Establishment and Application of Polymerase Spiral Reaction Amplification for *Salmonella* Detection in Food

Wenli Xu¹, Jun Gao¹, Haoyue Zheng¹, Chaowen Yuan¹, Jinlong Hou¹, Liguang Zhang², and Guoqing Wang^{1*}

¹College of Life and Health Sciences, Northeastern University, Shenyang 110001, P.R. China

²Center for Animal Disease Emergency of Liaoning Province, Shenyang 110161, P.R. China

Received: June 13, 2019
Revised: August 21, 2019
Accepted: September 2, 2019

First published online:
September 9, 2019

*Corresponding author
Phone: +86-024-24245954
E-mail: wangguoqing@mail.neu.edu.cn

pISSN 1017-7825, eISSN 1738-8872

Copyright© 2019 by
The Korean Society for Microbiology
and Biotechnology

Salmonella is a common zoonotic and foodborne pathogen that causes high morbidity and mortality in developing countries. In this study, we established and validated a polymerase spiral reaction (PSR) assay which targeted the conserved invasion gene (*invA*) of *Salmonella* by SYBR Green I indicator methods. Subsequently, assays for determination of the optimal conditions for optimal specificity and sensitivity of PSR were performed. We performed comprehensive evaluations using loop-mediated isothermal amplification (LAMP) and real-time PCR. A total number of 532 samples of daily food were analyzed by PSR. Twenty-seven bacterial strains were tested in the specificity assay, from which positive results were obtained only for 14-*Salmonella* strains. However, none of the 13 non-*Salmonella* strains was amplified. Similarly with LAMP and real-time PCR, the detection limit of the PSR assay was 50 CFU/ml. The PSR method was also successfully applied to evaluate the contamination with *Salmonella* in 532 samples of daily food, corroborating traditional culture method data. The novel PSR method is simple, sensitive, and rapid and provides new insights into the prevention and detection of foodborne diseases.

Keywords: *Salmonella*, invasion gene A, isothermal amplification, rapid detection, food samples

Introduction

Salmonella is a common gram-negative bacterium belonging to the *Enterobacteriaceae* family, which can cause serious foodborne diseases (FBDs) in humans and animals [1, 2]. Currently, FBDs caused by *Salmonella* are a serious food safety problem, leading to high morbidity and mortality rates, and huge economic losses in developing countries [3, 4]. Infection usually occurs when food is contaminated by feces infected with *Salmonella* or through carriers, such as eggs, beef, milk, and water [5, 6]. Once *Salmonella* colonization in the intestine is completed, it can contaminate new food and water sources via the fecal-oral route [7]. Therefore, the development of methods for rapid and effective *Salmonella* detection, as well as for the identification and subsequent mitigation of related foodborne illnesses is critically important due to the considerable health risk and economic impacts of these infections [6, 8].

The conventional bacterial culture method for *Salmonella*

detection and identification consists of steps including bacteria isolation, biochemical identification, and serological confirmation tests. This approach is reliable but time-consuming and laboratory-intensive (requires from 5 to 7 days); furthermore, biochemical cross-reaction can occur between different species [4, 9, 10]. Various molecular-based methods, including PCR, real-time quantitative PCR (qPCR), and DNA microarray, have been successfully used to detect *Salmonella* in a range of food and feed products [11–13]. Despite their high rapidity, specificity, and sensitivity, these techniques require expensive equipment and special reagents, which makes them inappropriate for primary food quarantine analyses, particularly in developing countries [6, 7].

In recent years, various isothermal nucleic acid amplification techniques (INAATs) have been successfully applied to detect *Salmonella* and other pathogens [14]. Among these, loop-mediated isothermal amplification (LAMP) has been established as a valuable method, in which a strand

displacement DNA polymerase is employed to synthesize large amounts of DNA hairpin structure during the constant temperature step [15]. This diagnostic technique is used in studies of infectious diseases, such as *Yersinia pseudotuberculosis* [16], *Shigella* [17], Verotoxigenic *Escherichia coli* O157 [9], and *Salmonella* [18]. Polymerase spiral reaction (PSR) was also recently developed as a nucleotide amplification method with high specificity and sensitivity, performed at a constant temperature, whose positive results are determined through a visual color change [19]. Different from LAMP, which needs three pairs of special primers to recognize eight distinct sequences on the target DNA, PSR is performed with two primers that contain an unrelated exogenous sequence and finally form large amounts of spiral structures [20]. PSR has been successfully used for the detection of infectious pathogens genes and antibiotic resistance genes [21].

The current study aimed to develop a PSR method targeting the chromosomally located invasion gene (*invA*), which is the most frequently conserved targeted gene that exists in all *Salmonella* isolates [8, 22]. Additionally, the specificity and sensitivity of this PSR assay were systematically validated by comparison of LAMP and real-time PCR. The effectiveness of the PSR system and its applicability in the food industry were also evaluated by analysis of daily food samples.

Materials and Methods

Bacterial Origin and DNA Extraction

To standardize and evaluate the PSR system, we used a total of 27 bacterial strains in this study, including 23 standard strains and 4 clinical isolates (Table 1). *Salmonella enteritidis* ATCC 13076 was employed as a reference strain. The clinical isolates of *Salmonella* strains were grown on Mac-Conkey agar (Mast Group Ltd., UK), and identified by cultural and biochemical characteristics (Biolog Inc., USA) [18]. DNA template for all bacterial strains was extracted from the enrichment culture broth by boiling method as previously described [23]. All DNA templates were stored in Luria-Bertani (LB) broth, containing 20% glycerol at -80°C until use.

Primer Design

All specific primers were designed based on the nucleotide sequence of the *invA* gene region obtained from the GenBank database (No. U43273). The design principle of PSR-specific primers was described previously. As can be observed in Fig. 1A, the spiral primers (Ft and Bt) that targeted the *invA* gene sequence consisted of a forward primer (F) and a reverse primer (B), whose Nr and N sequences were abstracted from a botanic gene [19]. Additionally, two auxiliary accelerated primers IF and IB were designed by Primer Premier 5 (PREMIER Biosoft International

Table 1. Bacterial strains used in this study.

Bacterial strain	Source	PSR	LAMP	qPCR
<i>Salmonella</i> Enteritidis,	ATCC 13076	+	+	+
<i>Salmonella</i> Paratyphi A	CMCC 50001	+	+	+
<i>Salmonella</i> Paratyphi B	CMCC 50004	+	+	+
<i>Salmonella</i> Paratyphi C	CMCC 50334	+	+	+
<i>Salmonella</i> Typhimurium	ATCC 14028	+	+	+
<i>Salmonella bongori</i>	ATCC 43975	+	+	+
<i>Salmonella</i> Pullorum	CMCC 50771	+	+	+
<i>Salmonella</i> Choleraesuis	CMCC 50018	+	+	+
<i>Salmonella</i> Diarizonae	CMCC 50165	+	+	+
<i>Salmonella</i> Arizonae	CMCC 50166	+	+	+
<i>Salmonella</i> M14-A	Clinical-isolate	+	+	+
<i>Salmonella</i> M25-A	Clinical-isolate	+	+	+
<i>Salmonella</i> W04-B	Clinical-isolate	+	+	+
<i>Salmonella</i> W09-A	Clinical-isolate	+	+	+
<i>Escherichia coli</i>	ATCC 25922	-	-	-
<i>Escherichia coli</i>	CMCC 83930	-	-	-
<i>Proteus mirabilis</i>	ATCC 12453	-	-	-
<i>Aeromonas hydrophila</i>	ATCC 7966	-	-	-
<i>Staphylococcus aureus</i>	ATCC 25923	-	-	-
<i>Staphylococcus aureus</i>	ATCC 29213	-	-	-
<i>Listeria monocytogenes</i>	ATCC 19115	-	-	-
<i>Yersinia enterocolitica</i>	ATCC 23715	-	-	-
<i>Vibrio parahaemolyticus</i>	ATCC 27519	-	-	-
<i>Shigella sonnei</i>	ATCC 9290	-	-	-
<i>Klebsiella pneumoniae</i>	ATCC 70603	-	-	-
<i>Campylobacter jejuni</i>	ATCC 33560	-	-	-
<i>Cronobacter sakazakii</i>	ATCC 12868	-	-	-

ATCC: American Type Culture Collection. CMCC: National Center for Medical Culture Collections. Clinical-isolates were preserved in our lab. +: positive result. -: negative result.

Co., USA) to enhance the reaction utilized in this study. The specificity of the designed primers was checked by BLAST, available in the NCBI sequence database. For the LAMP assay (Fig. 1B), a set of four primers comprising two inner primers (FIP and BIP) and two outer primers (Fig.3 to F3 and B3) were designed as previously specified by Hara-Kudo et al., and a pair of loop primers was also used to accelerate the amplification reaction [24]. A pair of qPCR primers and a probe were designed, which were then used as a standard reference for the sensitivity assays and food sample detection [25]. All primer sequences are listed in Table 2.

Reaction System

Both PSR and LAMP assays were set up in a final volume of 25 μl containing the following components: 2.5 μl of $10 \times$ ThermolPol

Fig. 1. Schematic presentation of PSR and LAMP assays.

(A) Schematic showing the mechanism of PSR. (B) Schematic showing the mechanism of LAMP.

reaction buffer, 1.4 mM dNTPs (Takara Bio, Japan), 3 mM MgSO_4 (Sigma, USA), 1.0 M betaine (Sigma), 1 μl (8U) of *Bst* DNA polymerase (New England Biolabs, USA), and 2 μl of an appropriate concentration of target DNA. The primers for the PSR assay contained 1.6 μM for Ft and Bt and 0.8 μM for IF and IB, whereas for the LAMP assay, 1.6 μM for FIP and BIP primers, 0.2 μM for F3 and B3 primers, and 0.8 μM for LF and LB primers were utilized. The reactions were carried out for 60 min at 65°C, and then the samples were incubated for 5 min at 85°C. For analysis of the products, a special color under normal light or ultraviolet light was observed in an isothermal amplification tube following the addition of 1 μl of SYBR Green I (2,000 $\times$) (Solarbio, China). The bright green fluorescence was considered positive, whereas orange indicated a negative result. Subsequently, the products were also analyzed by electrophoresis on 2.0% agarose gel.

The qPCR assay was conducted in a final volume of 50 μl , according to the manufacturer's instructions for the specific reaction systems. The protocol consisted of a cycle of denaturation at 95°C for 3 min, followed by 40 cycles of 10 sec at 94°C and

30 sec at 60°C. The fluorescence generated in each reaction was recorded at the extension step of each cycle.

Optimization of the PSR Assay

A PSR assay was carried out with different constituents and a temperature gradient of 61°C–69°C for 60 min. The concentration of *Bst*-DNA polymerase was set at 6, 8, 10, and 12 U/tube; the concentration of MgSO_4 was set at 1.0, 2.0, 3.0, and 4.0 mM, respectively. The concentration of betaine ranged from 0.6 to 1.4 M. The concentration of dNTP was optimized at 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0 mM, respectively. The incubation time was optimized from 30 to 120 min at the established optimal temperature. After incubation, analysis was conducted of the products in the 2.0% agarose gels stained with ethidium bromide (0.5 $\mu\text{g}/\text{ml}$).

Specificity of PSR

The specificity of the PSR assay, compared with that of LAMP, was evaluated by using 27 different DNA extracts (14 *Salmonella* strains and 13 non-*Salmonella* strains as listed in Table 1) and

Table 2. Primers of PSR, LAMP, and qPCR assays.

Name	Primer	Primer sequences (5'-3')
PSR primers	Ft	gtcaaagcgatcccgcttac-TCAACTTGGGAGCGTCTA
	Bt	cattccgcctagcgaaactg-GACTTCATCGGAATAATTAC
	IF	TATTACTTGTGCCGAAGAGCC
	IB	TTACCCAGAAATACTGACTGCTAC
LAMP primers	FIP	GACGACTGGTACTGATCGATAGTTTTTCAACGTTTCCTGCGG
	BIP	CCGGTGAAATTATCGCCACACAAAACCCACCGCCAGG
	F3	GGCGATATTGGTGTATTATGGGG
	B3	AACGATAAACTGGACCACGG
	Loop-F	GACGAAAGAGCGTGGTAATTAAC
	Loop-B	GGGCAATTCGTTATTGGCGATAG
qPCR primers	<i>invA</i> -F	AGCGTACTGGAAAGGGAAAG
	<i>invA</i> -B	ATACCGCCAATAAAGTTCACAAAG
	<i>invA</i> -probe	6FAM/CGTCACCTTTGATAAACTTCATCGCA-BHQ1

sterilized deionized water as the blank control. The reactions were performed as described above under corresponding conditions; all tests were repeated three times. A volume of 5 µl of each product was subjected to 2.0% agarose gel electrophoresis, stained with SYBR Green I, and visualized under ultraviolet light.

Sensitivity of PSR

To evaluate the sensitivity of the PSR assay, the concentrations of *Salmonella* Enteritidis ATCC 13076 DNA, extracted from serially diluted bacterial culture, ranging from 10⁶ CFU/ml–5 CFU/ml, were subjected to PSR, LAMP, and qPCR amplification in triplicate. The reactions were performed under the corresponding aforementioned amplification conditions. Next, SYBR Green I (2,000 ×) was added to the LAMP and PSR reaction tubes and visualized under natural and ultraviolet light. Meanwhile, the PSR and LAMP products were assayed by 2.0% agarose gel electrophoresis.

Detection of *Salmonella* in a Diverse Range of Food Samples

To determine the validity and reliability of the PSR method detection of the *invA* gene for *Salmonella* identification in food samples, a total of 532 food samples were purchased randomly in several local markets in Shenyang as follows: 103 pork, 97 beef, 95 mutton, 76 fish, 87 chicken, and 74 fresh vegetable samples. To confirm the results of the CPA assay, all 532 samples were evaluated by the culture-based method as a reference for comparative analysis [26]. Briefly, each food sample (25 g) was homogenized in 225 ml of buffered peptone water (BPW) and pre-enriched for 16–24 h at 37°C. A volume of 1 ml of each culture was centrifuged for 5 min at 10,000 ×g, and the pellet was re-suspended in 1 ml of sterile deionized water, followed by heating at 100°C for 10 min and centrifugation at 10,000 ×g for 3 min. Then, 2 µl of each supernatant was directly used as a DNA template for the PSR assays; each of the DNA samples was tested at least in triplicate.

Results

Establishment of the PSR Assay for *Salmonella* Detection

Similarly to the LAMP assay, the positive amplification in the PSR assay also revealed a ladder-like pattern with

Fig. 2. Results of the PSR amplification of the *invA* gene. (A) Results of agarose gel electrophoresis; (B) Observation of amplification products with SYBR Green I under natural light; (C) Observation of amplification products with SYBR Green I under ultraviolet light. M: Trans2K Plus II DNA Marker; (1) Negative control (double-distilled water); (2) Detection of the *invA* gene.

Fig. 3. Optimization of the reaction conditions of PSR assay.

(A) PSR assay incubation temperature optimization; (B) PSR assay *Bst* DNA polymerase concentration optimization; (C) dNTP concentration optimization; (D) betaine concentration optimization; (E) Mg^{2+} concentration optimization; (F) PSR assay incubation time optimization. The optimum conditions have been marked in the figures; (M) Trans2k Plus II DNA Marker; (N) negative control (double-distilled water).

electrophoretic separation on a 2.0% agarose gel, whereas the negative samples did not show any laddering pattern. Addition of the chromogenic substrate SYBR Green I (2000 ×) to the reaction tubes induced bright green fluorescence in the positive amplified products under natural and ultraviolet light, whereas the color of the negative samples remained orange (Fig. 2).

Optimization of the PSR Assay

Optimal temperatures ranging from 61 to 69°C at 2°C increments were tested and compared for. The best results

of the PSR assay were obtained at 63°C for 60 min, which were considered as standardized optimal PSR assay conditions (Figs. 3A and 3F). Various concentrations of *Bst*-DNA polymerase, ranging from 6 U/tube to 12 U/tube, were tested; optimal results were obtained at a concentration of 8 U/tube (Fig. 3B). The optimal results of the dNTP concentrations are represented in Fig. 3C; 1.5, 2.0, and 2.5 mM had the same efficiency in the amplification of the *invA* gene. Therefore, 1.5 mM was chosen as an optimum concentration. Different concentrations of betaine were examined (0.6–1.4 M), of which 1.2 M was obviously the

Fig. 4. Specificity of PSR and LAMP assays for *Salmonella* detection.

(A) The specificity of PSR assay; (B) The specificity LAMP assays; (M) Trans2K Plus II DNA Marker; (N) negative control. 1: *S. Enteritidis* ATCC 13076; 2: *S. Paratyphi A* CMCC 50001; 3: *S. Paratyphi B* CMCC 50004; 4: *S. Paratyphi C* CMCC 50334; 5: *S. typhimurium* ATCC 14028; 6: *S. bongori* ATCC 43975; 7: *S. Pullorum* CMCC 50771; 8: *S. Choleraesuis* CMCC 50018; 9: *S. Diarizonae* CMCC 50165; 10: *S. Arizonae* CMCC 50166; 11: *S. M14-A*; 12: *S. M25-A*; 13: *S. W04-B*; 14: *S. W09-A*; 15: *E. coli* ATCC 25922; 16: *E. coli* CMCC 83930; 17: *P. mirabilis* ATCC 12453; 18: *A. hydrophila* ATCC 7966; 19: *S. aureus* ATCC 25923; 20: *S. aureus* ATCC 29213; 21: *L. monocytogenes* ATCC 19115; 22: *Y. enterocolitica* ATCC 23715; 23: *V. parahaemolyticus* ATCC 27519; 24: *S. sonnei* ATCC 9290; 25: *K. pneumonia* ATCC 70603; 26: *C. jejuni* ATCC 33560; 27: *E. sakazakii* ATCC 12868.

optimum concentration (Fig. 3D). The optimum concentration of MgSO_4 was 3.0 mM (Fig. 3E).

Specificity of the PSR Assay

The results for the specificity of the *Salmonella invA*-PSR assay are presented in Table 1. Specifically, 14 *Salmonella* strains (including 10 standard reference bacterial and 4 self-isolated strains) and 13 non-*Salmonella* strains were examined by the PSR and LAMP methods, respectively. Of the 27 bacterial DNA samples, the 14 positive *Salmonella* strains showed a ladder-like pattern in 2.0% agarose electrophoresis and bright green fluorescence under ultraviolet light. In contrast, the 13 non-*Salmonella* strains and the blank control (sterilized deionized water) had no amplified products and remained orange under ultraviolet light (Fig. 4).

Sensitivity of the PSR Assay

We analyzed the sensitivity of the PSR method and compared it with that of two diagnostic methods (LAMP and qPCR) widely used for *Salmonella* detection. Serially diluted samples of *Salmonella enteritidis* ATCC 13076 DNA (from 1×10^6 CFU/ml to 5 CFU/ml) were applied. As can be seen in Fig. 5, the PSR, LAMP and qPCR methods had identical sensitivity, and the detection limit in the three methods was 50 CFU/ml (equivalent to 5 CFU/tube).

Detection of *Salmonella* in Artificially-Contaminated Food Samples

A total of 532 food samples (pork, beef, mutton, fish, chicken, and fresh vegetables) were analyzed using the reference culture-based method. All food samples (25 g) were enriched in 225 ml of buffered peptone water (BPW)

Fig. 5. The sensitivity of PSR, LAMP and qPCR assays.

(A) and (D) Analysis of the amplification products of PSR and LAMP by agarose gel electrophoresis; (B) and (E) Observation of the PSR and LAMP amplification products with SYBR Green I under natural light; (C) and (F) Observation of the PSR and LAMP amplification products with SYBR Green I under UV light; (G) Amplification results of qPCR; (M) Trans2K Plus II DNA Marker; (N) negative control (double-distilled water). 1–9: 1×10^6 , 1×10^5 , 1×10^4 , 1×10^3 , 1×10^2 , 50, 25, 15 and 5 CFU/ml, respectively.

for 16 h at 37°C. Positive results were obtained in 14 samples, whereas 518 samples had negative results (Fig. 6A). All DNA samples were extracted and then simultaneously analyzed by PSR. The amplified products were observed under UV light. Fourteen samples were positive in the PSR assay and 518 were PSR-negative, as confirmed by qPCR (data not shown). The relative diagnostic accuracy was 2.63% (14/532), with no false-negative or false-positive results (Fig. 6B).

Discussion

According to statistics, food-borne diseases caused by *Salmonella* are a major health issue, with high morbidity and mortality in humans and animals, especially in developing countries [23, 27]. To ensure effective control of *Salmonella* infections and improve the disease resistance of animals, antibiotics are commonly added to daily animal feed. However, the extensive abuse of antibiotics has resulted in

Fig. 6. Detection results of *Salmonella* by culture-based method and PSR. (A) Detection results of food samples by culture-based method and PSR; (B) Partial results of the PSR method applied in the testing of daily food samples (under UV light). 1–14: All fourteen positive products of PSR assays in daily food samples; 15–28: Partial results of PSR-negative amplification.

significant resistance. Epidemiological investigations have shown that the *Salmonella* infections have become particularly difficult to treat due to the emergence of multidrug-resistant strains; the pathogenic bacteria are usually transferred among different species [28, 29]. In recent years, the research on new *Salmonella* vaccines has continued uninterrupted, but the vaccines have not achieved ideal application effect. Therefore, reliable, rapid, specific and sensitive detection methods are still the main way to effectively control and prevent *Salmonella* infection.

Currently, the classical official methods for *Salmonella* detection and identification include mainly bacterial isolation and biochemical identification, which are time-consuming and can seriously delay the acquisition of detection results [30]. Although the PCR-based methods have been successfully established as a valuable method for *Salmonella* detection, their complicated procedures and expensive equipment, especially for the PSR method,

decrease their suitability for extensive practical use [6, 31]. A number of isothermal amplification methods have been successfully used to detect *Salmonella* spp. In the current study, the PSR method we developed for the rapid detection of *Salmonella* supplements and enriches the group of isothermal amplification methods.

In this study, we established a PSR method targeting the invasion gene (*invA*), considered to trigger the invasion of *Salmonella* into cultured epithelial cells and detected in all *Salmonella* isolates. The primer design in PSR was simpler than that in LAMP, as the sequence of the target region was selected in the same way as in conventional PCR. An exogenous sequence from an unrelated species was added to the primer sequence at the 5' end, which induced a spiral shape of the primers. Additionally, two accelerated primers were designed to improve the reaction velocity. Optimal PSR reactions were achieved at 62°C for 60 min, with the addition of 8 U *Bst*-DNA polymerase, 1.5 mM of dNTP, and 3.0 mM of MgSO₄. PSR successfully amplified all 14 *Salmonella* strains of different serotypes and 13 non-*Salmonella* strains tested. Moreover, it was *Salmonella*-specific, as it did not detect any other common non-*Salmonella* strains. This level of specificity was identical to that of an *invA*-based LAMP assay, developed by Hara-Kudo *et al.* [24]. In addition, the performance of PSR may need to be further evaluated in a larger number of *Salmonella* strains. The sensitivity of the PSR method was equal with that of real-time PCR, achieving a high sensitivity level (5 CFU/tube) and two-fold higher sensitivity than those in the LAMP method. The evaluation of PSR in 532 food samples further confirmed its promising potential for the detection of *Salmonella*. The novel method correctly identified 14 samples as positive with no false-positive or false-negative results. All aforementioned evidence suggests that PSR can be used as a new effective technique for *Salmonella* detection in foods.

Insoluble by-products (Mg₂P₂O₇) are formed after the end of the reactions in several isothermal amplification methods. Thus, they can be used to directly determine if the result of a given particular reaction is positive or negative by naked eye observation. However, this naked eye technique is not a completely accurate way to judge the reaction results as the use of fluorescent dyes is generally far more credible. However, finding ways to effectively reduce the pollution risk caused by the large number of aerosols generated during amplification still remains a major problem to be solved in the future.

In conclusion, the *invA*-based PSR method developed and described in current study has many advantages, for

detection of *Salmonella* in food, including rapidity, reliability, sensitivity, and specificity, not to mention lacking a requirement for expensive equipment and reagents.

Acknowledgments

This research was financed by the Natural Science Foundation of China (No.31272615).

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

- McClelland M, Sanderson KE, Spieth J, Clifton SW, Latreille P, Courtney L, et al. 2001. Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. *Nature* **413**: 852-856.
- Carvajal-Restrepo H, Sanchez-Jimenez MM, Diaz-Rodriguez S, Cardona-Castro N. 2017. Detection of *Salmonella* human carriers in Colombian outbreak areas. *J. Infect. Dev. Ctries* **11**: 228-233.
- Lopes ATS, Albuquerque GR, Maciel BM. 2018. Multiplex real-time polymerase chain reaction for simultaneous quantification of *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus* in different food matrices: advantages and disadvantages. *Biomed. Res. Int.* **2018**: 6104015.
- Vinayaka AC, Ngo TA, Kant K, Engelsmann P, Dave VP, Shahbazi MA, et al. 2019. Rapid detection of *Salmonella enterica* in food samples by a novel approach with combination of sample concentration and direct PCR. *Biosens. Bioelectron.* **129**: 224-230.
- Hendriksen SW, Orsel K, Wagenaar JA, Miko A, van Duikeren E. 2004. Animal-to-human transmission of *Salmonella* Typhimurium DT104A variant. *Emerg. Infect. Dis.* **10**: 2225-2227.
- Srisawat M, Panbangred W. 2015. Efficient and specific detection of *Salmonella* in food samples using a stn-based loop-mediated isothermal amplification method. *Biomed. Res. Int.* **2015**: 356401.
- Abdullah J, Saffie N, Sjasri FA, Husin A, Abdul-Rahman Z, Ismail A, et al. 2014. Rapid detection of *Salmonella* Typhi by loop-mediated isothermal amplification (LAMP) method. *Braz. J. Microbiol.* **45**: 1385-1391.
- Yang Q, Domesle KJ, Ge B. 2018. Loop-mediated isothermal amplification for *Salmonella* detection in food and feed: Current applications and future directions. *Foodborne Pathog. Dis.* **15**: 309-331.
- Carloni E, Rotundo L, Brandi G, Amagliani G. 2018. Rapid and simultaneous detection of *Salmonella* spp., *Escherichia coli* O157, and *Listeria monocytogenes* by magnetic capture hybridization and multiplex real-time PCR. *Folia Microbiol (Praha)*. **63**: 735-742.
- Yang Q, Domesle KJ, Wang F, Ge B. 2016. Rapid detection of *Salmonella* in food and feed by coupling loop-mediated isothermal amplification with bioluminescent assay in real-time. *BMC Microbiol.* **16**: 112.
- Balachandran P, Friberg M, Vanlandingham V, Kozak K, Manolis A, Brevnov M, et al. 2012. Rapid detection of *Salmonella* in pet food: design and evaluation of integrated methods based on real-time PCR detection. *J. Food Prot.* **75**: 347-352.
- Li X, Zhang S, Zhang H, Zhang L, Tao H, Yu J, et al. 2009. A loop-mediated isothermal amplification method targets the phoP gene for the detection of *Salmonella* in food samples. *Int. J. Food Microbiol.* **133**: 252-258.
- Lofstrom C, Schelin J, Norling B, Vigre H, Hoorfar J, Radstrom P. 2011. Culture-independent quantification of *Salmonella enterica* in carcass gauze swabs by flotation prior to real-time PCR. *Int. J. Food Microbiol.* **145 (Suppl 1)**: S103-109.
- Mashooq M, Kumar D, Niranjana AK, Agarwal RK, Rathore R. 2016. Development and evaluation of probe based real time loop mediated isothermal amplification for *Salmonella*: a new tool for DNA quantification. *J. Microbiol. Methods* **126**: 24-29.
- Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* **28**: E63.
- Horisaka T, Fujita K, Iwata T, Nakadai A, Okatani AT, Horikita T, et al. 2004. Sensitive and specific detection of *Yersinia pseudotuberculosis* by loop-mediated isothermal amplification. *J. Clin. Microbiol.* **42**: 5349-5352.
- Soli KW, Kas M, Maure T, Umezaki M, Morita A, Siba PM, et al. 2013. Evaluation of colorimetric detection methods for *Shigella*, *Salmonella*, and *Vibrio cholerae* by loop-mediated isothermal amplification. *Diagn. Microbiol. Infect. Dis.* **77**: 321-323.
- Zhuang L, Gong J, Li Q, Zhu C, Yu Y, Dou X, et al. 2014. Detection of *Salmonella* spp. by a loop-mediated isothermal amplification (LAMP) method targeting bcfD gene. *Lett. Appl. Microbiol.* **59**: 658-664.
- Liu W, Dong D, Yang Z, Zou D, Chen Z, Yuan J, et al. 2015. Polymerase Spiral Reaction (PSR): a novel isothermal nucleic acid amplification method. *Sci. Rep.* **5**: 12723.
- Gupta V, Chakravarti S, Chander V, Majumder S, Bhat SA, Gupta VK, et al. 2017. Polymerase spiral reaction (PSR): a novel, visual isothermal amplification method for detection of canine parvovirus 2 genomic DNA. *Arch. Virol.* **162**: 1995-2001.
- Jiang X, Dong D, Bian L, Zou D, He X, Ao D, et al. 2016. Rapid detection of *Candida albicans* by polymerase spiral reaction assay in clinical blood samples. *Front Microbiol.* **7**: 916.

22. Galan JE, Ginocchio C, Costeas P. 1992. Molecular and functional characterization of the *Salmonella* invasion gene *invA*: homology of InvA to members of a new protein family. *J. Bacteriol.* **174**: 4338-4349.
23. Garrido-Maestu A, Azinheiro S, Carvalho J, Abalde-Cela S, Carbo-Argibay E, Dieguez L, *et al.* 2017. Combination of microfluidic loop-mediated isothermal amplification with gold nanoparticles for rapid detection of *Salmonella* spp. in food samples. *Front Microbiol.* **8**: 2159.
24. Hara-Kudo Y, Yoshino M, Kojima T, Ikedo M. 2005. Loop-mediated isothermal amplification for the rapid detection of *Salmonella*. *FEMS Microbiol. Lett.* **253**: 155-161.
25. Kasturi KN, Drgon T. 2017. Real-Time PCR method for detection of *Salmonella* spp. in environmental samples. *Appl. Environ. Microbiol.* **83**:14.
26. Malorny B, Bunge C, Helmuth R. 2007. A real-time PCR for the detection of *Salmonella* Enteritidis in poultry meat and consumption eggs. *J. Microbiol. Methods.* **70**: 245-251.
27. Sanchez-Vargas FM, Abu-El-Haija MA, Gomez-Duarte OG. 2011. *Salmonella* infections: an update on epidemiology, management, and prevention. *Travel Med. Infect. Dis.* **9**: 263-277.
28. Bai L, Zhao J, Gan X, Wang J, Zhang X, Cui S, *et al.* 2016. Emergence and diversity of *Salmonella* enterica serovar indiana isolates with concurrent resistance to ciprofloxacin and cefotaxime from patients and food-producing animals in China. *Antimicrob. Agents Chemother.* **60**: 3365-3371.
29. Gong J, Kelly P, Wang C. 2017. Prevalence and antimicrobial resistance of *Salmonella* enterica serovar indiana in China (1984-2016). *Zoonoses Public Health* **64**: 239-251.
30. Liu Z, Zhang Q, Yang NN, Xu MG, Xu JF, Jing ML, *et al.* 2019. Rapid and sensitive detection of *Salmonella* in chickens using loop-mediated isothermal amplification combined with a lateral flow dipstick. *J. Microbiol. Biotechnol.* **29**: 454-464.
31. Wang YX, Zhang AY, Yang YQ, Lei CW, Cheng GY, Zou WC, *et al.* 2018. Sensitive and rapid detection of *Salmonella* enterica serovar Indiana by cross-priming amplification. *J. Microbiol. Methods.* **153**: 24-30.