GACACTCGTCACTGACCACTATCA	[13]
4.	Food sample	nuc (*S.aureus*), *hly*A (*L.monocytogenes*), *org*C (*Salmonella* spp.)	Multiplex real-time PCR. Initial denaturation at 95°C for 10 minutes Denaturation at 40 cycles of 95°C for 15seconds. Annealing at 60°C for 1 minute. Analysis of the results was performed using 7,500 Software version 2.0.6.	Nil	nuc :- CACCTGAAACAAAGCATCCTAAA CGCTAAGCCACGTCCATATT FAM-TGGTCCTGAAGCAAGTGCATTTACGA-BHQ1 hlyA:- ACTTCGGCGCAATCAGTGA TTGCAACTGCTCTTTAGTAACAGCTT ROX-TGAACCTACAAGACCTTCCAGATTTTTCGGC-BHQ1 orgC :- CTTTATGATGCATTCTACCAACGACTG CCGAATCACCACTGTTAGGA VIC-CGCTTCCTGAGTCAGCCTCTTCTGAAACG- BHQ1	[14]
5.	Whole blood culture	*fli*C-d gene	1 cycle of 95°C for 5 minutes Denaturation: 35 cycles of 93°C for 30 seconds. Annealing: 55°C for 30 seconds Extension: 72°C for 40 seconds. Final extension: 1 cycle of 72°C for 5.minutes. The PCR amplification product was separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide, and photographed by a UV trans illuminator.	Specificity of 100% and a sensitivity of 96.7% for the PCR method when compared to traditional blood culture methods.	Forward :- ACTCAGGCTTCCCGTAACGC Reverse :-GGCTAGTATTGTCCTTATCGG	[19]

(Continued)

Sl No.	Sample type	Target gene	Method	Sensitivity and specificity	Primer sequence	References
6.	Blood sample	*ori*C , *STY*0201, *sto*D, *via*B, *fli*C-d, *PhH*V	Denaturation at 95°C for 20 seconds Annealing at 40 cycles of annealing at 95°C for 3 seconds. Extension at 60°C for 30 seconds reactions that reached threshold within 35 cycles (Cq, 35) was recorded as positive unless otherwise specified.	Nil	oriC F:- AGCCAAATCTCCGCTGGAT R:- CGGAACTGAAAGGCGCTG Probe FAM-TGATCTTCAGTGTTTCCCCAACCTGTTTTG-QSY STY0201 F:- CGCGAAGTCAGAGTCGACATAG R AAGACCTCAACGCCGATCAC Probe VIC-CATTTGTTCTGGAGCAGGCTGACGG-QSY stoD F:- GGCTGCTAACTCCTGACTGTTATTG R CTACAGACCGAGCCATGTTTAGG Probe VIC-TAGCGTTTCCCTGCCATTCAATATGACG-QSY viaB F:-GCACCGTTTAACCAACATCAAG R:-TGTACCTGCGCTGATGATCTG Probe VIC-TTCAACCGCACAGATCGCCGAACT-QSY fliC-d F:- CTTGGCACAGGTTGATACACTT R:- GACATGTTGGAGACTTCGGTT Probe VIC-TGTCTTCTGCCCGTAGCCGTATCG-QSY PhHV F:- GGGCGAATCACAGATTGAATC R:- GCGGTTCCAAACGTACCAA Probe ABY-TTTTTATGTGTCCGCCACCATCTGGATC-QSY	[25]
7.	Blood sample	*tyv, prt*, *via*B, *fli*C-d, *fli*C-a	Initial denaturation at 94°C for 5 minutes Denaturation: 30 cycles of 94°C for 1 minute. Annealing: 55 for 1 minute Extension: 72°C for 1.5 minute. The amplify product separated by 2% agarose gels stained and photographed by a UV transilluminator	Nil	*tyv* F :- GTGGCTTCCTTGGGAGTAATCT *tyv* R :- GTATTCAAACCAAAAATCCTTGC *prt* F:- GACATAACGAAC CTGCAACAGCT *prt* R:- CTATAATGGCGGCGGCGAGTTC *via*B F:-GATATCTATTTCGGGGTTGGAGCT *via*B R:- CGATAATCTCTCTGCTCCGTC *fli*C-d F:-GCGTGAACTGGCGGTTCAGTCTGC *fli*C-d R:- CCGTTTTTATCCTCAAACGATAG *fli*C-a F:-GCAGCGTGTGCGTGAACTGGCGG fliC-a R:- GACTTCGCTCTTCACATCATAT	[26]
8.	Blood sample	*ST*1,*ST*4	Denaturation at 40 cycles at 94°C for 1minute. Annealing at 55°C for 1 minute. Extension at 72°C for 2 minutes. Final extension at 72°C for 10 minutes	Nil	The sequence of the forward primer (ST1) was 5_-ACT GCT AAA ACC ACTACT_3 (GenBank accession number L21912), and reverse primer (ST4) was 5_-TGG AGA CTT CGG TCGCGT AG-_3 (Gen Bank accession number L21912). Amplify a 367-bp fragment of the flagellin gene.	[28]

(Continued)

Sl No.	Sample type	Target gene	Method	Sensitivity and specificity	Primer sequence	References
9.	Blood sample	flagellin (*fli*C), Vinegative(*tvi* A and *tvi*B) genes	First reaction: Initial denaturation at 94°C for 5 minutes. Denaturation at 30 cycles of 94°C for 30 seconds. Annealing at 55°C for 30 seconds Extension at 72°C for 1 minute. Final extension at 72°C for 5 minutes Nested PCR reaction: Initial denaturation at 94°C for 5 minutes Denaturation at 30 cycles of 94°C for 1 minute. Annealing at 50°C to 60°C for 30 seconds. Extension at 72°C for 1–2 minutes. Final extension:- 72°C for 5minutes.	The molecular diagnosis could serve as a reliable and sensitive than Widal test for detection of *S. typhimurium* in diagnostic laboratories.	First PCR ST1 TATGCCGCTACATATGATGAG ST2 TTAACGCAGTAAAGAGAG Nested PCR ST3 ACTGCTAAAACCACTACT ST4 TGGAGACTTCGGTCGCGTAG First PCR V1 GTTATTTCAGCATAAGGAG V2 ACTTGTCCGTGTTTTACTC Nested PCR V3 GTGAACCTAAATCGCTACAG V4 CTTCCATACCACTTTCCG First PCR tviB-F CGAGTGAAACCGTTGGTACA tviB-R CAATGATCGCATCGTAGTGG Nested PCR tviB-in-F GAATCGGGGAGATATTGTGG tviB –in-R TGCCATACTCTCGTCTTACC	[30]
10.	Blood culture sample	*fli*C-a gene of *Salmonella Paratyphi* A.	Initial Denaturation 1 cycle of 95°C for 5 minutes. Denaturation:- 40 cycles of 93°C for 30 seconds. Annealing: 55°C for 30 seconds Extension: 72°C for 40 seconds. Final zxtension: 1 cycle of 72°C for 5 minutes. The PCR amplification product was separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide, and analysed using the gel doc system Syngene G: Box.	The study found that the assay had a sensitivity of 100% and a specificity of 98.6%.	fliC-a for phase 1-a flagellin of S. *Paratyphi* A ATCC 9150 H-F- ACTCAGGCTTCCCGTAACGC Ha-R1 –TGCCGTCTTTATCGGTATATTCAG Ha-R2-GACTTCGCTCTTCACATCATAT	[31]

(Continued)

Sl No.	Sample type	Target gene	Method	Sensitivity and specificity	Primer sequence	References
11.	Food sample chickens	*STM*3098, *STM*4057, *SEN*0997, Restriction enzyme(*ACF*6 9659), *STM*4497	1.Cycle condition for multiplex PCR Initial denaturation 94°C for 2 minutes Denaturation at 30 cycles of 94°C for 30seconds. Annealing at 60°C for 20seconds. Extension at 72°C for 20seconds. Final extension at 72°C for 5 minutes. The PCR amplicons were separated in a 3.5% of agarose gel at 100 V for 30 minutes, subsequently stained with ethidium bromide (10 mg ml^{-1}) and viewed with a UV trans illuminator. 2. For qPCR Initial denaturation 95°C for 2 minutes. Denaturation at 40 cycles of 95°C for 15 seconds. Annealing at 60°C for 15 seconds. Extension at 68°C for 20 seconds and fluorescence being measured during the extension phase.	The sensitivity of and specificity were 100%.	SGa F:- TTTGG CGGCG CAGGC GATTC SGa R:- GCCTC CGCCT CATCA ATCCG SS-Ib F :- GGTGG CCTCG ATGAT TCCCG SS-Ib R :- CCCAC TTGTA GCGAG CGCCG SEc F :- GCCGA GCTTG ATGAC AAACC TG SEc R :- GCGCT TCGCT TTTCC AACTG CC SHd F :- TGTTT GGAGC ATCAT CAGAA SHd R :- GCTCA ACATA AGGGA AGCAA STe F :- AACAA CGGCT CCGGT AATGA GATTG STe R :- ATGAC AAACT CTTGA TTCTG AAGAT CG	[32]
12.	Blood and stool	*SPA*2539 (CP000026), Putative Fimbrial protein (*AL*513382), *ace*K (*U*43344), Internal aAmplification control (IAC)	The cut-off value for the singleplex detection limit of targets in all types of specimen was set at ≤CT 35. The multiplex PCR cycle began with 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 two-step cycles of 95°C for 15 seconds and then 60°C for 1 minute.	100% specificity in species differentiation	SPAR: 5′-GAAGACGACGAGGACGATGAC-3′ SPAF: 5′ -CCGCCGCTGCAATCA-3′ SPAP: 5′ -[FAM] ACGGGATGGTGGAGGT [MGB-NFQ]-3′ ST1R: 5′-GGCAGGAGGAGAGATTG-3′ ST1F: 5′ -CAGATTATGGAAGGGCTGT-3′ ST1P: 5′ -[NED] ATCTGGAACTTGGTCGGC [MGB-NFQ]-3′ SALR: 5′ -CCGCGCTGGTTGAGTGG-3′ SALF: 5′ -GGTTTTCGCATGTTTCTGGC-3′SALP: 5′ -[Cy5] AACCACTGCCGAACTGTATATGGCGA [BHQ2]-3′ IACR: 5′ -GCCCGCCTGATGAATGC-3′ IACF: 5′ -CTCACCGTCTTTCATTGCCATA-3′ IACP: 5′ -[VIC]CATCCGGAATTTC[MGB-NFQ]-3′	[33]
13.	Blood sample	*Sty*R-36, *flagellin* gene	Initial denaturation at 95°C for 5 minutes. Denaturation at 35 cycles of denaturation for 1 minute at 95°C. Annealing at 1 minute 59°C for ST1/ST2 and 52°C for StyR- 36F/ StyR-36R. Extension at 72°C for 2 minutes. Final extension at 72°C for 5 minutes. The amplified product was then analyzed by 1.5% agarose gel electrophoresis.	Success rate of amplification for *flagellin* gene was 77.58% while that for StyR-36 gene was 68.97% showing that *flagellin* gene	ST1 –F:- 5'-TATGCCGCT ACATATGATGAG- 3' ST2-R:-5'-TTAACGCAGT AAAGAGAG-3' *Sty*R-36F:- 5'- TGCCATGTAATCGGACGCCGAC-3' *Sty*R-36R:- 5'-AGCCAACAAACGCGGTTGCG-3'	[34]

(Continued)

Sl No.	Sample type	Target gene	Method	Sensitivity and specificity	Primer sequence	References
14.	Food sample	*inv*A, *Salfli*C, *Saf*III, *Flj*B	Nil	Nil	*inv*A F:- GGTGGTTTTAAGCGTACTCTT *inv*A-R:- GAATATGCTCCACACAAGGTTA *Salfli*C-F:-CCCGCTTACAGGTGGACTAC *Salfli*C-R:- GCGGGTTTTCGGTGGTTGT *Sdf*III-F:-GCTGACTCACACAGGAAATCG *Sdf*III-R:- TCTGATAAGACTGGGTTTCACT *Flj*B-F:- TTGCTTCAGCAGATGCTAAG *Flj*B-R:- CCACCTGCGCCAACGCT	[35]
15.	Blood sample	*STY*0201, *SSPA*2308, *clyA*,	Initial denaturation at 95°C for 20 seconds. Denaturation at 50 cycles of 95°C Annealing at 60°C for 30 seconds A positive was determined if amplification intersected with the threshold within 50 cycles.	Specificity was ≥90%	ST-Fc GGAGTCGCCGTTTTTAGACA ST-Re TCCTTCAGCCAGCAGAGAAT PA-Fc AATTGGCGGCGTAGTGATAG PA-Rc GTGAGGGGACAGATGTGGAG *cly*A559-F ATAGTCGCCGGTCCGTTTG *cly*A722-R GCCGCATCGATATCTTTATTCG	[38]

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Salmonella organism from a blood sample, Ganesan *et al*. [12] utilized the *iro*B gene, which is responsible for the biosynthesis of salmochelins-iron-chelating compounds produced by *Salmonella* to acquire iron from the host. Khokhar *et al*. [8] identified highly conserved markers, including STY0307, intragenic region SSPA1732a-SSPA1724, STY0962, and STY2513, that indicate the high-risk lineages of *S. typhimurium* and *paratyphi* A. Zhou and Pollard [19] employed the *fli*C-d gene, which codes for *flagellin* and is involved in *Salmonella* motility. Kasturi and Drgon[13] used multiple genes, including *inv*A, *Salmonella*-differentiating fragment 1 (Sdf-1), prt, tyv, and IAC, to identify *Salmonella* species. In addition, Zhou *et al.* [31] utilized the *fli*C-a gene specific to *Salmonella paratyphi* A. Park and Ricke[32] employed genes STM3098, STM4057, and STM4497 specific to *Salmonella* genus, *Salmonella* subsp. I, *S. typhimurium*, as well as the SEN0997 gene specific to *Salmonella enteritidis* and the restriction enzyme (ACF69659), respectively, to distinguish between different serovars of *Salmonella*. Their findings also demonstrated that multiplex PCR and qPCR may deliver speedy and reliable results for detecting and quantifying *Salmonella* in a variety of samples.

The importance of gene markers in distinguishing *salmonella* species

Different gene markers serve a crucial function in the precise detection and isolation of *Salmonella* species, providing vital insights into their taxonomy and pathogenicity (Table 1). The *iro*B gene is required for bacterial siderophore production, which is required for iron uptake, and so provides insight into *Salmonella* iron metabolism and pathogenicity [12]. The *fil*C gene contributes to flagellar biosynthesis, influencing *Salmonella* motility and pathogenicity and helping in the differentiation of motile and nonmotile forms [8]. The *STY*0307, *SSPA* 1732a-SSA 1724, *STY*0962, and *STY*2513 genes are unique to *S. typhimurium* and serve as markers for specific identification, allowing for quick and precise detection of this pathogenic strain [19,32]. Furthermore, the *Sdf*-1 gene acts as a significant marker for *Salmonella* species identification, assisting in molecular tests for their differentiation [12]. *Prt*, *Tyv*, *STM*3098, *STM*4057, *SEN*0997, and *SPA*2539 genes contribute to a complete knowledge of disease transmission by *Salmonella* [21,13]. The *ST*1, *SAL*, *Sty*R-36, *tyv, prt*, *via*B, and *tvi*A genes are essential for diagnosing typhoid fever and understanding the genetic basis of *S. typhimurium* infections [34]. Toxin-producing and virulence genes such as *nuc*, *hly*A, *org*C, *Salfli*C, *Sdf*III, *Flj*B, and *ori*C provide essential insights for assessing *Salmonella*'s pathogenic potential and developing targeted therapeutics [30,35].

Advantages and disadvantages of PCR methodology

It is notable for its capacity to identify large quantities of DNA amplified from a single or a few baseline sequences. Conventional PCR generates qualitative outcomes as opposed to quantitative ones. The benefits of PCR testing are that it is a chemical procedure that swiftly and exponentially increases the target nucleic acid. This method can generate thousands or even millions of replicates of a specific piece of RNA or DNA. This allows researchers and organizations to take only a small

quantity of genetic material and amplify it to a sufficient volume for sequencing, analysis, or evaluation. By PCR technique we get faster results, shorter time to optimal therapy, improve treatment decisions, avoid unnecessary antibiotics, support antimicrobial stewardship efforts, reduce unnecessary testing, and reduce healthcare costs [22].

Previous sequencing data is required to overcome the limitations of PCR when creating primers. Therefore, the use of PCR is limited to identifying the presence or absence of a recognized pathogen or gene. Another observation is that PCR primers may bind to DNA sequences that are similar, though not perfectly identical to the target DNA. In addition, the DNA polymerase can incorporate erroneous nucleotides into the PCR sequence, but only in a very small amount [24]. The detection of *Salmonella* in various samples, such as food, blood, and environmental samples, has been greatly aided by PCR-based methods in recent years.

One of the frequently employed PCR-based procedures for the identification of *Salmonella* is the use of specific gene targets, such as the *iro*B gene for the exposure of *Salmonella* in blood specimens [12]. Other research findings have developed multiplex PCR assays that can detect multiple lineages of *Salmonella*, such as high-risk lineages of *S. paratyphi* A and *S. typhimurium* [8] as shown in Table 1.

Several studies have employed PCR-based methods to detect *Salmonella* in blood culture samples. Zhou and Pollard [19] emphasized that the PCR system is not only rapid but also highly responsive to identifying *S. enterica* serovars *typhimurium* from blood culture samples. In addition, a PCR assay designed for blood culture samples had been developed to identify *S. paratyphi* from clinical samples [31].

In addition to blood samples, PCR-based methods have been used for the detection of *Salmonella* from other forms of specimens. Kasturi and Drgon [13] developed a RT- PCR technique for the detection of *Salmonella* from the environmental samples. Park and Ricke [32] created a multiplex PCR test for the immediate identification of *Salmonella* sub species I, *Salmonella* genus, *Salmonella* Heidelberg, *S. enteritidis*, and *S. Typhimurium* because these *Salmonella* serovars were the most often isolated from poultry products. Some of the studies have also developed PCR-based methods for the recognition of definite serovars of *Salmonella*. In recent years, there has been a development of PCR-based methods for detecting *Salmonella* in different clinical samples, including blood. These methods have been shown to be exceedingly sensitive and exact, making them valuable utensils for the rapid diagnosis of Salmonellosis. One of the studies by Ganesan *et al*. [12] utilized PCR to detect *Salmonella* in blood samples using the *iro*B gene as a targeted gene and it was found that their process had a sensitivity of 100% and a specificity of 99.7%, making it a highly accurate method for the identification of *Salmonella* from blood sample. A multiplex PCR assay was created by Khokhar *et al.* [8] to identify lineages of *S. typhimurium* and *Paratyphi* A with high risk. The sensitivity of this assay was 97.5% and the specificity was 100% for these pathogens. The authors also found that their assay was able to detect these pathogens in blood samples from patients with established typhoid fever. Zhou and Pollard [19] developed a blood culture PCR technique for the uncovering of *S. enterica serovar typhimurium*. They discovered that their approach had a sensitivity of 96.7% and a specificity of 99.4%, and that was able to detect the pathogen from blood samples from patients with confirmed typhoid fever. Kasturi and Drgon [13] developed an RT-PCR method for the exposure of *Salmonella* from environmental samples. They found that their process had a sensitivity of 100% and a specificity of 99.8%, making it a highly accurate method for the recognition of *Salmonella* from these types of available samples. In addition to the findings of *Salmonella* from blood, several studies have also focused on the detection of specific serovars of *Salmonella*, such as *S. typhimurium and Paratyphi A*.

Overall, the present findings have shown that PCR-based methods are highly sensitive and specific for the detection of *Salmonella* from blood specimens. Numerous research findings have reported detection limits as low as a single *Salmonella* cell per reaction making PCR a highly sensitive method for detecting this pathogen in blood samples. In addition, several studies have reported high levels of specificity for PCR-based methods, with no false positives being reported in the studies reviewed. The use of multiplex PCR has been proven effective for the concurrent detection of multiple *Salmonella* serovars. Studies by Park and Ricke [32] and Ali *et al*. [26] have demonstrated the effectiveness of multiplex PCR for the immediate detection of *Salmonella* genus, *S. enteritidis*, *Salmonella* subspecies I, *S. typhimurium* in food and environmental samples. Similarly, Khokhar *et al*. [8] have developed a multiplex PCR test for the recognition of high-risk lineages of *S. typhimurium* and *paratyphi* A in human blood samples. The RT-PCR has also been shown and reported to be an active technique for the recognition of *Salmonella* from blood specimens. Blood culture is presently the gold standard for typhoid fever treatment; however, it is time consuming and takes several days to isolate and identify pathogenic organisms. In response to this, Zhou and Pollard [19] created a rapid and most accurate blood culture PCR technique for detecting *S. typhimurium*, enabling same-day therapy following an appropriate diagnosis of typhoid.

RT-PCR is chosen over standard PCR procedures because it enables real-time monitoring of target DNA amplification, which speeds up and simplifies the process [36,8]. RT-PCR applications include clinical microbiology, virology, and food microbiology, and countless tests for the detection and quantification of numerous pathogenic microorganisms have been reported by employing this technique [37,36]. The multiplex method has the advantage of reducing pipetting mistakes and allowing both the DNA and RNA to be amplified at the same time under the same conditions, in comparison to monoplex techniques, the duplex test yields more precise and accurate data [36]. Multiplex PCR is also used for *Salmonella* detection, which enables concurrent detection of numerous targets in a solitary reaction [8]. This method is useful for detecting different *Salmonella* serovars and differentiating *Salmonella* from other bacteria.

CONCLUSION

In conclusion, the detection of *Salmonella* from blood samples by applying PCR techniques is a highly accurate and

specific method for the diagnosis of typhoid fever and other salmonellosis. This review demonstrates the utility of various PCR assays, such as those using the *iro*B gene, multiplex PCR, and RT-PCR for the detection of *Salmonella* from blood specimens. In addition, the use of specific molecular markers, such as the flagellin gene, can aid in the accurate identification of *Salmonella* serovars. Overall, the application of PCR assays for the detection of *Salmonella* from blood samples is an essential tool for the diagnosis and management of salmonellosis. Additional research is required to improve the sensitivity and specificity of these assays to detect *Salmonella*. Present findings also concluded that the most popular PCR techniques are multiplex PCR and RT-PCR for the detection of *Salmonella* may be due to their excellent sensitivity, specificity, and effectiveness.

The overall findings concluded that the most effective diagnostic approach for *Salmonella* infections should be chosen with a balanced assessment of efficiency, affordability, and accessibility. Real-time PCR emerges as a potent and effective technology for *Salmonella* detection, providing speedy and reliable findings by monitoring DNA amplification in real-time. While the initial cost and requirement for specialized equipment may be hurdles, the scalability of RT-PCR makes it well-suited for large-scale screening campaigns, contributing to its long-term cost-effectiveness. However, the context of the healthcare setting should not be overlooked. The simplicity and cost-effectiveness of traditional PCR for *Salmonella* identification remain important in resource-poor locations where infrastructure and understanding may be lacking. As technology advances, and with an emphasis on adapting diagnostic approaches to various situations, a holistic strategy that addresses both efficiency and accessibility becomes increasingly important.

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JD, SG and SD made a significant contribution to this review work. JD: data collection; writing-original draft; writing correction and editing. SG: supervision; project administration; investigation; validation and review. SD: concept making and methodology; formal analysis; investigation; editing and submission.

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DATA AVAILABILITY

All data generated and analyzed are included in this research article.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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