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Toward the Adoption of Loop-Mediated Isothermal Amplification for *Salmonella* Screening at the National Antimicrobial Resistance Monitoring System's Retail Meat Sites

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Abstract

The National Antimicrobial Resistance Monitoring System (NARMS) is a One Health program in the United States that collects data on antimicrobial resistance in enteric bacteria from humans, animals, and the environment. *Salmonella* is a major pathogen tracked by the NARMS retail meat arm but currently lacks a uniform screening method. We evaluated a loop-mediated isothermal amplification (LAMP) assay for the rapid screening of *Salmonella* from 69 NARMS retail meat and poultry samples. All samples were processed side by side for culture isolation using two protocols, one from NARMS and the other one described in the U.S. Food and Drug Administration's *Bacteriological Analytical Manual* (BAM). Overall, 10 (14.5%) samples screened positive by the *Salmonella* LAMP assay. Of those, six were culture-confirmed by the NARMS protocol and six by the BAM method with overlap on four samples. No *Salmonella* isolates were recovered from samples that screened negative with LAMP. These results suggested 100% sensitivity for LAMP in reference to culture. Antimicrobial susceptibility testing and whole-genome sequencing analysis confirmed identities of these isolates. Using the BAM protocol, all *Salmonella* isolates were recovered from samples undergoing Rappaport-Vassiliadis medium selective enrichment and presumptive colonies ($n = 130$) were dominated by *Hafnia alvei* (44.6%), *Proteus mirabilis* (22.3%), and *Morganella morganii* (9.9%) based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. This method comparison study clearly demonstrated the benefit of a rapid, robust, and highly sensitive molecular screening method in streamlining the laboratory workflow. Fourteen NARMS retail meat sites further verified the performance of this assay using a portion of their routine samples, reporting an overall specificity of 98.8% and sensitivity of 90%. As of July 2022, the vast majority of NARMS retail meat sites have adopted the *Salmonella* LAMP assay for rapid screening of *Salmonella* in all samples.

Keywords: antimicrobial resistance, LAMP, NARMS, *Salmonella*, screening, monitoring

Introduction

Antimicrobial resistance (AMR) is widely recognized as a leading public health threat around the world (Antimicrobial Resistance Collaborators, 2022; CDC, 2019; WHO, 2021) and requires a global coordinated action plan (WHO, 2015a). Effective surveillance plays an essential role in the combat against AMR (Federal Task Force on Combating Antibiotic-Resistant Bacteria, 2020; WHO, 2015b). Established in 1996, the National Antimicrobial Resistance

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Monitoring System (NARMS) is a collaborative program of the U.S. Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), the U.S. Department of Agriculture (USDA), and state and local public health and agriculture departments and universities (FDA, 2022; Karp et al, 2017). Operating under the new One Health paradigm, NARMS now tracks resistance in enteric bacteria from humans (clinical samples), animals (cecal, slaughter, retail meats, and veterinary), and the environment (surface water) in the United States.

Since its inception, the NARMS retail meat arm has been monitoring AMR trends in *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus* from retail beef, chicken, pork, and turkey products for more than two decades (Nyirabahizi et al, 2020; Tadesse et al, 2018; Tyson et al, 2018; Whitehouse et al, 2018; Yin et al, 2021). Pilot studies targeting additional bacteria and/or commodities have been carried out (Ge et al, 2017; Tate et al, 2021). Throughout these testing efforts, *Salmonella* remains a key pathogen tracked by the NARMS retail meat arm. Understandably, *Salmonella* is a ubiquitous zoonotic pathogen of significant food safety concern worldwide (WHO, 2018) and AMR issues in *Salmonella* are constantly evolving (Kim et al, 2020; Li et al, 2021; Tate et al, 2017; Tyson et al, 2017).

Performed at >20 NARMS retail meat sites/states, the *Salmonella* testing protocol relies on culture isolation followed by whole-genome sequencing (WGS) and phenotypic antimicrobial susceptibility testing (AST) (FDA, 2022). Considered the gold standard, culture methods are time-consuming and labor-intensive, demanding days to weeks of intensive work for a definitive result (Andrews et al, 2022; USDA, 2021). Previously, NARMS retail meat sites used the TECRA *Salmonella* Visual Immunoassay (3M Food Safety, St. Paul, MN) for screening *Salmonella*, which was discontinued in 2016. Some NARMS retail meat sites have since adopted new screening methods, including VIDAS (bioMérieux, Hazelwood, MO), 3M Molecular Detection Assay (MDA) 2–*Salmonella*, or BAX System Real-time PCR Assay *Salmonella* (Hygiena, Camarillo, CA). However, most sites do not use any screening methods before culture isolation for *Salmonella*.

Loop-mediated isothermal amplification (LAMP) has emerged as a powerful alternative to polymerase chain reaction (PCR) for detecting numerous bacterial, fungal, parasitic, and viral agents (Kumar et al, 2017; Mansour et al, 2015; Mori and Notomi, 2020). We have developed an *invA*-based *Salmonella* LAMP assay that was rapid, reliable, and robust in multiple food matrices (Chen et al, 2011; Domesle et al, 2021; Yang et al, 2013, 2015, 2016). Upon the completion of single laboratory and multilaboratory validations in animal food (Domesle et al, 2018; Ge et al, 2019), this LAMP assay was incorporated into the FDA's *Bacteriological Analytical Manual* (BAM) Chapter 5 in February 2020, as a regulatory method to screen for *Salmonella* in animal food and confirm presumptive *Salmonella* isolates from either animal food or human food (Andrews et al, 2022).

Given the compendium status of the *Salmonella* LAMP assay, we proposed for all NARMS retail meat sites to adopt this rapid screening method to improve laboratory workflow, efficiency, and *Salmonella* isolation rates. This method comparison study aimed to demonstrate LAMP's utility by comparing it with two culture protocols, one from NARMS (FDA, 2022) and another in the FDA's BAM (Andrews et al, 2022). The assay efficacy was verified at additional NARMS retail meat sites before wider adoption as a universal *Salmonella* screening method.

Materials and Methods

Sample collection and processing

Figure 1 shows a schematic diagram of this study. In September 2021, two NARMS retail meat sites (Maryland and North Carolina) obtained a total of 69 retail meat and poultry samples (Table 1) from local grocery stores as part of the routine NARMS sample collection (FDA, 2022). For each sample, a 50-g test portion was added to 250 mL of buffered peptone water (BPW; BD Diagnostic Systems, Sparks, MD) in a filtered plastic bag. After homogenization and overnight enrichment, sample pre-enrichment broths were processed for *Salmonella* isolation at the NARMS sites and aliquots (40 mL) from the filtered side of the bag were shipped frozen to the FDA's Center for Veterinary Medicine (CVM).

NARMS protocol

Fresh overnight sample pre-enrichment broths at the sites were subjected to selective enrichment in Rappaport Vassiliadis R10 broth (RVR10) and selective plating on xylose lysine Tergitol 4 agar plate (XLT4), followed by biochemical confirmation on VITEK 2 Compact system (bioMérieux) or matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry such as MALDI Biotyper (Bruker, Billerica, MA).

LAMP screening

Frozen sample pre-enrichment broths received at CVM were thawed at 4°C overnight and 1-mL aliquots were subjected to DNA extraction using the PrepMan ultra sample preparation reagent (Thermo Fisher Scientific, Waltham, MA) (Ge et al, 2019). Sample DNA extracts were stored at –20°C until use. Positive and negative extraction controls, that is, BPW with and without inoculation of *Salmonella enterica* serovar Typhimurium ATCC 14028 supplied in BioBall SingleShot (bioMérieux) at ~ 30 CFU, were included. A positive control (*Salmonella* Typhimurium ATCC 19585 [LT2] at $\sim 10^3$ CFU/reaction) and a no-template control (molecular grade water) were included in each LAMP run.

The LAMP assay was performed as described previously (Domesle et al, 2020). In brief, the reaction mix (25 μL total) contained $1\times$GspSSD2.0 isothermal master mix (OptiGene Ltd.), $1\times$primer mix (0.1 μM each outer primer Sal4-F3 [GAACGTGTCGCGGAAGTC] and Sal4-B3 [CGGCAATAGCGTCACCTT], 1.8 μM each inner primer Sal4-FIP [GCGCGGCATCCGCATCAATATCTGGATGGTATGCCCGG] and Sal4-BIP [GCGAACGGCGAAGCGTACTGTCGCACCGTCAAAGGAAC], and 1 μM each loop primer Sal4-LF [TCAAATCGGCATCAATACTCATCTG] and Sal4-LB [AAAGGGAAAGCCAGCTTTACG]; Integrated DNA Technologies, Coralville, IA), and 2 μL of DNA template.

The reaction was carried out at 65°C for 30 min followed by 98°C to 80°C with 0.05°C decrement per second in Genie II (OptiGene, Ltd.). Time-to-peak values (T_p; min) were obtained when fluorescence ratios reached peak amplification rates and annealing temperatures (T_a; °C) were determined

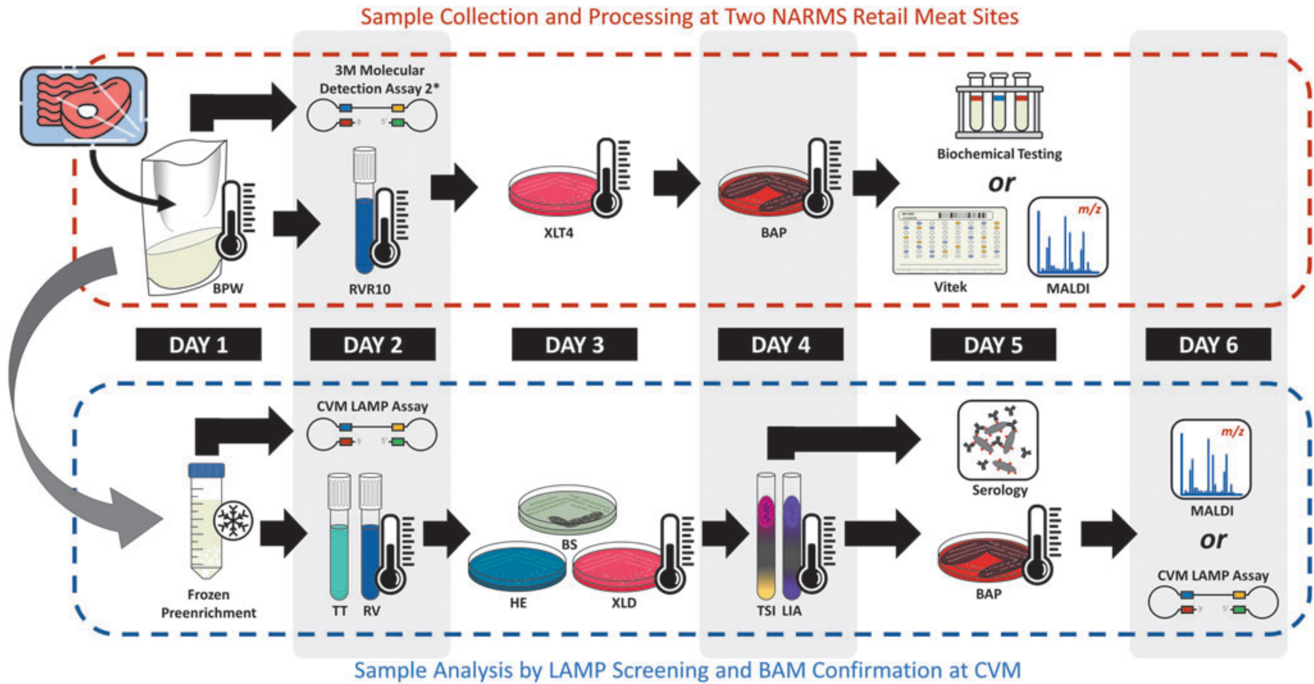

FIG. 1. A schematic diagram of the design and workflow of this method comparison study. *Maryland site used the 3M Molecular Detection Assay 2—*Salmonella* as a screening method. BAP, blood agar plate; BPW, buffered peptone water; BS, bismuth sulfite agar; CVM, U.S. Food and Drug Administration's Center for Veterinary Medicine; HE, Hektoen enteric agar; LAMP, loop-mediated isothermal amplification; LIA, lysine iron agar slant; MALDI, matrix-assisted laser desorption/ionization; NARMS, National Antimicrobial Resistance Monitoring System; RV, Rappaport-Vassiliadis medium; RVR10, Rappaport-Vassiliadis R10 broth; TSI, triple sugar iron slant; TT, tetrathionate broth; XLD, xylose lysine desoxycholate agar; XLT4, xylose lysine Tergitol 4 agar.

from peaks of anneal derivative curves. Samples were considered screen-positive for *Salmonella* with $T_p \leq 20$ min and T_a at 89°C ± 2°C. The LAMP assay was repeated once for each sample independently using separately prepared reaction master mixes.

BAM confirmation

Regardless of LAMP screening results, all samples including extraction controls were subjected to BAM culture confirmation (Andrews et al, 2022). These included selective enrichment in Rappaport-Vassiliadis (RV) medium and tetrathionate (TT) broth, selective plating on bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar, and biochemical confirmation on triple sugar iron agar and lysine iron agar slants. Bacterial identities were confirmed by serology and MALDI Biotyper (Bruker) or LAMP.

WGS characterization

All confirmed *Salmonella* isolates were sequenced on MiSeq using the v3 reagent kit (Illumina, Inc., San Diego, CA) (Domesle et al, 2021). *Salmonella* serotypes were determined using SeqSero2 (Zhang et al, 2019), whereas AMR genes were identified using AMRFinderPlus (Feldgarden

Table 1. Overview of Sample Testing Results from the Method Comparison Study

	Samples collected in Maryland[a]				*Samples collected in North Carolina*[a]			
Sample type	*Total*	*Site+*	*LAMP+*	*BAM+*	*Total*	*Site+*	*LAMP+*	*BAM+*
Chicken	8	1	1	1	8	0	0	0
Ground turkey	8	1	2	2	8	0	1	1
Ground beef	8	0	1[b]	0	8	0	1	0
Pork	8	0	0	0	8	1	1	0
Chicken gizzard	1	1	1	1	1	0	0	0
Chicken heart	1	1	1	1	1	1	1	0
Chicken liver	0	N/A	N/A	N/A	1	0	0	0
Subtotal	34	4	6	5	35	2	4	1

[a]Site+ means that the samples were tested at the NARMS sites following the NARMS protocol and yielded positive cultures. LAMP+ means that the LAMP screening assays performed on the samples at CVM were positive. BAM+ means the BAM confirmations performed on the samples at CVM yielded positive cultures.
[b]Positive LAMP results in the second replicate only.
BAM, *Bacteriological Analytical Manual*; CVM, U.S. Food and Drug Administration's Center for Veterinary Medicine; LAMP, loop-mediated isothermal amplification; N/A, not applicable; NARMS, National Antimicrobial Resistance Monitoring System.

Table 2. Interpretation of Results for This Paired Method Comparison Study per ISO Guidelines

Interpretation	*Reference method (NARMS)*	*Alternative method (LAMP)*	*Confirmed alternative method (BAM)*	*This study (based on duplicate LAMP)*	*This study (based on single LAMP)*
Positive agreement (PA)	+	+	Not needed	6	6
Negative agreement (NA)	–	–	Not needed	59	60
Negative deviation due to false negative alternative-method result (ND)	+	–	Not needed	0	0
Positive deviation (PD)	–	+	+	2	2
Negative agreement due to false positive alternative-method result (NA)	–	+	–	2	1

The first four columns follow definitions used by ISO 16140-2 in a method comparison study (ISO, 2016).

et al, 2019). Phylogenetic analysis was performed based on single nucleotide polymorphism (SNP) (Davis et al, 2015). All WGS data were submitted to the National Center for Biotechnology Information (NCBI) under BioProject PRJNA292661.

Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MICs) for *Salmonella* isolates were determined by broth microdilution using Sensititre NARMS plate CMV5AGNF (Thermo Fisher Scientific) and interpreted following guidelines from the Clinical and Laboratory Standards Institute (CLSI, 2018, 2020), except for azithromycin and streptomycin, which have no CLSI breakpoints.

Data analysis

The analysis was performed as outlined in ISO 16140-2 section 5.1.3 for a paired sensitivity study (ISO, 2016). Positive agreement (PA), negative agreement (NA), positive deviation (PD), and negative deviation (ND) were calculated (Table 2) followed by sensitivity, relative trueness, and false positive (FP) ratio calculations. The acceptability limits of 4 for ND-PD and 8 for ND+PD were used for the two categories (raw meat and raw poultry) tested.

LAMP performance verification before adoption

Fourteen NARMS retail meat sites performed further verification of the *Salmonella* LAMP screening assay when incorporated into their respective laboratory workflows using a portion of routine NARMS samples. Agreement (%) between the NARMS protocol and LAMP was calculated followed by Cohen's Kappa calculation (Microsoft Excel, Redmond, WA). FP, true negative (TN), false negative (FN), and true positive (TP) numbers were used to calculate FP rate (FP/[FP+TN]) and FN rate (FN/[FN+TP]), along with specificity and sensitivity outputs (FDA, 2019).

Results

Salmonella *positive rates differed between methods*

Overall, 10 (14.5%) samples screened positive with LAMP (Table 1). Six (8.7%) were culture-confirmed by the NARMS protocol and six by the BAM method with overlap on four samples. No *Salmonella* isolates were recovered from samples screening negative with LAMP. Of the 34 Maryland samples, 6 (17.6%) screened positive with LAMP and 3M MDA 2—*Salmonella* performed at the site. Of those samples, 5 (14.7%) and 4 (11.8%) were confirmed by BAM and NARMS, respectively. One ground beef sample, screened positive with LAMP in one replicate and positive with the 3M assay, was negative by both culture methods. Of the 35 North Carolina samples, 4 (11.4%) screened positive with LAMP, with 1 (2.9%) and 2 (5.7%) of them also confirmed by BAM and NARMS methods, respectively. One ground beef sample, screened positive with LAMP (in both replicates), was negative by both culture methods.

Method metrics showed 100% LAMP sensitivity

Based on duplicate LAMP testing (Table 2), sensitivity ([PA+PD]/[PA+ND+PD]) was 100% (8/8) for the alternative LAMP method and 75% (6/8) for the reference NARMS method. Relative trueness (i.e., [PA+NA]/N = 67/69) was 97.1%, and FP ratio for LAMP (i.e., FP/NA = 2/61) was 3.3%. Based on single LAMP testing (differing by one ground beef sample from Maryland), sensitivity remained 100% for LAMP and 75% for NARMS, the relative trueness stayed at 97.1%, whereas the FP ratio for LAMP decreased to 1.6% (1/61). Considering the testing efficiency, we recommended single LAMP testing for implementation at NARMS retail meat sites. Both ND-PD and ND+PD were within the acceptability limits.

Table 3. Distribution of Non-*Salmonella* Isolates Recovered Using the *Bacteriological Analytical Manual* Protocol

Genus	*Species*	*No. (%) of isolates*
Hafnia	*alvei*	54 (44.6)
Proteus	*mirabilis*	27 (22.3)
Morganella	*morganii*	12 (9.9)
Citrobacter	*braakii*	6 (5.0)
Citrobacter	*freundii*	4 (3.3)
Enterobacter	*cloacae*	3 (2.5)
Proteus	*vulgaris*	3 (2.5)
Providencia	*alcalifaciens*	3 (2.5)
Aeromonas	*veronii*	2 (1.7)
Other[a]	Other[a]	7 (5.8)
Combined	Combined	121 (100)

[a]Other genus/species identified by MALDI Biotyper include one isolate each of *Acinetobacter dijkshoorniae*, *Alcaligenes faecalis*, *Citrobacter gillenii*, *Escherichia coli*, and *Proteus hauseri*, and two undetermined.

LAMP improved laboratory workflows

For samples screening positive with LAMP, T_p values averaged 8.8 ± 2.6 min (range 6.0–14.5 min) and T_a values averaged 90.0°C ± 0.4°C (range 88.7–90.5°C). Following the BAM protocol, repeated efforts were made to pick typical or atypical colonies on multiple occasions. This resulted in numerous presumptive *Salmonella* isolates ($n = 121$), which were not confirmed to be *Salmonella* by MALDI or LAMP. They were primarily *Enterobacterales*, including *Hafnia alvei* (44.6%), *Proteus mirabilis* (22.3%), and *Morganella morganii* (9.9%) (Table 3).

Salmonella *isolate WGS and AST profiles matched within samples*

A total of five serovars were identified among *Salmonella* isolates ($n = 18$), which were Anatum, Infantis, Kentucky, Meleagridis, and Senftenberg (Table 4). All isolates ($n = 6$) recovered using the NARMS protocol had RVR10 selective enrichment and XLT4 selective plating. Using the BAM protocol, all *Salmonella* isolates ($n = 12$) were recovered from samples undergoing RV medium selective enrichment, and most were from XLD agar (6/12) as the selective plating medium followed by BS (4/12) and HE (2/12) agars.

Where multiple *Salmonella* isolates were recovered by NARMS and/or BAM protocols, the isolates matched in serovar, phenotypic AST, and WGS-predicted AMR genes/mutations (Table 4). Multidrug resistance to three or more antimicrobial classes were identified for *Salmonella* Infantis from MD8 and *Salmonella* Anatum from MD13. *Salmonella* Kentucky isolates from MD33 and MD34 carried aminoglycoside resistance genes *aph(3″)-Ib* and *aph(6)-Id* conferring resistance to kanamycin and/or streptomycin and *Salmonella* Meleagridis from NC30 carried *aac(2′)-IIa* and *fosA7.4* conferring resistance to kasugamycin (an aminoglycoside) and fosfomycin, but none of these antimicrobials were in the NARMS CMV5AGNF plates. Phylogenetic analysis confirmed isolates from the same samples having less than five SNP differences (data not shown).

Multiple NARMS retail meat sites verified LAMP performance

From 14 NARMS retail meat sites, an overall agreement of 97% between LAMP screening and the NARMS culture method was achieved, ranging from 87.5% to 100% by site (Table 5). The Cohen's Kappa statistic was 0.905, suggesting excellent agreement. The overall FP rate was 1.2% (2/161) and FN rate was 10% (4/40), thus a specificity of 98.8% and sensitivity of 90%.

Discussion

This collaborative study highlighted the highly sensitive nature of *Salmonella* LAMP with 100% sensitivity demonstrated in raw meat and raw poultry. BAM and NARMS protocols were time-consuming and labor-intensive whereas trailing in sensitivity. The benefit of incorporating the rapid, robust, and highly sensitive LAMP screening method into NARMS to prioritize *Salmonella* isolation from presumptive positive samples was clearly demonstrated. LAMP could also serve as a reliable method to confirm all presumptive *Salmonella* isolates.

Table 4. Characteristics of *Salmonella* Isolates Recovered from the Method Comparison Study

Sample ID[a]	*Sample type*	*Isolation method*	*Selective media*	*No. of isolates*	*Serovar*	*Antimicrobial susceptibility profile*	*Antimicrobial resistance gene/mutation*[b]
MD8	Chicken	NARMS	RVR10-XLT4	1	Infantis	CHL-GEN-NAL-TET	*aac(3)-IVa*, *aph(4)-Ia*, *floR*, *sul1*, *tet*(A), and *gyrA*_D87Y
		BAM	RV-XLD	1	Infantis	CHL-GEN-NAL-TET	*aac(3)-IVa*, *aph(4)-Ia*, *floR*, *sul1*, *tet*(A), and *gyrA*_D87Y
MD13	Ground turkey	BAM	RV-BS, RV-HE, RV-XLD	6	Anatum	AMP-GEN-FIS	*aac(3)-VIa*, *aadA1*, bla_{HER-3}, and *sul1*
MD16	Ground turkey	NARMS	RVR10-XLT4	1	Senftenberg	None	None
		BAM	RV-HE, RV-XLD	2	Senftenberg	None	None
MD33	Chicken gizzard	NARMS	RVR10-XLT4	1	Kentucky	None	*aph(3″)-Ib* and *aph(6)-Id*
		BAM	RV-XLD	1	Kentucky	None	*aph(3″)-Ib* and *aph(6)-Id*
MD34	Chicken heart	NARMS	RVR10-XLT4	1	Kentucky	None	*aph(3″)-Ib* and *aph(6)-Id*
		BAM	RV-XLD	1	Kentucky	None	*aph(3″)-Ib* and *aph(6)-Id*
NC10	Ground turkey	BAM	RV-XLD	1	Senftenberg	None	None
NC30	Pork	NARMS	RVR10-XLT4	1	Meleagridis	None	*aac(2′)-IIa* and *fosA7.4*
NC35	Chicken heart	NARMS	RVR10-XLT4	1	Kentucky	Tet	*aph(3″)-Ib*, *aph(6)-Id*, and *tet*(B)

[a]No isolates were recovered from samples MD23 or NC24, which were loop-mediated isothermal amplification (LAMP)-positive in one and both replicates, respectively.
[b]Predicted from whole-genome sequencing data.

Table 5. Loop-Mediated Isothermal Amplification Performance Verification at 14 National Antimicrobial Resistance Monitoring System Retail Meat Sites Using a Portion of Routine National Antimicrobial Resistance Monitoring System Samples

		LAMP positive		*LAMP negative*		
NARMS retail meat site	*No. of samples tested*	*Culture positive (true positive)*	*Culture negative (false positive)*	*Culture positive (false negative)*	*Culture negative (true negative)*	*Agreement between LAMP and culture (%)*
A	8	1	0	0	7	100
B	26	8	0	0	18	100
C	14	2	0	0	12	100
D	8	1	0	1	6	87.5
E	28	4	1	0	23	96.4
F	8	1	0	1	6	87.5
G	8	0	0	0	8	100
H	8	1	0	0	7	100
I	8	3	0	0	5	100
J	8	4	0	1	3	87.5
K	26	7	0	1	18	96.2
L	12	1	1	0	10	91.7
M	26	1	0	0	25	100
N	13	2	0	0	11	100
Combined	201	36	2	4	159	97

It is of the highest priority to select naturally contaminated samples for use in method comparison studies (ISO, 2016). However, few studies have compared the performance of *Salmonella* LAMP assays and culture methods using such samples (Yang et al, 2018). Several studies in Asian Countries reported >90% *Salmonella* LAMP sensitivity testing naturally contaminated meat and poultry. For example, one study in China (Zhang et al, 2012) tested 160 fresh chicken and pork samples by a *hisJ*-based LAMP, PCR, and culture, with positivity rates of 17.5%, 16.3%, and 18.8%, respectively, and an overall sensitivity of 93.6% for LAMP and 87.1% for PCR. Another study in Thailand (Srisawat and Panbangred, 2015) compared an *stn*-based LAMP and BAM in 60 chicken meat and minced pork samples and showed both methods having an 88.3% positivity rate.

Studies also compared the LAMP-based 3M MDA—*Salmonella* with culture. One study in Italy (Bonardi et al, 2013) reported low levels of *Salmonella* contamination (<0.3–2.1 most-probable-number [MPN]/g) in 10.5% of 200 meat samples with a relative sensitivity of 78.9% for 3M MDA compared with ISO 6579:2002 (Bonardi et al, 2013). Another study in Poland (Sarowska et al, 2016) tested 107 meat samples and reported 100% sensitivity of the 3M MDA assay compared with ISO 6579, both detecting four positive samples.

We previously evaluated the sensitivity of this *Salmonella* LAMP assay run on a bioluminescent-based platform in comparison with 3M MDA in spiked ground beef and ground turkey (Yang et al, 2016). Without pre-enrichment, LAMP could detect 10^5 CFU/25 g in both matrices, whereas 3M MDA required 10^6 CFU/25 g in ground beef and 10^8 CFU/g in ground turkey. With 24-h pre-enrichment, both assays accurately detected 1 to 3 CFU/25 g of *Salmonella* within 20 min. We also verified the LAMP performance in raw pet food, that is, raw meat-based diets for pets, in comparison with BAM (Domesle et al, 2021). LAMP consistently detected low-level (<30 CFU/25 g) *Salmonella* spiked in five raw pet food matrices after pre-enrichment in BPW and lactose broth. In this study, LAMP agreed 100% with 3M MDA 2 in screening *Salmonella* from Maryland.

As a premier AMR monitoring program, NARMS publishes resistance data on a timely basis with online integrated reports/summaries, visual displays, and open access raw data (FDA, 2022). The prevalence of *Salmonella* in retail chickens ranged from 3% (270/8302) in 2017 to 21% (272/1320) in 2009, whereas that in ground turkey ranged from 5% (152/2907) in 2016 to 19% (246/1309) in 2008. During the same period, *Salmonella* prevalence in retail beef and pork remained <2% (number of samples ranged from 613 to 2204) (FDA, 2022). With such historical prevalence, implementing a rapid, reliable, and robust screening method could significantly reduce the number of samples needed for downstream culture work.

With LAMP, laboratory personnel can quickly identify presumptive positive samples and focus effort on culturing from samples likely to generate *Salmonella* isolates, a critical component of the NARMS program. As shown in Table 5, initial LAMP trials in 14 NARMS retail meat laboratories showed excellent agreement with NARMS culture. The method was regarded as simple and straightforward, requiring little hands-on time, and user-friendly. As laboratory personnel further develop proficiency with the LAMP method, the value of this rapid screening method would be even more appreciated.

Although this study tested a limited number of retail meat and poultry samples, it was apparent that RV medium was the superior selective enrichment broth for BAM compared with TT. An earlier study (Hughes et al, 2003) did a pairwise comparison of RVR10 and TT for the TECRA *Salmonella* Visual Immunoassay and did not find a significant difference ($p > 0.05$). A recent study (Broadway et al, 2021) reported a 1.4% *Salmonella* prevalence rate (out of 865 retail samples)

using the USDA protocol (USDA, 2021), much more effective compared with BAM. Further evaluation of the BAM protocol in meat and poultry may be warranted.

We recently completed a method extension study (Domesle et al, 2022), expanding the *Salmonella* LAMP assay to 7500 Fast (Applied Biosystems), a widely used real-time quantitative PCR platform. Both Genie II and 7500 Fast generated reliable results against an extensive collection of inclusivity and exclusivity templates and in seven animal food matrices. GspSSD2.0 master mix had the fastest time-to-positive results (as early as 3.5 min). The cost of LAMP assay per sample using GspSSD2.0 was less than 2 U.S. dollars, whereas the cost of the small and portable Genie II instrument was competitive (10,000 U.S. dollars).

Adoption of this rapid and versatile screening method by the NARMS retail meat sites for screening *Salmonella* and confirming presumptive *Salmonella* isolates will enhance the program's mission to promote and protect public health by providing real-time information about emerging bacterial resistance, limit the spread of resistance, and aid the FDA in making regulatory decisions designed to preserve the effectiveness of antibiotics for humans and animals.

Conclusions

Salmonella detection from 69 NARMS retail meat and poultry samples using the NARMS protocol, LAMP, and BAM were compared. LAMP was 100% sensitive in reference to culture. RV medium was the superior selective enrichment broth for BAM compared with TT. *Enterobacterales* recovered by BAM was dominated by *H. alvei* (44.6%), *P. mirabilis* (22.3%), and *M. morganii* (9.9%). This study clearly demonstrated the benefit of a rapid, robust, highly sensitive, and specific molecular screening method in streamlining the laboratory workflow. After verification at multiple NARMS retail meat sites, the *Salmonella* LAMP screening assay has been successfully adopted by the NARMS program.

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Disclaimer

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Authors' Contributions

Conceptualization, methodology, investigation, data curation, visualization, and writing—review and editing by S.R.Y. and K.J.D. Investigation, visualization, and writing—review and editing by R.C.M. Investigation and data curation by K.A.L. and E.H. Project administration and supervision by P.L. and S.T. Project administration and resources by C.K. Conceptualization and resources by E.A.S. Funding acquisition and resources by P.F.M. Conceptualization, methodology, project administration, validation, visualization, writing—original draft, and writing—review and editing by B.G.

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No competing financial interests exist.

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