


ORIGINAL RESEARCH

Investigation on *Salmonella enterica*, *Escherichia coli*, and coliforms in beef from Ethiopian abattoirs: A potential risk of meat safety

Andarge Zelalem¹  | Kebede Abegaz² | Ameha Kebede¹ | Yitagele Terefe³ |
Jessie L. Vipham⁴

¹School of Biological Sciences and Biotechnology, College of Natural and Computational Sciences, Haramaya University, Dire Dawa, Ethiopia

²Department of Food Science & Technology, College of Agriculture, Hawassa University, Hawassa, Ethiopia

³College of Veterinary Medicine, Haramaya University, Dire Dawa, Ethiopia

⁴Department of Animal Sciences and Industry, Kansas State University, Manhattan, Kansas, USA

Correspondence

Andarge Zelalem, School of Biological Sciences and Biotechnology, College of Natural and Computational Sciences, P.O. Box, 135, Haramaya University, Dire Dawa, Ethiopia.

Email: andselam2014@gmail.com

Funding information

The authors were funded by the United States Agency for International Development (USAID) Bureau for Food Security under Agreement # AID-OAA-L-15-00003 as part of Feed the Future Innovation Lab for Livestock Systems. Any opinions, findings, conclusions, or recommendations expressed here are those of the authors alone.

Abstract

The aims of this study were to determine the prevalence of *Salmonella enterica* (*S. enterica*) antimicrobial resistance based upon phenotypic assessment, and level of sanitation indicator organisms from 150 beef carcasses collected from three representative abattoirs in eastern, central, and southern Ethiopia. Samples were screened for *S. enterica* prevalence following the U.S. Department of Agriculture Microbiology Laboratory Guidebook (MLG) and confirmed by real-time PCR. The *S. enterica* isolates were phenotypically evaluated for susceptibility to a panel of 13 antimicrobials using disk diffusion method as described in Clinical and Laboratory Standard Institutes guidelines. In addition, the coliform, total coliform, and generic *E. coli* populations were quantified by plating onto *E. coli*/coliform petrifilms. A total of 45 isolates of *S. enterica* were confirmed using real-time PCR. The overall prevalence of *S. enterica* at the carcass level was 22.7% (95% CI, 16.0–30.0). However, based on the sampling points, *S. enterica* prevalence detected on hides was 13.3% (95% CI, 8.0–18.7), pre-evisceration was 12.0% (95% CI, 7.3–17.3), and post-evisceration was 4.7% (95% CI, 1.3–8.0). The prevalence of *S. enterica* was significantly lower on post-evisceration as compared to hides ($p = .009$) and pre-evisceration ($p = .022$). No differences were detected for the prevalence of *S. enterica* between abattoirs ($p = .346$). Finally, the prevalence of *S. enterica* was highest during the wet season ($p = .011$). The overall mean log CFU/cm² \pm SD of generic *E. coli*, coliform, and total coliform counts were 4.55 ± 0.99 , 4.91 ± 1.13 , and 4.98 ± 1.09 , respectively. About 20% of *S. enterica* exhibited phenotypic multidrug resistant. The most frequently detected resistance was to Tetracycline (28.9%), followed by Streptomycin (22.2%) and Sulfisoxazole (20.0%). The data from this study highlight the need to implement interventions on improvement of sanitary practices in abattoirs.

KEYWORDS

abattoir, antimicrobial resistance, meat safety, pathogens, *Salmonella enterica*

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. *Food Science & Nutrition* published by Wiley Periodicals LLC.

1 | INTRODUCTION

Foodborne disease (FBD) is a major global public health concern, as food remains a significant source of human disease around the world (Ahmed et al., 2014; Ferrari et al., 2019; Havelaar et al., 2015; Jacob et al., 2010). Estimates indicate that biological hazards are responsible for 70% of the global burden of foodborne diseases (Havelaar et al., 2015). The most frequent cause of foodborne illnesses are diarrheal disease agents, particularly *Salmonella enterica* (*S. enterica*) (Havelaar et al., 2015). In fact, in 2010, a study by Majowicz et al. (2010) found that *S. enterica* is responsible for an estimated 93.8 million cases of the global human gastroenteritis and 155,000 of the global fatalities on an annual basis. Additionally, *S. enterica* is the second most frequently reported zoonotic agent in the European Union (EU) after thermotolerant *Campylobacter* (EFSA & ECDC, 2015), whereas the estimates in the United State of America (USA) indicated that *S. enterica* causes 1.2 million cases of gastroenteritis, 23,000 hospitalizations, and 450 deaths each year (Scallan et al., 2011). Moreover, the burden of bacterial foodborne disease, including disease caused by *S. enterica*, is disproportionately higher in Africa compared with other parts of the world (Havelaar et al., 2015).

Foodborne illnesses are often attributed to the consumption of contaminated foods; however, animal source foods (ASFs) are the leading point of exposure for foodborne pathogens (Ferrari et al., 2019; Finstad et al., 2012; Grace, 2015a, 2015b; Hoffmann et al., 2017). Among the ASFs, meat is considered as a high-risk food due to its abundant nutrients that could favor the growth of microorganisms and the carriage of pathogenic microorganism by livestock (Bosilevac et al., 2019; Brichta-Harhay et al., 2008; Finstad et al., 2012; Laufer et al., 2015; Sallam et al., 2014; Scharff et al., 2009; Xu et al., 2019). Although data on key bacterial pathogens associated with meat and meat products are limited in Ethiopia, studies in the country have shown that *S. enterica* are highly prevalent in meat and meat products (Alemu & Zewde, 2012; Gebremedhin et al., 2021; Hiko et al., 2018; Kore et al., 2017; Zelalem et al., 2019).

The presence of *S. enterica* in meat carcasses has become a complex issue and a critical source of cross-contamination for meat products, for instance, ground beef (Xu et al., 2020). Bacterial contamination of carcasses during the slaughtering process can originate from multiple sources, including hide, feces and ingesta, equipment, environment, and personnel (Ali et al., 2010; Buncic et al., 2014; Hauge et al., 2012; Kh et al., 2012). However, the initial microbial load in the gastrointestinal tract and cross-contamination during the slaughtering process are the key contributors of carcass contamination (Akbar et al., 2014; Arthur et al., 2007; Brichta-Harhay et al., 2008; Vipham et al., 2015). Studies have also highlighted that microbial pathogen contamination and their occurrences in meat are affected by seasonal variation (Vipham et al., 2012; Xu et al., 2020).

The emergence and spread of antimicrobial resistance among microbial pathogens, including in NTS, has become a public health threat (Ribot et al., 2002; Sallam et al., 2014; Xu et al., 2020). In most developing countries, injudicious use of antibiotics is entirely

associated with increasing trend of multidrug resistance (MDR) in *Salmonella* (Ed-dra et al., 2017). The increased number of human salmonellosis cases are also linked with resistant strains of *Salmonella* spp. (Barbosa & Levy, 2000; Cui et al., 2016). Thus, antimicrobial-resistant *S. enterica* strains are classified as critical priority pathogens by the World Health Organization (Monte et al., 2019; Tacconelli et al., 2018).

In Ethiopia, poor sanitary conditions and abattoir facilities, lack of food safety standards, weak regulatory structure and enforcement, poor food safety knowledge and practices of meat handlers, and habits of raw meat consumption increase the risk of foodborne infections like salmonellosis (Amistu et al., 2017; Delesa, 2017; Girma, 2015; Zelalem et al., 2019). Generally, the food safety practices and microbiological contaminants present in abattoirs have been poorly investigated in low-income countries (Casas et al., 2020). Previous reports on the prevalence, distribution, and antimicrobial resistance of *Salmonella* serovars at Ethiopian beef abattoirs (Hiko et al., 2018; Kore et al., 2017; Wabeto et al., 2017) are an important source of data. However, to the best of our knowledge, there have been no published studies on the occurrence of *S. enterica* and level of hygiene indicator organisms on beef carcasses from abattoirs based on seasonal differences (wet vs. dry) and sampling points (hide, pre-evisceration, and post-evisceration). Thus, the aims of this study were to determine (i) the prevalence of *S. enterica*, (ii) its phenotypic evaluation of antimicrobial resistance, and (iii) the quantification of hygiene indicator organisms on beef carcasses from Ethiopian abattoirs.

2 | MATERIALS AND METHODS

2.1 | Study design

A cross-sectional study design was carried out and samples were collected from SH01, SH02, and SH03 abattoirs (*anonymous of company's business interest*) in Ethiopia. Collectively, these abattoirs are providing the highest harvest services in eastern, central, and southern regions of Ethiopia. The abattoirs are well situated to receive cattle representing different agro-ecological ranges across the major cattle supply locations in Ethiopia. The sample collection was conducted during the rainy (wet) season (June through September, 2019) and the dry season (October, 2019 through February, 2020) as identified elsewhere (Walke, 2016).

2.2 | Sample size

The sample size estimation was based on expected prevalence of *S. enterica* on beef carcasses determined according to Thrusfield (2007). The previous study by Zelalem et al. (2019) indicated a 10% pooled prevalence of *S. enterica* on beef samples in Ethiopia. Therefore, using 10% expected prevalence, 95% confidence interval, and 5% type I error, the number of animals required for sampling from beef

slaughtering facilities was estimated to be 138. The sample size was inflated to increase the precision of estimation. Thus, a grand total of 150 beef carcasses were sampled in the study. The sampling proportion allocation for each site was calculated based on abattoir's minimum beef slaughtering capacity per day. Thus, SH01 (78), SH02 (32), and SH03 (40) slaughtered animals were sampled from a total of 75 beef carcasses in each wet or dry season.

2.3 | Sample collection

Beef carcasses ($n = 150$) were collected following USDA-FSIS MLG. 4.10 method (USDA-FSIS, 2019). Sampling was carried out using Sani-stick sponges (LABPLAS) hydrated with 10 ml of buffered peptone water (BPW; Becton Dickinson) and sterile plastic templates covering 250 cm² area of the region (Narváez-Bravo et al., 2013). Each carcass was randomly sampled during the time of sample collection. Sample collection was carried out at three steps for each carcass in the processing line: hide (P1), pre-evisceration (P2), and post-evisceration (P3). In each sampling point, swabbing was carried out at three regions: midline, fore shank, and hind shank. After swabbing, only the sponge was aseptically detached from the handle placed back into the original sterile bag. The bags were labeled and transported using iceboxes containing frozen freezer packs (2–8°C) and samples were processed within 24 h.

2.4 | Detection and enumeration of *E. coli*, coliform, and total coliform

Each sample bag was hand stomached for 1 min and the homogenate was used for microbial analysis. An aliquot of 1 ml was used to create a tenfold serial dilution; which was plated onto *E. coli*/Coliform Count Plates (ECC; 3 M Petrifilm Count Plates) in duplicate. The inoculated plates were incubated at 35 ± 2.0°C for 24 ± 2.0 h. After incubation, petrifilm plates were examined for generic *E. coli*, coliform, and total coliform growth. According to AOAC Official Methods (998.08 and 991.14) (Curiale et al., 2002), blue to red-blue colonies on ECC associated with entrapped gas, regardless of size or intensity of color, were confirmed as generic *E. coli*. Red colonies closely associated with entrapped gas were counted as coliform, whereas both the red and blue colonies associated with gas at 24 h were counted as total coliform. The petrifilm plates were incubated for additional 24 ± 2 h to detect any further *E. coli* growth.

2.5 | Isolation and characterization of *S. enterica*

Samples were analyzed for the presence of *S. enterica* following USDA-FSIS MLG. 4.10 method (USDA-FSIS, 2019). Each bag with 50 ± 1 ml of Tryptic Soya Broth (TSB; Hardy Diagnostic) was aseptically added and the sample was hand-stomached for 1 min. Sponge bags were incubated at 42 ± 1°C for 15–24 h. An aliquot of

0.5 ± 0.05 ml of the sample was transferred into 10-ml Tetrathionate (TT) broth (HiMedia) and 0.1 ± 0.02 ml into 10-ml Rappaport-Vassiliadis soya (RVS) broth (HiMedia) and were incubated at 42 ± 0.5°C for 22–24 h.

Selective enrichments were incubated at 42 ± 0.5°C for 22–24 h. Ten µl loopful of the selective enrichment medium was streaked onto a 100 × 15 mm plate of Xylose Lysine Tergitol-4 agar (XLT-4, Hardy Diagnostic), and Brilliant Green Sulfur agar (BGS; HiMedia, LBS Marg). Plates were incubated at 35 ± 2°C for 18–24 h. After incubation, yellow and clear colonies with dark centers from XLT-4 agar, and pink and opaque colonies with a smooth appearance surrounded by a red color from BGS agar were tested for agglutination using *Salmonella* latex agglutination kit Wellcolex™ color (Remel). Colonies that tested positive in the agglutination test were recorded as presumptive positives for *S. enterica*.

2.6 | *Salmonella enterica* isolates collection

When agglutination reaction of a colony was positive, it was streaked onto 60 × 15 mm plate of Tryptic Soy Agar (TSA; Hardy Diagnostics), and incubated at 35 ± 2.0°C for 24 ± 2.0 h. The isolated colony was transferred into conical tube containing 10-ml Tryptic Soya Broth (TSB; Hardy Diagnostic) and incubated at 35 ± 2°C for 18–24 h. The TSB inoculated broth was then used to create a lawn of *S. enterica* culture onto TSA using a sterilized cotton swab. The TSA plates then were incubated at 35 ± 2.0°C for 18–24 h. After incubation, the lawn was harvested using a sterile 10 µl loop and transferred the growth into cryobeads (Key Scientific Products Inc.) following manufacturer's instruction. The cryobeads were stored at –80 ± 2.0°C at the National Animal Health Diagnostic and Investigation Center (NAHDIC), Ethiopia for further analysis.

2.7 | Confirmation of *S. enterica* isolates

The real-time PCR (RT-PCR) confirmation of isolates was completed at NAHDIC, Ethiopia. All isolates previously stored in cryobeads were streaked for growth onto a TSA plate. The plates were incubated at 37 ± 2.0°C for 24 ± 2.0 h. The DNA extraction was performed as previously described by Bonke et al. (2012). The DNA extracts were stored at –20°C for further use. Isolates confirmation by a real-time PCR assay was targeting the *invA* gene (5'd CGTGTTCGTCGCGTAATA 3' and 5'd GCCATTGGCGAATTTATG 3') using *invA*-Pr (5'd FAM-ATTATGGAAGCGCTCGCATT-BHQ-1 3') (Bai et al., 2018). In brief, the PCR reaction mixture (25 µl) contained 2.5 µl of DNA template, 0.5 µl *invA* probe, 1 µl *invA* Primer mix, 12.5 µl iQ Multiplex Powermix (2× mix contains dNTPs, 11 mM MgCl₂, iTaq DNA polymerase), and 8.5 µl dd H₂O. The mixture was then dispensed with the correct volume (25 µl) in each well into the plate. The PCR program was set with the following conditions: Initial denaturation at 95°C for 10 min, 45 cycles of denaturation at 95°C for 15 s, and annealing and extension at 60°C for 50 s. *Salmonella*

Typhimurium (ATCC 14028) was used as a positive control and nuclease-free distilled water (Integrated DNA Technologies) as a negative control. Finally, isolates were confirmed using RT-PCR (AB Applied Biosystem 7500 Fast System, Sequence Detection Software Version 1.4). Isolates with a C_t value <0 and <38 were considered as *S. enterica*.

2.8 | Antimicrobial susceptibility testing

The phenotypic evaluation of antimicrobial susceptibility test of *S. enterica* isolates ($n = 45$) was determined using a Kirby–Bauer disk diffusion assay as described by the Clinical and Laboratory Standards Institute CLSI M100, 30th ed (CLSI, 2020). Five colonies of each isolate were inoculated into a sterile tube containing 5-ml sterile distilled water to create a suspension. The suspensions were adjusted to a 0.5 McFarland turbidity and streaked onto Mueller Hinton II Agar (MHA, BD). Antimicrobial disks (Oxoid) were dispensed onto the surface of the inoculated agar plates and the plates were incubated at 37°C for 16–18 h. Thirteen antimicrobials were tested: Ampicillin (AMP, 10 µg), Amoxicillin–clavulanate (AMC, 20/10 µg), Gentamycin (CN, 10 µg), Streptomycin (S, 10 µg), Tetracycline (TE, 30 µg), Ciprofloxacin (CIP, 5 µg), Nalidixic acid (NA, 30 µg), Meropenem (MEN, 10 µg), Trimethoprim–sulfamethoxazole (SXT, 1.25/23.75 µg), Chloramphenicol (C, 30 µg), Trimethoprim (W, 5 µg), Sulfisoxazole (S3, 250/300 µg), and Ceftriaxone (CRO, 30 µg). The resistance levels were evaluated based on the interpretive standards proposed by CLSI as susceptible (S), intermediate resistant (I), or resistant (R). An isolate was considered multidrug resistant when it was resistance to three or more antimicrobials belonging to different antibiotic classes. The reference strain of *E. coli* ATCC 25922 was used as quality control to monitor the precision and accuracy of susceptibility testing.

2.9 | Data analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 23.0 (IBM Corp). The prevalence of *S. enterica* at 95% Confidence Interval was determined for all

samples, by sampling point (hides, pre-evisceration, and post-evisceration), season (wet and dry), and abattoirs (SH01, SH02, and SH03). The results of microbial counts (CFU/cm²) were converted into log₁₀, and descriptive statistics were used to determine the frequency of microbial count, mean, and standard deviation of the samples. For the bacteria detection level or prevalence on samples, a Chi-squared analysis (Fisher's exact test) was used to test for differences among abattoirs, sampling points, and seasons. The microbial counts were compared across the abattoirs using one-way ANOVA. The p value of ≤ 0.05 was considered statistically significant. The results for the microbiological counts of *E. coli* were compared with the Ethiopian standard (ES 1111:2019) of beef specification (ESA, 2019).

3 | RESULTS

3.1 | Prevalence of *S. enterica*

The overall prevalence of *S. enterica* on hides, pre-evisceration, and post-evisceration carcasses within and across abattoirs is shown in Table 1. A total of 450 sub-swab samples (three samples per carcass) were collected from 150 beef carcasses. Forty-five samples were positive for *S. enterica* and confirmed utilizing the detection of *invA* gene with C_t values ranging from 17.61 to 27.74 (provided as Supporting Information). The overall prevalence of *S. enterica* at the carcass level was 22.7% (95% CI, 16.0–30.0). Carcasses were considered positive for *S. enterica* if one of the three sub-samples was positive. However, *S. enterica* was detected from sampling points of hides 13.3% (95% CI, 8.0–18.7), pre-evisceration 12.0% (95% CI, 7.3–17.3), and post-evisceration 4.7% (95% CI, 1.3–8.0). Chi-square analysis indicated that no difference was detected for the prevalence of *S. enterica* on beef samples across abattoirs ($p = .346$). Also, no significance difference ($p = .728$) was observed between hide and pre-evisceration samples for the prevalence of *S. enterica*. However, the prevalence of *S. enterica* was significantly lower in post-evisceration samples as compared to hides ($p = .009$) and pre-evisceration ($p = .022$). This study also identified that the overall *S. enterica* prevalence on beef carcass samples was significantly higher ($p = .011$) during wet than dry season (Figure 1).

TABLE 1 The overall *S. enterica* prevalence for abattoirs and sampling points on beef carcass ($n = 150$)

Abattoir	N	Prevalence (%) of <i>S. enterica</i> (95% Confidence interval)			
		Carcass	Hide	Pre-evisceration	Post-evisceration
SH01	78	21.8 (12.8–32.1) ^a	10.3 (3.8–17.9)	15.4 (7.7–24.4)	6.4 (1.3–11.5)
SH02	40	27.5 (15.0–42.5) ^{ab}	20.0 (7.5–32.5)	12.5 (2.5–22.5)	2.5 (0.0–7.5)
SH03	32	18.8 (6.3–31.3) ^a	12.5 (3.1–25.0)	3.1 (0.0–9.4)	3.1 (0.0–9.4)
Total	150	22.7 (16.0–30.0)	13.3 (8.0–18.7) ^c	12.0 (7.3–17.3) ^{cd}	4.7 (1.3–8.0) ^e

Note: N, sample size. Overall prevalence of *S. enterica* across the sampling points that have a superscript in common is not significantly different from each other. The prevalence of *S. enterica* per carcass across abattoirs that have a superscript in common is not significantly different from each other.

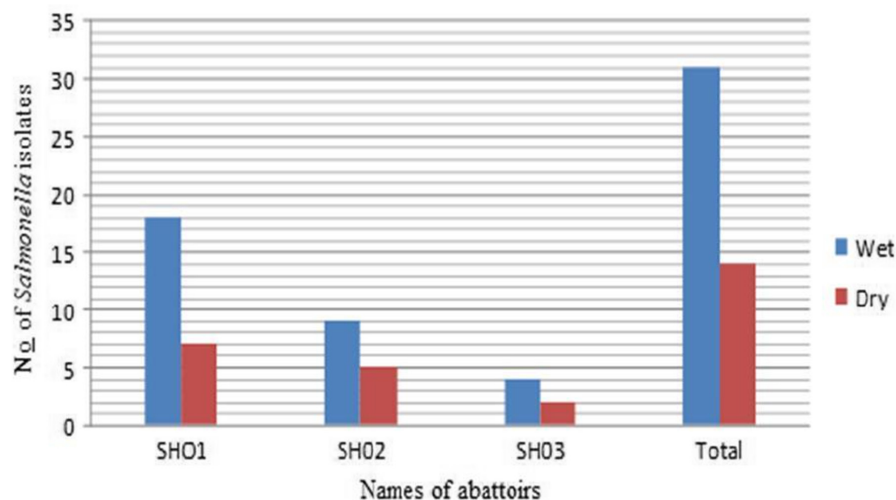


FIGURE 1 *Salmonella enterica* isolates distribution across different seasons and abattoirs

3.2 | Hygiene indicator organisms

The descriptive statistics of hygiene indicator organisms is presented in Table 2. The overall mean \pm SD (log CFU/cm²) of generic *E. coli*, coliform, and total coliform counts were 4.55 ± 0.99 , 4.91 ± 1.13 , and 4.98 ± 1.09 , respectively. The majority of the samples, 91.9% (87.2–96.0) were contaminated by generic *E. coli*. Moreover, approximately 95% (92.0–98.7) and 97% (93.3–99.3) of beef carcasses tested positive for coliform and total coliform, respectively.

According to the Ethiopian national standard of beef specification (ES 1111:2019), the microbiological limit of generic *E. coli* on beef is 2 log CFU/cm². Thus, in this study, the overall beef carcass samples of pre-evisceration (24.7%) and post-evisceration (25.3%) were within the acceptable range of microbiological limit for generic *E. coli*. The pre-evisceration samples in SH01 (41.1%), SH02 (35.0%), and SH03 (65.6%) had *E. coli* count ranged from ≥ 4 to < 6 log CFU/cm² which was comparable with contamination level of hide samples. The high level of *E. coli* contamination on beef indicates weaknesses of slaughtering procedures and sanitary practices at abattoirs. Likewise, most pre-evisceration samples from SH02 (70.0%) and SH03 (46.9%) had coliform counts ranging from ≥ 4 to < 6 log CFU/cm².

The level of generic *E. coli* contamination in beef was significantly lower at the SH02 abattoir as compared to SH01 (ANOVA, $p = .009$) and SH03 (ANOVA, $p = .000$). The contamination of coliforms in beef at the SH02 abattoir was significantly lower (ANOVA, $p = .048$) than SH01 abattoir. There was no significant difference in the level of generic *E. coli* (ANOVA, $p = .063$), coliform (ANOVA, $p = .925$), and total coliform (ANOVA, $p = .374$) contamination of beef carcasses between SH01 and SH03 abattoirs, whereas the beef carcasses from SH02 abattoir had significantly lower total coliform contamination than the SH03 abattoir (ANOVA, $p = .01$). Fisher's exact test analysis indicated that significantly higher number of samples were detected positive for generic *E. coli* during wet season ($p = .001$). Nevertheless, the coliform occurrence was significantly higher ($p = .027$) during dry season. No significant difference (ANOVA, $p = .456$) was observed in contamination of coliform between hides and post-evisceration carcasses. However, significant

difference (ANOVA, $p < .05$) in contamination of total coliform was observed among the sampling points in which hide samples showed the highest contamination. In this study, it was noted that about 76% of *S. enterica* were detected from *E. coli* positive samples, indicating a failure in process control that fecal contamination may pose a serious risk in beef processing.

3.3 | Antimicrobial resistance profile of *S. enterica*

Escherichia coli ATCC 25922 was used as the quality control for the phenotypic antimicrobial susceptibility testing. Phenotypic antimicrobial resistance was low among *S. enterica* isolates in this study as presented in Table 3. Approximately 44% of the isolates were susceptible to all antibiotics tested. All *S. enterica* isolates (100%) were phenotypically susceptible to five antibiotics tested, whereas 37.8% isolates were phenotypically resistant to at least one antibiotic and 11% to two antibiotics. The most frequently detected resistance was to Tetracycline (28.9%), followed by Streptomycin (22.2%), Sulfisoxazole (20.0%), and Ampicillin (17.8%). In addition, about 20% of the *S. enterica* isolates were found to be phenotypically multidrug resistant (MRD) with 15.6% to three antibiotics and 4.4% to five antibiotics (Table 4).

4 | DISCUSSION

This study demonstrates a prevalence of *S. enterica* in beef carcasses at different abattoir locations in Ethiopia to be 22.7%. Earlier studies in Ethiopian abattoir facilities (Alemu & Zewde, 2012; Kebede et al., 2016; Kore et al., 2017; Zelalem et al., 2019) have shown lower rates of *S. enterica* contamination of beef, with prevalence ranging from 2% to 10%. The potential differences in *S. enterica* contamination levels might be attributed to differences in sampling procedure, detection methods, and seasons as described by other study (Siriken et al., 2020).

Based upon previous research from our team (Zelalem et al., 2021) on abattoir workers' knowledge, attitudes, and

TABLE 2 Results from hygiene indicator organism assessment of beef carcasses at abattoirs (n = 150)

Abattoir	Description	E. coli			Coliform			Total coliform		
		P1	P2	P3	P1	P2	P3	P1	P2	P3
SH01	Prevalence (%)	80.8			78.2	65.4	66.7	85.9	84.6	85.9
	n (%) of logcfu/cm ²									
	≤2	15 (19.2)	16 (21.9) ^a	21 (26.9) ^a	17 (21.8)	29 (37.2)	26 (33.3)	11 (14.1)	12 (15.4)	11 (14.1)
	>2 to <4	13 (16.7)	22 (30.1)	20 (25.6)	8 (10.3)	20 (25.6)	13 (16.7)	7 (9.0)	16 (20.5)	15 (19.2)
	≥4 to <6	38 (48.7)	30 (41.1)	26 (33.3)	29 (37.2)	12 (15.4)	18 (23.1)	35 (44.9)	32 (41.0)	31 (39.7)
	≥6	12 (15.4)	5 (6.8)	11 (14.1)	24 (30.8)	17 (21.8)	21 (26.9)	25 (32.1)	18 (23.1)	21 (26.9)
	Mean ± SD logcfu/cm ²	4.72 ± 0.92	4.37 ± 0.91	4.61 ± 1.11	5.13 ± 1.13	4.65 ± 1.42	5.17 ± 1.18	5.20 ± 1.12	4.76 ± 1.20	5.08 ± 1.20
SH02	Prevalence (%)	80	70.0	75.0	87.5	82.5	82.5	95.0	92.5	90.0
	n (%) of logcfu/cm ²									
	≤2	8 (20.0)	12 (30.0) ^a	10 (25.0) ^a	5 (12.5)	7 (17.5)	7 (17.5)	2 (5.0)	3 (7.5)	4 (10.0)
	>2 to <4	12 (30.0)	14 (35.0)	14 (35.0)	2 (5.0)	5 (12.5)	5 (12.5)	4 (10.0)	5 (12.5)	5 (12.5)
	≥4 to <6	13 (32.5)	14 (35.0)	16 (40.0)	21 (52.5)	28 (70.0)	26 (65.0)	21 (52.5)	31 (77.5)	28 (70.0)
	≥6	7 (17.5)	-	-	12 (30.0)	-	2 (5.0)	13 (32.5)	1 (2.5)	3 (7.5)
	Mean ± SD logcfu/cm ²	4.66 ± 1.09	3.91 ± 0.83	3.99 ± 0.71	5.08 ± 1.03	4.42 ± 0.63	4.44 ± 0.73	5.16 ± 1.07	4.50 ± 0.67	4.57 ± 0.90
SH03	Prevalence (%)	90.6	87.5	78.1	78.1	81.3	71.9	96.9	93.8	90.6
	n (%) of logcfu/cm ²									
	≤2	3 (9.4)	4 (12.5) ^b	7 (21.9) ^b	7 (21.9)	6 (18.8)	9 (28.1)	1 (3.1)	2 (6.3)	3 (9.4)
	>2 to <4	4 (12.5)	4 (12.5)	3 (9.4)	1 (3.1)	4 (12.5)	3 (9.4)	3 (9.4)	2 (6.3)	3 (9.4)
	≥4 to <6	24 (75.0)	21 (65.6)	17 (53.1)	18 (56.3)	15 (46.9)	12 (37.5)	19 (59.4)	19 (59.4)	17 (53.1)
	≥6	1 (3.1)	3 (9.4)	5 (15.6)	6 (18.8)	7 (21.9)	8 (25.0)	9 (28.1)	9 (28.1)	9 (28.1)
	Mean ± SD logcfu/cm ²	4.80 ± 0.72	4.67 ± 0.15	5.15 ± 1.22	5.10 ± 0.93	4.93 ± 1.07	5.13 ± 1.34	5.24 ± 1.01	5.12 ± 0.96	5.24 ± 1.27
Overall		91.9% (87.2–96.0) ^b			95.3% (92.0–98.7) ^c			96.7% (93.3–99.3) ^d		
		4.55 ± 0.99 ^e			4.91 ± 1.13 ^f			4.98 ± 1.09 ^g		
					37 (24.7%) ^h			38 (25.3%) ⁱ		

Note: Sampling points (P1—hide, P2—Pre-evisceration, P3—Post-evisceration), n (%), number (percent).

^aNumber of samples within the acceptable ranges of E. coli limit on beef (ES1111:2019).

^bE. coli prevalence (95% CI).

^cColiform prevalence (95% CI).

^dTotal coliform prevalence (95% CI) at carcass level.

^eOverall mean ± standard deviation logCFU/cm² for E. coli count.

^fOverall mean ± standard deviation logCFU/cm² for coliform count.

^gOverall mean ± standard deviation logCFU/cm² for total coliform count.

^hNumber (percent) of pre-evisceration samples that were within the acceptable range of microbiological limit of E. coli (ES1111:2019).

ⁱNumber (percent) of post-evisceration samples that were within the acceptable range of microbiological limit of E. coli (ES 1111:2019).

Antibiotics	Antibiogram pattern of <i>S. enterica</i> (n = 45)		
	S n (%)	I n (%)	R n (%)
Ampicillin (AMP, 10 µg)	37 (82.2)	0 (0.0)	8 (17.8)
Amoxicillin-clavulanate (AMC, 20/10 µg)	45 (100)	0 (0.0)	0 (0.0)
Gentamycin (CN, 10 µg)	45 (100)	0 (0.0)	0 (0.0)
Streptomycin (S, 10 µg)	29 (64.4)	6 (13.3)	10 (22.2)
Tetracycline (TE, 30 µg)	32 (71.1)	0 (0.0)	13 (28.9)
Ciprofloxacin (CIP, 5 µg)	44 (97.8)	1 (2.2)	0 (0.0)
Nalidixic acid (NA, 30 µg)	43 (95.6)	2 (4.4)	0 (0.0)
Meropenem (MEN, 10 µg)	45 (100)	0 (0.0)	0 (0.0)
Trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg)	43 (95.6)	0 (0.0)	2 (4.4)
Chloramphenicol (C, 30 µg)	45 (100)	0 (0.0)	0 (0.0)
Trimethoprim (W, 5 µg)	42 (93.3)	0 (0.0)	3 (6.7)
Sulfisoxazole (S3, 250/300 µg)	36 (80.0)	0 (0.0)	9 (20.0)
Ceftriaxone (CRO, 30 µg)	45 (100)	0 (0.0)	0 (0.0)

Abbreviations: I, intermediate; n, number of isolates; R, resistant; S, susceptible.

TABLE 4 Phenotypic multidrug resistance of *S. enterica* isolates from samples of abattoirs

Antibiotics	No of isolates (%)
AMP, S, TE, SXT, W, S3	2 (4.4)
S, TE, S3	7 (15.6)

Abbreviations: AMP, Ampicillin; S, Streptomycin; S3, Sulfisoxazole; SXT, Trimethoprim-sulfamethoxazole; TE, Tetracycline; W, Trimethoprim.

practices from the same abattoirs, findings from this study may be due to cross-contamination and poor hygiene within abattoirs. For example, previous research has indicated that a majority (66.8%) of the respondents rarely cleaned their working space and equipment before and after work. These practices are not in compliance with hygiene requirement described by the ES 1111:2019 standard of Ethiopia and might contribute to the high contamination observed for indicator organisms of process control. These data paired with previous data may indicate that contamination of beef carcasses, at the abattoir level, can be reduced by employing good sanitary and hygienic practices as described by other study (Alhaji & Baiwa, 2015). In general, the assessment of Zelalem et al. (2021) revealed that the knowledge, attitude, and hygiene practices toward meat safety were not as advanced and expected. The knowledge and skills of personnel who were handling carcass were low that may potentially exacerbate beef contamination during process of slaughtering.

The occurrence of *S. enterica* on beef is a public health risk as study in southern Ethiopia indicated that 79% of respondents preferred to eat raw or undercooked beef (Amistu et al., 2017). *Salmonella enterica* was observed from hides (13.3%) and pre-evisceration (12.0%) with similar level of incidence. A related study

TABLE 3 Phenotypic antimicrobial resistance of *S. enterica* isolates from beef samples of abattoirs

in China isolated *Salmonella* from hides in 20% of the samples (Dong et al., 2014), and in Ethiopia 31% (Sibhat et al., 2011).

The prevalence of *S. enterica* in hides and pre-evisceration was not significantly different ($p = .728$) which may suggest poor practices during hide removal that entail high cross-contamination from hide to carcass. According to our previous research (Zelalem et al., 2021), 52.4% of abattoir workers did not know that animal hide is a source of bacterial contamination. It was also observed that the abattoir workers rub their knives on hide to remove pieces of the carcass and fats during hide removal. The workers continuously use the same knife for carcass dehiding that could transfer pathogens from hide to the carcass. Several studies have reported this pattern for *S. enterica* contamination of beef carcasses (Brichta-Harhay et al., 2011; Narvaez-Bravo et al., 2013). Significantly lower prevalence of *S. enterica* (4.7%) was recorded from post-evisceration samples. During sample collection, it was observed that part of the dehiding process performed in crowd on the floor before hanging the carcass and washing with tap water for evisceration. The wash step might contribute to the reduction of *S. enterica* prevalence during post-evisceration.

The presence of *S. enterica* during the wet season was significantly higher in this study. The aggravating factors for increased *S. enterica* prevalence during wet season might be due to the variation of fecal shedding, fresh wet feed and frequent slurred dung dropping, animal hygiene status, mobility in muddy barn contamination, mixed pasture, contaminated water source, etc., unlike the dry season. Different studies have indicated strong seasonal patterns of *S. enterica* prevalence in meat (Vipham et al., 2012; Xu et al., 2020) that corroborate to the present findings. Therefore, this finding indicates the need of seasonal based intervention to ensure meat safety along its supply chain. Nonetheless, the prevalence of other pathogens during different seasons needs to be studied to fully picture out their seasonal profiles in

the abattoirs. None of the abattoirs sought for this study had pathogen reduction interventions or decontamination treatments in place. Thus, abattoirs in Ethiopia require good hygiene practices, such as hide decontamination, employee training on safe meat handling, and improve the current good practice in abattoirs to reduce the prevalence of pathogens in meat contamination and its further processing.

The overall mean log CFU/cm² of generic *E. coli* and total coliform was 4.55 and 4.98, respectively, which indicated high fecal contamination and potential meat safety risk of public health interest. On the contrary, study conducted in Brazilian slaughterhouses reported a lower mean count (<3.1 log CFU/cm²) *E. coli* and (<3.21 log CFU/cm²) total coliform (Camargo et al., 2019). In the present study, it was observed that significantly higher number of samples were detected positive for *E. coli* during wet season ($p = .001$). The results were in accordance with those previously reported studies in Korea and Argentina, where seasonal variation was observed on the detection and load of generic *E. coli* and coliform from meat samples (Kim & Yim, 2016; Lasta et al., 1992).

In the present study, no significant difference ($p > .05$) in contamination of *E. coli* and coliform was observed among sampling points. The similarity of microbial loads over slaughtering steps was attributed to poor hygiene practices in abattoir environments, knives, and other cutting equipment (Buncic et al., 2014; Hauge et al., 2012). Moreover, the lack of strict hygiene practices during slaughter operations results in the accumulation of meat and fat residues on tools and meat contact surfaces that potentially cause an increase in microbial load and recontamination of carcasses on the beef processing continuum (Gill & McGinnis, 2004).

The generic *E. coli* testing on beef carcasses is used as an indicator of hygiene practices in different stages of beef chain (USDA-FSIS, 1996). In Ethiopia, the established national standard for beef specification (ES 1111:2019) sets a maximum limit of 2 log CFU/cm² for generic *E. coli*. Evaluation of beef carcasses based on the standard demonstrated that (24.7%) pre-evisceration and (25.3%) post-evisceration samples presented *E. coli* count within the range acceptable limit. The level of *E. coli* contamination in abattoirs highlighted the presence of poor hygiene practices and slaughtering operation. Further, the occurrence of *E. coli* on beef carcasses is a public health concern as some strains are pathogenic and are associated with the production of shiga toxins that cause severe illness in humans (Gyles, 2007; Pennington, 2010).

It is important to note that this study only conducted a phenotypic assessment of antimicrobial resistance. Although this is not an exhaustive assessment of the antimicrobial resistance of potential (i.e., genotypic and phenotypic evaluation) isolates collected in the study, it provides a preliminary view of the phenotypic response of these isolates to antimicrobials. Interestingly, in the present study, a very high percentage (>95%) of the *S. enterica* was sensitive to nalidixic acid, ciprofloxacin, and ceftriaxone. These antibiotics are commonly used to treat salmonellosis in humans (Chen et al., 2013; Chiappini et al., 2002). In similar trend, all isolates recovered from beef cattle feedlots in south Texas demonstrated susceptibility to ceftriaxone, ciprofloxacin, and nalidixic acid (Xie et al., 2016).

However, 37.8% *S. enterica* were resistant to at least one antibiotic. In contrast, a study conducted on imported beef in Jordan demonstrated a high percentage (93.0%) of resistant *S. enterica* to at least one antimicrobial (Obaidat, 2020).

In addition, 20% of the *S. enterica* isolates were found to be multidrug resistant. Nevertheless, 70% of multidrug resistant was exhibited by *S. enterica* isolates from beef cattle in Mexico (Delgado-Suárez et al., 2019). Although the number of MDR *S. enterica* is relatively low in this study compared to other literature (Li et al., 2018; Siriken et al., 2015), it is still a public health concern as MDR *Salmonella* serovars are considered to be more virulent than non-MDR *Salmonella* (Dong et al., 2014). The most frequently detected resistance was to Tetracycline (28.9%), followed by Streptomycin (22.2%), Sulfisoxazole (20.0%), and Ampicillin (17.8%). Similarly, the Centers for Disease Control and Prevention (CDC) reported that the most common multidrug-resistance pattern for *S. enterica* was observed against ampicillin, streptomycin, sulfamethoxazole, and tetracycline (CDC, 2007). Similarly, a systematic review and meta-analysis study in Ethiopia demonstrated 25% ampicillin resistance of *Salmonella* isolated from meat and its product (Zelalem et al., 2019).

5 | CONCLUSIONS

In conclusion, this study gives insight on the status of beef contamination at Ethiopian abattoirs and the prevalence of *S. enterica* and its phenotypic antibiotic susceptibility. The *S. enterica* were more prevalent on hides and pre-evisceration carcasses. Interestingly, reductions of *S. enterica* occurrence on beef carcasses were observed on post-evisceration carcasses, likely due to the wash practices being followed within abattoirs. This finding is promising, as it demonstrates that simple interventions are effective in reducing *S. enterica* within abattoir settings. However, it is important to note that cold chain is not often available in Ethiopia. Therefore, even small occurrences of *S. enterica* contamination on beef carcasses can pose a risk to public health, due to potential growth overtime. Additionally, these data demonstrate that some isolates exhibited multidrug resistance to commonly used antibiotics which may present a public health care challenge. Finally, the prevalence of *S. enterica* was seasonally affected, with an overall higher prevalence occurred during rainy season. The association of seasons and *S. enterica* prevalence on meat should be further investigated to design seasonal based mitigation strategies.

The study also demonstrated a high contamination level of indicator organisms which signifies that the process control and hygiene practices of abattoirs were poor. Therefore, there is a need to implement good hygiene practices and appropriate food safety management to reduce pathogenic contamination of beef carcasses at Ethiopian abattoirs. Further research is recommended to evaluate the *S. enterica* serotype diversity and their genotypic antimicrobial resistance using whole-genome sequencing to demonstrate the levels of carcass contamination and meat safety profile to initiate policy advice on national standard setting.

ACKNOWLEDGMENTS

The authors would like to express their thanks to staff of the National Animal Health Diagnostic and Investigation Center (NAHDIC), Haramaya University, Hawassa University, and Kansas State University for the technical support and allowing use of laboratory facilities during the research process. The authors are also equally thankful for the management and technical staff of abattoirs for the cooperation and assistance during sample collection.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Andarge Zelalem  <https://orcid.org/0000-0003-4854-0463>

REFERENCES

- Ahmed, A. M., Shimamoto, T., & Shimamoto, T. (2014). Characterization of integrons and resistance genes in multidrug-resistant *Salmonella enterica* isolated from meat and dairy products in Egypt. *International Journal of Food Microbiology*, 189, 39–44. <https://doi.org/10.1016/j.ijfoodmicro.2014.07.031>
- Akbar, A., Sitara, U., Khan, S. A., Ali, I., Khan, M. I., Phadungchob, T., & Anal, A. K. (2014). Presence of *Escherichia coli* in poultry meat: A potential food safety threat. *International Food Research Journal*, 21(3), 941–945. <http://www.ifrj.upm.edu.my>
- Alemu, S., & Zewde, B. M. (2012). Prevalence and antimicrobial resistance profiles of *Salmonella enterica* serovars isolated from slaughtered cattle in Bahir Dar, Ethiopia. *Tropical Animal Health Production*, 44, 595–600. <https://doi.org/10.1007/s11250-011-9941-y>
- Alhaji, N. B., & Baiwa, M. (2015). Factors affecting workers' delivery of good hygienic and sanitary operations in slaughterhouses in north-central Nigeria. *Sokoto Journal of Veterinary Sciences*, 13(1), 29–37. <http://dx.doi.org/10.4314/sokjvs.v13i1.5>
- Ali, N. H., Farooqui, A., Khan, A., Khan, A. Y., & Kazmi, S. U. (2010). Microbial contamination of raw meat and its environment in retail shops in. *Journal of Infection in Developing Countries*, 4(6), 382–388.
- Amistu, K., Ermias, B., & Asrat, A. (2017). Consumer preference of raw beef ("Kurt") in Wolaita Sodo Town, Southern Ethiopia. *Research & Reviews: Journal of Food and Dairy Technology*, 5(4), 7–13.
- Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2007). Effects of a minimal hide wash cabinet on the levels and prevalence of *Escherichia coli* O157:H7 and *Salmonella* on the hides of beef cattle at slaughter. *Journal of Food Protection*, 70(5), 1076–1079. <https://doi.org/10.4315/0362-028X-70.5.1076>
- Bai, J., Trinetta, V., Shi, X., Noll, L. W., Magossi, G., Zheng, W., Porter, E. P., Cernicchiaro, N., Renter, D. G., & Nagaraja, T. G. (2018). A multiplex real-time PCR assay, based on *invA* and *pagC* genes, for the detection and quantification of *Salmonella enterica* from cattle lymph nodes. *Journal of Microbiological Methods*, 148, 110–116. <https://doi.org/10.1016/j.mimet.2018.03.019>
- Barbosa, T. M., & Levy, S. B. (2000). The impact of antibiotic use on resistance development and persistence. *Drug Resistance Updates*, 3, 303–311. <https://doi.org/10.1054/drup.2000.0167>
- Bonke, R., Wacheck, S., Bumann, C., Thum, C., Stüber, E., König, M., Stephan, R., & Fredriksson-Ahomaa, M. (2012). High prevalence of *Salmonella enterica* subsp. *diarizonae* in tonsils of sheep at slaughter. *Food Research International*, 45(2), 880–884. <https://doi.org/10.1016/j.foodres.2011.01.050>
- Bosilevac, J. M., Zhilyaev, S., Wang, R., Luedtke, B. E., Wheeler, T. L., & Koohmaraie, M. (2019). Prevalence and characterization of *Salmonella* present during veal harvest. *Journal of Food Protection*, 82(5), 775–784. <https://doi.org/10.4315/0362-028X.JFP-18-478>
- Brichta-Harhay, D. M., Arthur, T. M., Bosilevac, J. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2011). Diversity of multidrug-resistant *Salmonella enterica* strains associated with cattle at harvest in the United States. *Applied and Environmental Microbiology*, 77(5), 1783–1796. <https://doi.org/10.1128/AEM.01885-10>
- Brichta-Harhay, D. M., Guerini, M. N., Terrance, M., Bosilevac, J. M., Kalchayanand, N., Shackelford, S. D., & Koohmaraie, M. (2008). *Salmonella* and *Escherichia coli* O157:H7 contamination on hides and Carcasses of Cull cattle presented for slaughter in the United States: An evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Applied and Environmental Microbiology*, 74(20), 6289–6297. <https://doi.org/10.1128/AEM.00700-08>
- Buncic, S., Nychas, G., Lee, M. R. F., Koutsoumanis, K., Hébraud, M., Desvaux, M., Chorianopoulos, N., Bolton, D., Blagojevic, B., & Antic, D. (2014). Microbial pathogen control in the beef chain: Recent research advances. *Meat Science*, 97(2014), 288–297. <https://doi.org/10.1016/j.meatsci.2013.04.040>
- Camargo, A., Cossi, M., Silva, W., Bersot, L., Landgraf, M., Baranyi, J., Franco, B., & Luís Augusto, N. (2019). Microbiological testing for the proper assessment of the hygiene status of beef carcasses. *Microorganisms*, 7(3), 1–11. <https://doi.org/10.3390/microorganisms7030086>
- Casas, D., Calle, A., Bueso, M., Huerta-Leidenz, N., Miller, M. F., & Brashears, M. M. (2020). Multiple interventions for improving food safety practices in 2 small beef Abattoirs of Honduras and associated impacts on risk-mitigation management. *Environmental Health Insights*, 14, <https://doi.org/10.1177/1178630220914596>
- CDC (2007). *National antimicrobial resistance monitoring system for enteric Bacteria (NARMS): Human isolates final report, 2004*. <https://www.cdc.gov/narms/annual/2007/NARMSAnnualReport2007.pdf>
- Chen, H. M., Wang, Y., Su, L. H., & Chiu, C. H. (2013). Nontyphoid *Salmonella* infection: Microbiology, clinical features, and antimicrobial therapy. *Pediatrics and Neonatology*, 54(3), 147–152. <https://doi.org/10.1016/j.pedneo.2013.01.010>
- Chiappini, E., Galli, L., Pecile, P., Vierucci, A., & De Martino, M. (2002). Results of a 5-year prospective surveillance study of antibiotic resistance among *Salmonella enterica* isolates and ceftriaxone therapy among children hospitalized for acute diarrhea. *Clinical Therapeutics*, 24(10), 1585–1594. [https://doi.org/10.1016/S0149-2918\(02\)80062-5](https://doi.org/10.1016/S0149-2918(02)80062-5)
- CLSI (2020). *Performance standards for antimicrobial susceptibility testing* (30th ed., vol. , 8). CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. standard@clsi.org
- Cui, M., Xie, M., Qu, Z., Zhao, S., Wang, J., Wang, Y., He, T., Wang, H., Zuo, Z., & Wu, C. (2016). Prevalence and antimicrobial resistance of *Salmonella* isolated from an integrated broiler chicken supply chain in Qingdao, China. *Food Control*, 62(2016), 270–276. <https://doi.org/10.1016/j.foodcont.2015.10.036>
- Curiale, M., Sons, T., McIver, D., McAllister, J., Halsey, B., Roblee, D., & Fox, T. (2002). Official Method 991.14 Coliform and *Escherichia coli* counts in foods. *Journal of AOAC International*, 74(4), 635–648.
- Delesa, D. A. (2017). Standards related to foods and food products. *International Journal of Advanced Research in Biological Sciences*, 4(12), 201–206. <https://doi.org/10.22192/ijarbs>

- Delgado-Suárez, E. J., Ortiz-López, R., Gebreyes, W. A., Allard, M. W., Barona-Gómez, F., & Rubio-Lozano, M. S. (2019). Genomic surveillance links livestock production with the emergence and spread of multi-drug resistant non-typhoidal Salmonella in Mexico. *Journal of Microbiology*, 57(4), 271–280. <https://doi.org/10.1007/s12275-019-8421-3>
- Dong, P., Zhu, L., Mao, Y., Liang, R., Niu, L., Zhang, Y., Li, K. E., & Luo, X. (2014). Prevalence and profile of Salmonella from samples along the production line in Chinese beef processing plants. *Food Control*, 38(2014), 54–60. <https://doi.org/10.1016/j.foodcont.2013.09.066>
- Ed-dra, A., Filali, F. R., Karraouan, B., Allaoui, A. E., Aboulkacem, A., & Bouchrif, B. (2017). Prevalence, molecular and antimicrobial resistance of Salmonella isolated from sausages in Meknes, Morocco. *Microbial Pathogenesis*, 105, 340–345. <https://doi.org/10.1016/j.micpath.2017.02.042>
- EFSA and ECDC (2015). The European Union summary report on trends of zoonoses, zoonotic agents and food-borne outbreaks in 2013. *EFSA Journal*, 13(1), 3991. www.efsa.europa.eu/efsajournal
- ESA (2019). *Ethiopian national standard on beef specification* (3rd ed.). ES 1111: 2019.
- Ferrari, R. G., Rosario, D. K. A., Cunha-neto, A., Mano, S. B., & Figueiredo, E. E. S. (2019). Worldwide Epidemiology of Salmonella Serovars in animal-based foods: A meta-analysis. *Applied and Environmental Microbiology*, 85(14), 1–21. <https://doi.org/10.1128/AEM.00591-19>
- Finstad, S., O'Bryan, C. A., Marcy, J. A., Crandall, P. G., & Rieke, S. C. (2012). Salmonella and broiler processing in the United States: Relationship to foodborne salmonellosis. *Food Research International*, 45(2), 789–794. <https://doi.org/10.1016/j.foodres.2011.03.057>
- Gebremedhin, E. Z., Soboka, G. T., Borana, B. M., Marami, L. M., Sarba, E. J., Tadesse, N. D., & Ambecha, H. A. (2021). Prevalence, risk factors, and antibiogram of nontyphoidal salmonella from beef in Ambo and Holeta Towns, Oromia Region, Ethiopia. *International Journal of Microbiology*, 2021, Article ID 6626373, 13 pages. <https://doi.org/10.1155/2021/6626373>
- Gill, C. O., & McGinnis, J. C. (2004). Microbiological conditions of air knives before and after maintenance at a beef packing plant. *Meat Science*, 68(2), 333–337. <https://doi.org/10.1016/j.meatsci.2004.03.007>
- Girma, G. (2015). Prevalence, antibiogram and growth potential of Salmonella and Shigella in Ethiopia: Implications for public health. A review. *Advances in Life Sciences and Technology*, 33, 74–88.
- Grace, D. (2015a). Food safety in low and middle income countries. *International Journal of Environmental Research and Public Health*, 12(9), 10490–10507. <https://doi.org/10.3390/ijerph120910490>
- Grace, D. (2015b). *Food safety in developing countries: Research gaps and opportunities*. <https://cgspace.cgiar.org/bitstream/handle/10568/81515/White%2520paper%2520food%2520safety.pdf?sequence=1>
- Gyles, C. L. (2007). Shiga toxin-producing *Escherichia coli*: An overview. *Journal of Animal Science*, 85(13 Suppl), E45–E62. <https://doi.org/10.2527/jas.2006-508>
- Hauge, S. J., Nafstad, O., Røtterud, O., & Nesbakken, T. (2012). The hygienic impact of categorisation of cattle by hide cleanliness in the abattoir. *Food Control*, 27(1), 100–107. <https://doi.org/10.1016/j.foodcont.2012.03.004>
- Havelaar, A. H., Kirk, M. D., Torgerson, P. R., Gibb, H. J., Hald, T., Lake, R. J., De Silva, N. R. (2015). World Health Organization Global Estimates and Regional comparisons of the burden of foodborne disease in 2010. *PLoS Medicine*, 12(12), e1001923. <https://doi.org/10.1371/journal.pmed.1001923>
- Hiko, A., Irsigler, H., Bräutigam, L., Ameni, G., Fries, R., & Maximilian, B. (2018). Antimicrobial resistance and genotypic profiles of Salmonella Saintpaul isolated along beef processing and distribution continuum. *Heliyon*, 4(12), e01025. <https://doi.org/10.1016/j.heliyon.2018.e01025>
- Hoffmann, S., Devleeschauwer, B., Aspinall, W., Cooke, R., Corrigan, T., Havelaar, A., Angulo, F., Gibb, H., Kirk, M., Lake, R., Speybroeck, N., Torgerson, P., & Hald, T. (2017). Attribution of global foodborne disease to specific foods: Findings from a World Health Organization structured expert elicitation. *PLoS One*, 12(9), 1–26. <https://doi.org/10.1371/journal.pone.0183641>
- Jacob, C., Mathiasen, L., & Powell, D. (2010). Designing effective messages for microbial food safety hazards. *Food Control*, 21(1), 1–6. <https://doi.org/10.1016/j.foodcont.2009.04.011>
- Kebede, A., Kemal, J., Alemayehu, H., & Mariam, S. H. (2016). Isolation, identification, and antibiotic susceptibility testing of salmonella from slaughtered bovines and ovines in Addis Ababa Abattoir Enterprise, Ethiopia: A cross-sectional study. *International Journal of Bacteriology*, 2016, Article ID 3714785, 8. <https://doi.org/10.1155/2016/3714785>
- Kh, H., Boukhors, K. T., Dahmani, A., Zenia, S., & Aissi, M. (2012). Survey of hygiene in ovine slaughterhouses of Algiers region by bacteriological analysis of carcasses. *Africa Journal of Microbiology Research*, 6(22), 4722–4726. <https://doi.org/10.5897/AJMR12.023>
- Kim, J. H., & Yim, D. G. (2016). Assessment of the microbial level for livestock products in retail meat shops implementing HACCP system. *Korean Journal for Food Science of Animal Resources*, 36(5), 594–600. <https://doi.org/10.5851/kosfa.2016.36.5.594>
- Kore, K., Asrade, B., Demissie, K., & Aragaw, K. (2017). Characterization of Salmonella isolated from apparently healthy slaughtered cattle and retail beef in Hawassa, southern Ethiopia. *Preventive Veterinary Medicine*, 147(2017), 11–16. <https://doi.org/10.1016/j.prevetmed.2017.08.018>
- Lasta, J. A., Rodríguez, R., Zanelli, M., & Margaría, C. A. (1992). Bacterial count from bovine carcasses as an indicator of hygiene at slaughtering places: A proposal for sampling. *Journal of Food Protection*, 55(4), 271–278. <https://doi.org/10.4315/0362-028X-55.4.271>
- Laufer, A. S., Grass, J., Holt, K., Whichard, J. M., Griffin, P. M., & Gould, L. H. (2015). Outbreaks of Salmonella infections attributed to beef – United States, 1973–2011. *Epidemiology and Infection*, 143, 2003–2013. <https://doi.org/10.1017/S0950268814003112>
- Li, Y., Yang, Q., Cao, C., Cui, S., Wu, Y., Yang, H., Xiao, Y., & Yang, B. (2018). Prevalence and characteristics of Salmonella isolates recovered from retail raw chickens in Shaanxi Province, China. *Poultry Science*, 99(11), 6031–6044. <https://doi.org/10.1016/j.psj.2020.07.038>
- Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'Brien, S. J., Jones, T. F., Fazil, A., & Hoekstra, R. M. (2010). The global burden of nontyphoidal Salmonella gastroenteritis. *Food Safety*, 50, 882–889. <https://doi.org/10.1086/650733>
- Monte, D. F., Lincopan, N., Fedorka-cray, P. J., & Landgraf, M. (2019). Current insights on high priority antibiotic-resistant Salmonella enterica in food and foodstuffs: A review. *Current Opinion in Food Science*, 26(2019), 35–46. <https://doi.org/10.1016/j.cofs.2019.03.004>
- Narvaez-bravo, C., Miller, M. F., Jackson, T., Jackson, S., Rodas-gonzalez, A., Pond, K., Echeverry, A., & Brashears, M. M. (2013). Salmonella and Escherichia coli O157:H7 Prevalence in cattle and on carcasses in a vertically integrated feedlot and harvest plant in Mexico. *Journal of Food Protection*, 76(5), 786–795. <https://doi.org/10.4315/0362-028X.JFP-12-079>
- Narvaez-Bravo, C., Rodas-González, A., Fuenmayor, Y., Flores-Rondón, C., Carruyo, G., Moreno, M., Perozo-Mena, A., & Hoet, A. E. (2013). Salmonella on feces, hides and carcasses in beef slaughter facilities in Venezuela. *International Journal of Food Microbiology*, 166(2), 226–230. <https://doi.org/10.1016/j.ijfoodmicro.2013.07.009>
- Obaidat, M. M. (2020). Prevalence and antimicrobial resistance of *Listeria monocytogenes*, *Salmonella enterica* and *Escherichia coli* O157:H7 in imported beef cattle in Jordan. *Comparative Immunology, Microbiology and Infectious Diseases*, 70, 101447. <https://doi.org/10.1016/j.cimid.2020.101447>

- Pennington, H. (2010). *Escherichia coli* O157. *The Lancet*, 376(9750), 1428–1435. [https://doi.org/10.1016/S0140-6736\(10\)60963-4](https://doi.org/10.1016/S0140-6736(10)60963-4)
- Ribot, E. M., Wierzbza, R. K., Angulo, F. J., & Barrett, T. J. (2002). *Salmonella enterica* Serotype Typhimurium DT104 isolated from humans, United States, 1985, 1990, and 1995. *Emerging Infectious Diseases*, 8(4), 387–391. <https://doi.org/10.3201/eid0804.010202>
- Sallam, K. I., Mohammed, M. A., Hassan, M. A., & Tamura, T. (2014). Prevalence, molecular identification and antimicrobial resistance profile of *Salmonella* serovars isolated from retail beef products in Mansoura, Egypt. *Food Control*, 38(2014), 209–214. <https://doi.org/10.1016/j.foodcont.2013.10.027>
- Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M.-A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases*, 17(1), 7–15. <https://doi.org/10.3201/eid1701.P11101>
- Scharff, R. L., McDowell, J., & Medeiros, L. (2009). Economic cost of foodborne illness in Ohio. *Journal of Food Protection*, 72(1), 128–136. <https://doi.org/10.4315/0362-028X-72.1.128>
- Sibhat, B., Molla Zewde, B., Zerihun, A., Muckle, A., Cole, L., Boerlin, P., Wilkie, E., Perets, A., Mistry, K., & Gebreyes, W. A. (2011). *Salmonella* Serovars and antimicrobial resistance profiles in beef cattle, slaughterhouse personnel and slaughterhouse environment in Ethiopia. *Zoonoses and Public Health*, 58(2), 102–109. <https://doi.org/10.1111/j.1863-2378.2009.01305.x>
- Siriken, B., Al, G., & Erol, I. (2020). Prevalence and antibiotic resistance of *Salmonella* Enteritidis and *Salmonella* Typhimurium in ground beef and meatball samples in Samsun, Turkey. *Microbial Drug Resistance*, 26(2), 136–144. <https://doi.org/10.1089/mdr.2018.0481>
- Siriken, B., Turk, H., Yildirim, T., Durupinar, B., & Erol, I. (2015). Prevalence and characterization of *Salmonella* isolated from chicken meat in Turkey. *Journal of Food Science*, 80(5), M1044–1050. <https://doi.org/10.1111/1750-3841.12829>
- Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E. M., Houchens, C. R., Grayson, M. L., Hansen, P., Singh, N., Zorzet, A. (2018). Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases*, 18(3), 318–327. [https://doi.org/10.1016/S1473-3099\(17\)30753-3](https://doi.org/10.1016/S1473-3099(17)30753-3)
- Thrusfield, M. (2007). *Veterinary epidemiology* (3rd ed.). Blackwell Science.
- USDA-FSIS (1996). *National Federal Plant Raw Ground Beef Microbiological Survey (1993–1994)*.
- USDA-FSIS (2019). *Isolation and identification of Salmonella from meat, poultry, pasteurized egg, and Siluriformes (fish) products and carcass and environmental sponges*.
- Vipham, J. L., Brashears, M. M., Loneragan, G. H., Echeverry, A., Brooks, J. C., Evan Chaney, W. A., & Miller, M. F. (2012). *Salmonella* and *Campylobacter* baseline in retail ground beef and whole-muscle cuts purchased during 2010 in the United States. *Journal of Food Protection*, 75(12), 2110–2115. <https://doi.org/10.4315/0362-028X.JFP-12-077>
- Vipham, J. L., Loneragan, G. H., Guillen, L. M., Brooks, J. C., Johnson, B. J., Pond, A., Pond, N., & Brashears, M. M. (2015). Reduced burden of *Salmonella enterica* in bovine subiliac lymph nodes associated with administration of a direct-fed microbial. *Zoonoses and Public Health*, 62(775), 599–608. <https://doi.org/10.1111/zph.12187>
- Wabeto, W., Abraham, Y., & Anjulo, A. A. (2017). Detection and identification of antimicrobial-resistant *Salmonella* in raw beef at Wolaita Sodo municipal abattoir, Southern Ethiopia. *Journal of Health, Population and Nutrition*, 2017, 36–52. <https://doi.org/10.1186/s41043-017-0131-z>
- Walke, B. (2016). *Seasonal weather assessment for Ethiopia*. <http://www.metoffice.gov.uk/research/climate/seasonal-to-decadal/gpc-outlooks/glob-seas-prob>
- Xie, Y., Savell, J. W., Arnold, A. N., Gehring, K. B., Gill, J. J., & Taylor, T. M. (2016). Prevalence and characterization of *salmonella enterica* and *salmonella* bacteriophages recovered from beef cattle feedlots in south Texas. *Journal of Food Protection*, 79(8), 1332–1340. <https://doi.org/10.4315/0362-028X.JFP-15-526>
- Xu, H., Zhang, W., Guo, C., Xiong, H., Chen, X., Jiao, X., & Li, Q. (2019). Prevalence, serotypes, and antimicrobial resistance profiles among *Salmonella* isolated from food catering workers in Nantong, China. *Foodborne Pathogens and Disease*, 16(5), 1–6. <https://doi.org/10.1089/fpd.2018.2584>
- Xu, Z., Wang, M., Zhou, C., Gu, G., Liang, J., Hou, X., Wang, M., & Wei, P. (2020). Prevalence and antimicrobial resistance of retail-meatborne *Salmonella* in southern China during the years 2009–2016: The diversity of contamination and the resistance evolution of multidrug-resistant isolates. *International Journal of Food Microbiology*, 333(2020), 108790. <https://doi.org/10.1016/j.ijfoodmicro.2020.108790>
- Zelalem, A., Abegaz, K., Kebede, A., Terefe, Y., Schwan, C. L., & Vipham, J. L. (2021). Food safety knowledge, attitudes, and hygienic practices of abattoir workers in Ethiopia: A cross-sectional study. *Food Protection Trends*, 41(5), 501–509. <https://doi.org/10.4315/1541-9576-41.5.501>
- Zelalem, A., Sisay, M., Vipham, J. L., Abegaz, K., Kebede, A., & Terefe, Y. (2019). The prevalence and antimicrobial resistance profiles of bacterial isolates from meat and meat products in Ethiopia: A systematic review and meta-analysis. *International Journal of Food Contamination*, 6(1), 1–14. <https://doi.org/10.1186/s40550-019-0071-z>

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Zelalem, A., Abegaz, K., Kebede, A., Terefe, Y., & Vipham, J. L. (2022). Investigation on *Salmonella enterica*, *Escherichia coli*, and coliforms in beef from Ethiopian abattoirs: A potential risk of meat safety. *Food Science & Nutrition*, 10, 1714–1724. <https://doi.org/10.1002/fsn3.2752>