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Evaluation of Different Genetic Targets for *Salmonella enterica* Serovar Enteriditis and Typhimurium, Using Loop-Mediated Isothermal AMPlification for Detection in Food Samples

Sarah Azinheiro, Joana Carvalho, Marta Prado and Alejandro Garrido-Maestu**

Department of Life Sciences, Nano4Food Unit, Food Quality and Safety Research Group, International Iberian Nanotechnology Laboratory, Braga, Portugal

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***Correspondence:**
Marta Prado
marta.prado@inl.int;
Alejandro Garrido-Maestu
alejandro.garrido@inl.int

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Salmonella Enteritidis and *Salmonella* Typhimurium continue to be the most frequently identified serovars among confirmed cases of salmonellosis. In the current study, different genetic targets (*safA*, *sdf I*, STM4497, and *typh*) were compared, attending to their specificity and sensitivity in pure cultures and in spiked samples, in order to determine their capacity to accurately identify them by loop-mediated isothermal amplification (LAMP). For the genes selected to detect Enteritidis, both performed equally well regarding their specificity, but *safA* proved more sensitive than *Sdf I*; minor differences were observed among these genes when analyzing spiked food samples. Regarding the targets for Typhimurium, STM4497, and *typh*, the former demonstrated to be more specific and sensitive, both when analyzing pure cultures as well as spiked samples. These results highlight the importance of an adequate evaluation of the genetic targets selected, before their implementation for routine analyses.

Keywords: ***Salmonella*** **Enteritidis,** ***Salmonella*** **Typhimurium, LAMP, characterization,** ***safA*****,** ***Sdf I*****,** ***typh*****, STM4497**

INTRODUCTION

Salmonella continues to be a major health issue in Europe, as well as in the rest of the world. This is supported by the figures reported by the European Food Safety Authority, who indicated that in 2015 a total of 94,625 salmonellosis cases were confirmed, representing a 1.9% increase compared to the previous year. In addition, it was also highlighted that the most prevalent serovar continues to be *Salmonella* Enteritidis (*S*E) and *Salmonella* Typhimurium (*S*T), representing 45.7 and 15.8% of all reported serovars confirmed in human cases (EFSA, 2015).

It has been extensively reported that classical microbiological methods, even though reliable, are lengthy and tedious. In this sense molecular applications have greatly allowed the reduction in the time needed to achieve the final result (Chapela et al., 2015; Law et al., 2015). One of the most popular methodologies, as evidenced by the increased number of publications in many different fields of microbiology, relies on the detection of specific genes by PCR/qPCR (Malorny et al., 2004; Park et al., 2013; Gianfranceschi et al., 2014; Garrido-Maestu et al., 2015; Maurischat et al., 2015). Novel technologies are continually being developed in order to further

reduce costs and ease of implementation. Gaining increasing interest are the isothermal amplification approaches. Among them, we can find helicase-dependent amplification, nucleic acid sequence-based amplification or Recombinase Polymerase Amplification, among others (Fykse et al., 2007; Kuchta et al., 2014; Kim and Lee, 2016; Garrido-Maestu et al., 2018). One of the isothermal methods that has caught great attention in recent years is Loop-mediated isothermal AMPlification (LAMP), which was originally developed by Notomi et al. (2000). It relies on an auto-cycling strand displacement DNA synthesis performed by the *Bst* DNA polymerase large fragment. LAMP presents several advantages over PCR/qPCR, such as higher specificity due to the use of six primers instead of two, the reaction is performed at one single temperature, thus not needing a thermocycler; in addition, during the reaction, an insoluble product is formed (magnesium pyrophosphate) generating an increase in turbidity detectable with a naked eye, which can even be correlated with DNA concentration making the technique quantitative (Mori et al., 2001, 2004; Hara-Kudo et al., 2007). New approaches have been developed in recent years with the aim of improving naked eye detection of positive amplification, such as the use of gold nanoparticles (Arunrut et al., 2016; Kong et al., 2018). All these approaches are very attractive for the development of miniaturized devices due to their simplicity in temperature setup, and feasibility for *in situ* performance of the assays (Garrido-Maestu et al., 2017a).

Along with the increase in methodologies, many different genetic targets have been reported for different foodborne pathogen detection and characterization, including *Salmonella* (Zhang et al., 2011; Liu et al., 2012; Tang et al., 2012; Zhuang et al., 2014; Chen et al., 2015). With so many options, difficulties in selecting the most appropriate target have occurred. In the present study, two sets of genetic targets were tested for each serovar. For *S*E genes, *safA* and *Sdf I*, were selected. *safA* encodes for the major subunit of *S. enterica* atypical fimbriae and is involved in host-restricted colonization of the porcine ileum (Maurischat et al., 2015). *Sdf I* was reported to be specific for *S*E and is chromosomally encoded, but its function is unknown (Agron et al., 2001; Regan et al., 2008). Similarly, two gene targets specific for *S*T are represented by STM4497 and *typh*. STM4497 encodes for a putative cytoplasmic protein (Kim et al., 2006). The gene target *typh*, was described previously to be Typhimurium specific, but no specific biological function has been assigned (Olsen et al., 1995; Alvarez et al., 2004). The selection of these targets was based on the fact that LAMP assays have been previously published for most of them, and the inclusivity/exclusivity tests reported several target and non-target strains from the genus *Salmonella* and nearest phylogenetically related species. The aim of the present study was to evaluate these gene targets for the specific identification of *S*E and *S*T in foods, based on LAMP.

MATERIALS AND METHODS

Bacterial Strains and Culture Media

S1400 and CECT 4594 were considered the reference strains for *S*E and *S*T, respectively. These strains were selected for the sensitivity assays, as well as for all spiking experiments. In addition to these, a total of 34 strains were included in the evaluation of the specificity. Information regarding the strains tested is provided in **Table 1**.

All pure bacterial cultures were performed by inoculating 1 single colony into 4 mL of buffered peptone water or tryptic soy broth (BPW, TSB, Biokar diagnostics S.A., France) and incubated at 37°C overnight. Whenever the cultures were used for food sample inoculation, 10-fold serial dilutions were performed in BPW, and then plated on TSB +15 g/L of agar. The plates were incubated as detailed previously. Finally, for food sample analysis, the enrichment broth selected was mTA10, as described by Garrido et al. (2013), and the incubation was performed at 37°C for 18–24 h.

DNA Extraction

Pure Bacterial Cultures

One milliliter of an overnight enrichment culture was centrifuged at 4,000 × *g* for 5 min. The supernatant was discarded, and the pellet resuspended in 1 mL of Tris-EDTA 1× buffer (TE 1×,

TABLE 1 | Strain list selected for the evaluation of the specificity of the Loop-mediated isothermal AMPlification (LAMP) assays.

Strain	Source	*N*	*Sdf I*	*safA*	STM4497	*typh*		Source	*N*	*Sdf I*	*safA*	STM4497	*typh*
S. Typhimurium	CECT 4594	1	–	–	+	+	*Salmonella* spp.	Mollusk AMC 253	1	–	–	–	+[a]
S. Typhimurium	P.T. AMC 96	1	–	–	+	–[a]	*Salmonella* spp.	Mollusk AMC 90, 255	2	–	–	–	+[a]
S. Typhimurium	Mollusk AMC 238	1	–	–	+	+	*Salmonella* spp.	Unknown AMC 260, 261	2	–	–	–	–
S. Enteritidis	UB (S1400)	1	+	+	–	–	*L. innocua*	CECT 910, 5376, 4030; CUP 1141, 1325, 2110	6	–	–	–	–
S. Enteritidis	P.T. AMC 82	1	+	+	–	–	*L. monocytogenes*	CECT 935	1	–	–	–	–
S. Veneziana	Mollusk AMC 200	1	–	–	–	–	*L. monocytogenes*	Mollusk	8	–	–	–	–
S. Wentworth	P.T. AMC 84	1	–	–	–	+[a]	*L. monocytogenes*	Chicken	1	–	–	–	–
S. Oranienburg	Mollusk AMC 28	1	–	–	–	+[a]	*L. seeligeri*	CECT 917	1	–	–	–	–
S. Anatum	P.T. AMC 60	1	–	–	–	–	*L. ivanovii*	CECT 913	1	–	–	–	–
S. Liverpool	P.T. AMC 198	1	–	–	–	–	*C. coli*	UM	1	–	–	–	–

N, number of strains; CECT, Spanish Type Culture Collection; P.T, Proficiency Test; UB, University of Bristol's collection; CUP, Catholic University of Porto; AMC, collection from the Institute of Applied Microbiology-ASMECRUZ; UM, University of Minho collection.
[a]*Strain misidentified.*
A "+" and "–" sign mean exponential amplification or non-exponential amplification detected in the Loopamp Realtime Turbidimeter LA-500.

10 mM Tris–HCl, 1 mM EDTA, Sigma–Aldrich, St. Louis, MS, USA) and centrifuged using the same conditions as described above. The pellet was resuspended in 300 µL of TE 1× and heated at 99°C for 15 min with constant agitation (1,000 rpm) in a shaker Thermomixer comfort heating block (Eppendorf AG, Germany). Finally, the bacterial cultures were centrifuged as detailed above, and the supernatant, containing bacterial DNA, was transferred to a clean tube and stored at −20°C. DNA concentration was measured with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). DNA extracted from pure bacterial cultures of each reference strain was 10-fold serially diluted in TE 1×, this was performed for the determination of the lowest detectable concentration.

Food Samples

In order to avoid PCR inhibitors which may be associated with the food matrices, 1 mL of all food samples was centrifuged at 380 × *g* for 2 min to remove food debris, the supernatant was collected and centrifuged at 16,000 × *g* for 5 min. The pellet was resuspended in 1 mL of PBS and centrifuged again using the same conditions as previously described. After eliminating the PBS, the pellet was resuspended in 300 µL of 6% Chelex®100 (Bio-Rad Laboratories, Inc., USA), the samples were incubated at 56°C for 15 min with constant agitation (1,000 rpm). This step was followed by a thermal lysis at 99°C for 10 min. Finally, the samples were centrifuged at 16,000 × *g* for 5 min, and the supernatant was collected, and stored as described for pure cultures.

Genes Selected and LAMP Reaction

Two sets of genetic targets were tested for each serovar, *safA* and *Sdf I* for *S*E; and STM4497 and *typh* for *S*T. The evaluation of *safA* and STM4497 was based on the primers designed by Garrido-Maestu et al. (2017b) with the addition of newly designed loop primers, while for *Sdf I* and *typh* those designed by Yang et al. (2010) and Pavan Kumar et al. (2014), respectively, were chosen. The selection of the primers for *safA* and STM4497, was based on the fact that they exhibited excellent performance (accurate, sensitive, and specific detection) in food samples including chicken and turkey (Garrido-Maestu et al., 2017b). On the other hand, regarding those targeting *Sdf I* and *typh*, it was based on the fact that their specificity was tested against a reasonable panel of strains (5 *S*E and 8 non-*S*E, including 1 *S*T with *Sdf I*; and 28 *S*T, 22 non-*S*T and 6 non-*Salmonella* for *typh*). Further details regarding the sequences of all primers, are provided in **Table 2**.

All LAMP experiments were performed in a Loopamp Realtime Turbidimeter LA-500 (Eiken Chemical Co., Ltd., Japan). The reactions were prepared in a final volume of 25 µL, with 1 M Betaine (Sigma–Aldrich, St. Louis, MS, USA), 12.5 µL of Isothermal Master Mix (OptiGene Ltd., Horsham, UK) and the corresponding amount of primers (see **Table 2**), and 3 µL of template DNA. The amplification was accomplished at 65°C for 1 h. Even though the amplification was performed in 1 h, only those food samples reporting positive within the first 30 min were considered as such for *safA*, *Sdf I*, and STM4497, while for *typh*, due to the lack of loop primers, up to 40 min were considered acceptable.

TABLE 2 | Primer list.

Bacterium	Gene		Sequence 5′-3′	Concentration[a]	Reference
S. Enteritidis	*safA*	SEN-F3	GTT GCT AAC ACG ACA CTG GAC	100	Garrido-Maestu et al. (2017b)
		SEN-B3	GTG GGA TAT TCT GAG CCC CTA T		
		SEN-FIP	AGC CCA CAG TGA GTA TCG TG-CGC TGC TGG TAG TGC ATG G	600	
		SEN-BIP	CAG AGG TCA TGG CGC GCA AAT-GGC ATT GGT ATC AAA GGT GA		
		SEN-LB	GTG GAA TGG GAG GAG CTG GT	300	
S. Typhimurium	STM4497	STM-F3	AGC CGC ATT AGC GAA GAG	100	Garrido-Maestu et al. (2017b)
		STM-B3	GCG GTC AAA TAA CCC ACG T		
		STM-FIP	ACC TGC AGC TCA TTC TGA GCA G-TCA AAA ACA ACG GCT CCG G	400	
		STM-BIP	GAA AAG GAC CAC AAG TTC GCG C-TCA GTG AGC ATG TCG ACG AT		
		STM-LF	TCA AAA ATC CAG AAC CCA ATC TCA	200	
S. Enteritidis	*Sdf I*	F3	GGG AGG AGC TTT AGC CAA	200	Yang et al. (2010)
		B3	ATG GTG AGC AGA CAA CAG		
		FIP	CAT GCT CGC TGC ACA AAA G C-GAG AGG CGG TTT GAT GTG	800	
		BIP	CTG GAA AGC CTC TTT ATA TAG CTC A-TGA TAT ACT CCC TGA ATC TGA GA		
		LF	GCC TAA AAA ATC AGT GAC GAA CCA A	400	
		LB	CTG ACC TCT AAG CCG GTC AAT G		
S. Typhimurium	*typh*	Typh F3	CAT CGT TGC GCA ATA GCT	400	Pavan Kumar et al. (2014)
		Typh B3	GTT TTT CAA CAC CAT TTT TCA AC		
		Typh FIP	TGC TGC TGT GCT TAT TAC TTT GTA AGT ATT TGT TCA CTT TTT ACC CCT	1,600	
		Typh BIP	GAT GCG CAG TGC CTA TTA AAC CTT AAG GCA ACG TAT CCT CTC		

F3/B3: outer primers, FIP/BIP: inner primers, LF/LB: loop primers.
[a]*Concentration units are nM.*

Food Sample Contamination and Analysis

A total of 87 food samples were spiked with different concentrations of both serovars, *SE* and *ST*. The types of foods selected covered those of high risk (raw chicken, turkey, raw, and cooked egg products). Twenty-five grams of each food sample were weighed and 225 mL of mTA10 broth were added and mixed for 30 s, then 1 mL of the appropriate bacterial dilution was added and incubated as detailed in the Section "Materials and Methods"

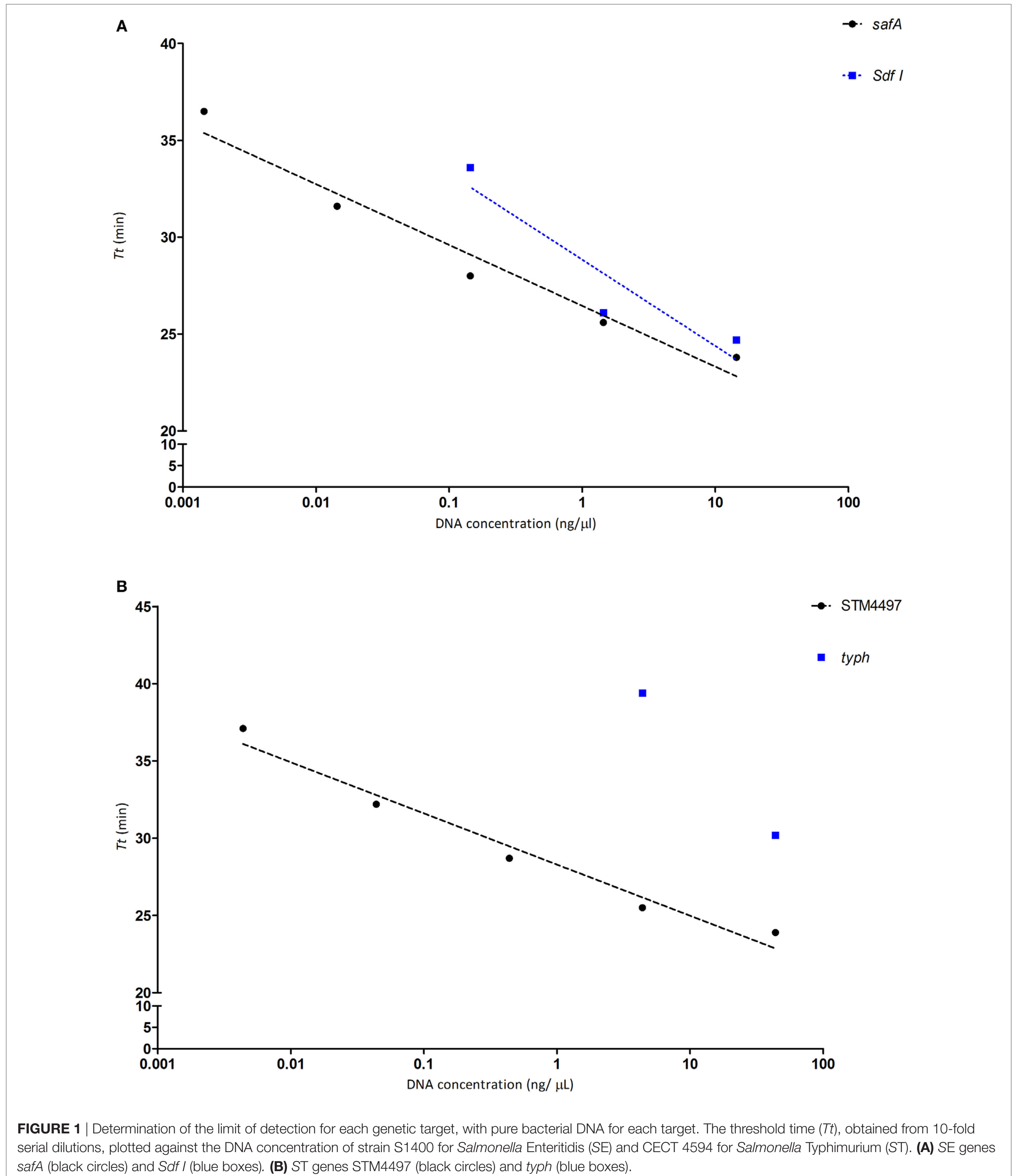

FIGURE 1 | Determination of the limit of detection for each genetic target, with pure bacterial DNA for each target. The threshold time (*Tt*), obtained from 10-fold serial dilutions, plotted against the DNA concentration of strain S1400 for *Salmonella* Enteritidis (*SE*) and CECT 4594 for *Salmonella* Typhimurium (*ST*). **(A)** *SE* genes *safA* (black circles) and *Sdf I* (blue boxes). **(B)** *ST* genes STM4497 (black circles) and *typh* (blue boxes).

"Bacterial Strains And Culture Media." After enrichment, DNA was extracted as described in the Section "Materials and Methods" "Food Samples."

Evaluation of the Capacity to Detect *SE* and *ST* in Food Samples

The results obtained after the inoculation, and analysis of the different food samples were classified as Positive or Negative agreement (PA and NA) if matched those expected, while as Positive or Negative Deviations (PD and ND) if the results did not match. With these values, the following parameters were calculated: relative sensitivity, specificity and accuracy (SE/SP/AC), positive and negative predictive values (PPV/NPV), index kappa of concordance (κ), and the acceptability limit (AL); as described elsewhere (Malorny et al., 2003; Tomas et al., 2009; Anderson et al., 2011; Garrido et al., 2013; D'Agostino et al., 2016).

RESULTS

Specificity of the Assays and Limit of Detection (LoD)

The evaluation of the specificity against a panel of 34 strains gave the expected results with both genes (*safA* and *Sdf I*) for *S*E. Both target strains were positive, while the 32 resulted negative. However, late amplification occurred with *safA* and we noticed that the threshold time (*Tt*) values higher than 35 min (positive strains reported *Tt* values below 30 min), occurred with certain strains, while this deviation in *Tt* values were not observed for *Sdf I*.

Regarding the identification of *S*T, all three target strains were detected with STM4497, without any interference due to non-target species. However, even the selection of *typh* gene, resulted in correct identification of all non-*Salmonella* strains, three *Salmonella* strains were misidentified as *S*T, AMC 28 (*S*. Oranienburg), 84 (*S*. Wentworth), and 253 (*Salmonella* non-Enteritidis/non-Typhimurium); while isolate AMC 96, previously confirmed as *S*T by qPCR with F3/B3 primers (Garrido-Maestu et al., 2017b) was not detected.

Regarding the LoD (lowest detectable concentration), for *S*E with *safA* it was possible to detect 0.00144 ng/µL over 5 consecutive 10-fold dilutions, while with *Sdf I* could only cover 3 dilutions reaching 0.144 ng/µL, as depicted in **Figure 1A**. Regarding *S*T, the lowest LoD was achieved with STM4497 reaching 0.00438 ng/µL, likewise *safA* over five consecutive dilutions; while with *typh* a higher value was detected as could only detect 4.38 ng/µL, see **Figure 1B**.

Evaluation and Comparison of the Different Assays to Detect *SE* and *ST* in Food Samples

Different types of foods, i.e., chicken, turkey, eggs, and egg products, were spiked at different concentrations, and with different combination levels of each *S*E/*S*T strain. A total of 87 samples were analyzed. Both *S*E targets gave similar positive results where all but two samples were correctly identified. However, with the *S*T STM4497 gene target, again only two deviations were detected, while with the *typh* gene target, 16 of the 87 samples were misidentified. These results are summarized in **Table 3**.

Based on the results obtained from the spiked food samples, it was determined that both genetic targets for *S*E performed well, with minor differences among them. This was not the case for *S*T, as major differences were observed when targeting STM4497 and *typh*. The values obtained by the later were the worst of all the genes evaluated. It is worth highlighting that the *k* value obtained was of 0.62, which can be interpreted as "substantial agreement," while for the rest, values higher than 0.9 were obtained. This is interpreted as "almost complete concordance." The results are summarized in **Table 4**.

DISCUSSION

The increased acceptance of molecular methods for the detection of different bacterial pathogens in foods has led to the appearance of a great number of approaches for this purpose. In the current study, a set of four genes, two for *S*E and two for *S*T, were systematically compared in order to evaluate their adequacy to

TABLE 3 | Spiked samples summary.

Sample	*N*	SE	ST	*Sdf I*	*safA*	STM4497	*typh*	Observations[a]
Egg	5	–	–	–	–	–	+[a]	4 PD
	3	<10	<10	+	+	+	+[a]	1 ND
	1	–	10–10^2	–		+	+	
	2	–	10^2–10^3				+[a]	1 ND
	1	–	10^8	–	–	+	+	
	1	10^2–10^3	–	+	+	–	+[a]	1 PD
	1	10^8	–	+	+	–	+[a]	1 PD
	1	10–10^2	10^3–10^4	+	+	+	+	
	1	10^2–10^3	10^2–10^3	+	+	+	+	
	1	10^3–10^4	10^3–10^4	+	+	+	+	
	1	10^3–10^4	10–10^2	+	+	+	+	
	1	10^4–10^5	10^4–10^5	+	+	–[a]	+	1 ND
	1	10^8	10^8	+	+	+	+	
Omelet	3	–	–	–	–	–	+[a]	1 PD
	1	10–10^2	–	+	+	–	–	
	1	10^2–10^3	–	+	+	–	+[a]	1 PD
Chicken	9	–	–	–	–	–	–	
	3	–	<10	–	–	+	+	
	13	<10	–	+	+	–	–	
	2	<10	<10	+	+	+	+[a]	1 ND
	1	<10	10^8	–[a]	–[a]	+	+	1ND
	1	10^8	<10	+	+	–[a]	–[a]	1 ND
Turkey	5	–	–	–	–	–	–	
	11	–	<10	–	–	+	+[a]	3 ND
	1	–	10–10^2	–	+[a]	+	+	1PD
	1	–	10^2–10^3	–	–	+	+	
	10	<10	–	+[a]	+	–	–	1 ND
	2	10–10^2	–	+	+	–	–	
	1	10^2–10^3	–	+	+	–	–	
	1	10–10^2	<10	+	+	+	+	
	1	<10	10–10^2	+	+	+	–[a]	1 ND

[a]*Deviations obtained per sample type and inoculation range. ND and PD stand for Negative and Positive Deviation respectively.*

TABLE 4 | Genetic target comparison.

Gene	*N*	PA	PD	NA	ND	SE	SP	AC	PPV	NPV	κ	AL
safA	87	44	1	41	1	97.8	97.6	97.7	97.8	97.6	0.95	0–2
Sdf I	87	45	0	40	2	95.7	100.0	97.7	100.0	95.2	0.95	0–2
STM4497	87	45	0	40	2	95.7	100.0	97.7	100.0	95.2	0.95	2–2
typh	87	27	8	44	8	77.1	84.6	81.6	77.1	84.6	0.62	0–16

N, number of samples; PA, Positive Agreement; PD, Positive Deviation; NA, Negative Agreement; ND, Negative Deviation; SE, relative sensitivity; SP, relative specificity; AC, relative accuracy; PPV, positive predictive value; NPV, negative predictive value; κ, index kappa of concordance [interpretation: 0.61–0.8 substantial agreement; 0.81–1.00 almost complete concordance according to Altman (1991) and Anderson et al. (2011)]. AL acceptable with values below 3–6 (D'Agostino et al., 2016).

detect these, particularly problematic *Salmonella* serovars, in food samples.

Overall, it was observed that both genes evaluated targeting *S*E (*safA* and *Sdf I*) performed well, with minor differences. In the specificity test, all bacterial strains were correctly identified with both genes. Regarding the LoD with DNA extracted from pure bacterial cultures, *safA* was 100 times more sensitive than *Sdf I* (0.00144 ng/μL, compared to 0.144 ng/μL). The results obtained after the analysis of spiked samples were similar, being only detected one PD with *safA* and one ND associated with a sample co-inoculated with *S*T but with a concentration 10^7 times higher. This sample was also misidentified with *Sdf I*. With this second gene, no PD were detected but a second ND was obtained. This was associated with a sample with <10 cfu/25 g of *S*E. Overall, in spiked samples, both genes obtained values higher than 95% in all the parameters evaluated and the Acceptability Limit (AL) below 3 and 6, as recommended (D'Agostino et al., 2016).

Greater differences were observed in the evaluation of the genetic targets selected for *S*T. While STM4497 obtained very good results, *typh* proved more difficult. Under the conditions tested the lowest DNA concentration that was detectable with STM4497 was 0.00438 ng/μL, while with the *typh* target only two consecutive dilutions were positive and reached 4.38 ng/μL. In addition, while all pure culture strains were correctly identified with STM4497, a total of four strains were misidentified targeting *typh* gene (one *S*T was not detected, and three non-*S*T obtained positive results). It is worth considering that the specificity problems were only associated with *Salmonella* strains, as all the 19 non-*Salmonella* isolates were correctly identified as negative.

The differences in performance detected when testing pure cultures matched what was observed after sample inoculation. For STM4497, only two ND were obtained (these were associated with two samples co-inoculated with *S*E, thus it seems that this second strain grew more than *S*T). Regarding *typh*, a total of 16 deviations were detected. Out of these, six ND were associated with inoculation levels of <10 cfu/25 g, 1 ND was a sample co-inoculated with 10^7 times more *S*E, and one with an inoculum in the range of 10^2–10^3 cfu/25 g. As commented above, these results correlate with those obtained with DNA isolated from pure bacterial cultures, where STM4497 proved more sensitive than *typh*. Finally, eight PD were detected, five associated with non-spiked samples, and three more with samples inoculated with *S*E. All these erroneous results obtained in food samples, corroborated the specificity data obtained with pure bacterial cultures, and ended up in unacceptable AL values (0–16).

When the results obtained were compared with those previously published for each target, some differences were observed. For *safA* and STM4497 it was possible to reach a lower LoD, with DNA from pure cultures, than that reported by Garrido-Maestu et al. (2017b); but minor differences were observed with spiked samples. These discrepancies may be associated with small changes in the method, such as the inclusion of loop primers, which were not used in the original study.

The specificity results obtained with *Sdf I* matched those previously published by Yang et al., but the LoD was higher in the current study (Yang et al., 2010). Greater differences were observed for *typh*, with respect to the study of Pavan Kumar et al., who reported excellent specificity (Pavan Kumar et al., 2014). In our experiments, their primers were not able to correctly discriminate all the strains tested. This is in agreement with the fact that BLAST testing of these primers reported same results for *S*T as for other serovars. Regarding the LoD, once more, in the present study, the results were worse than those reported in the original paper, as we could only detect 4.3 ng/μL, while it was indicated that 0.002 ng/μL could be reached. As mentioned previously, the discrepancies found among this and the original studies may be related with slight differences in the methodology followed, i.e., small differences in the amplification temperature, end-point results with respect to real-time turbidity tracking, application of gel electrophoresis, among others.

The reasons behind the overall differences in performance obtained by these four genetic targets may be of diverse origin, from the quality of the selected sequences, the primer design process, to specific assay optimization. Thus, caution must be taken when attempting to directly implement previously published studies in routine laboratory testing.

CONCLUSION

The comparison of four genetic targets for the specific detection of *S*E and *S*T, in food samples, reported minor differences among all them, except for *typh* gene. In this sense either *safA* or *Sdf I* can be implemented for the detection of *S*E (the assays were equally specific, *safA* was 100 times more sensitive than *sdf I*, but after enrichment, both obtained optimal results in food samples). It is advised to select STM4497 over *typh* (more deviations were obtained during the evaluation of the specificity with *typh*, in addition to being 1,000 times less sensitive, and with the lowest quality parameters after evaluation in food samples).

AUTHOR CONTRIBUTIONS

AG-M designed the experiments and wrote the manuscript. SA and JC performed the experiments and helped writing the manuscript. MP helped in the design of the experiments and proofread the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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