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

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**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 ± 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*
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 (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 States 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 microorganisms 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 (Alelu & 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).

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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) placed back into the original sterile bag. The bags were labeled and swabbing, only the sponge was aseptically detached from the handle placed back into the original sterile bag. The bags were labeled and swabbed onto 60 × 15 mm plate of Tryptic Soy Agar (TSA; Hardy Diagnostics), and incubated at 35 ± 2.0°C for 24 ± 2 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.

2.6 | 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 h. The isolated colony was transferred into conical tube containing 10 ml Tryptic Soy 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 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 CGTGTTTCCGTCGTAATA 3′ and 5′d GCCATTGGCGAATTGTG 3′) using invA-Pr (5′d FAM-ATTATGGAAGCGCTCGATT-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 (2x 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 antibiotics were tested: Ampicillin (AMP, 10 \( \mu \)g), Gentamicin (CN, 10 \( \mu \)g), Streptomycin (S, 10 \( \mu \)g), Tetracycline (TE, 30 \( \mu \)g), Ciprofloxacin (CIP, 5 \( \mu \)g), Nalidixic acid (NA, 30 \( \mu \)g), Meropenem (MEN, 10 \( \mu \)g), Trimethoprim–sulfamethoxazole (SXT, 1.25/23.75 \( \mu \)g), Chloramphenicol (C, 30 \( \mu \)g), Trimethoprim (W, 5 \( \mu \)g), Sulfisoxazole (S3, 250/300 \( \mu \)g), and Ceftriaxone (CRO, 30 \( \mu \)g). The resistance levels were evaluated based on the interpretive accuracy of susceptibility testing.

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).

| Abattoir | N | Carcass Prevalence (%) of \( S. \) enterica (95% Confidence interval) | Hide Pre-evisceration (95% CI) | Post-evisceration (95% CI) |
|----------|---|------------------------------------------------------|--------------------------|--------------------------|
| 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)\(^b\) | 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)\(^d\) | 4.7 (1.3–8.0)\(^d\) |

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.

### 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).
3.2 | Hygiene indicator organisms

The descriptive statistics of hygiene indicator organisms is presented in Table 2. The overall mean ± 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 (MDR) 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
| Abattoir | Description | E. coli | | | Coliform | | | Total coliform | | |
|---|---|---|--|--|--|---|---|---|---|---|
| | | P1 | P2 | P3 | P1 | P2 | P3 | P1 | P2 | P3 |
| SH01 | Prevalence (%) | n (%) of logcfu/cm² | | | | | | | | |
| | 80.8 | 73.1 | 73.1 | | 78.2 | 65.4 | 66.7 | | 85.9 | 84.6 | 85.9 |
| | ≤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/cm² | 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 (%) | n (%) of logcfu/cm² | | | | | | | | |
| | 80 | 70.0 | 75.0 | | 87.5 | 82.5 | 82.5 | | 95.0 | 92.5 | 90.0 |
| | ≤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/cm² | 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 (%) | n (%) of logcfu/cm² | | | | | | | | |
| | 90.6 | 87.5 | 78.1 | | 78.1 | 81.3 | 71.9 | | 96.9 | 93.8 | 90.6 |
| | ≤2 | 3 (9.4) | 4 (12.5)$^a$ | 7 (21.9)$^a$ | | 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/cm² | 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)$^b$ | 4.55 ± 0.99$^e$ | 95.3% (92.0–98.7)$^f$ | 4.91 ± 1.13$^f$ | 37 (24.7%)$^f$ | 96.7% (93.3–99.3)$^d$ | 4.98 ± 1.09$^g$ | 38 (25.3%)$^f$ |

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

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

$^b$E. coli prevalence (95% CI).

$^c$Coliform prevalence (95% CI).

$^d$Total coliform prevalence (95% CI) at carcass level.

$^e$Overall mean ± standard deviation logCFU/cm² for E. coli count.

$^f$Overall mean ± standard deviation logCFU/cm² for coliform count.

$^g$Overall mean ± standard deviation logCFU/cm² for total coliform count.

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

$^i$Number (percent) of post-evisceration samples that were within the acceptable range of microbiological limit of E. coli (ES1111:2019).
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 equip-
ment before and after work. These practices are not in compliance
with hygiene requirement described by the ES 1111:2019 stan-
dard of Ethiopia and might contribute to the high contamination
observed for indicator organisms of process control. These data
revealed that the knowledge, attitude, and hygiene practices to-
ward meat safety were not as advanced and expected. The knowl-
dge of personnel who were handling carcass were low
edge 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
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 patho-
gens from hide to the carcass. Several studies have reported this
pattern for S. enterica contamination of beef carcasses (Bricha-
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 stud-
ies 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 sea-
sonal based intervention to ensure meat safety along its supply chain.
Nonetheless, the prevalence of other pathogens during different sea-
sons needs to be studied to fully picture out their seasonal profiles in

| Antibiotics | Antibiogram pattern of S. enterica (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) |

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

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SUPPORTING INFORMATION

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