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ORIGINAL RESEARCH

Magnitude and Antimicrobial Susceptibility Profile of *Salmonella* Recovered from Export Abattoirs Located in East Shewa, Ethiopia

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Background: *Salmonella* is one of the most common foodborne pathogens globally, and it remains a major public health concern with the increasing concern of the emergence and spread of antimicrobial-resistant strains. In Ethiopia, the information on the prevalence of *Salmonella* is scarce in export abattoirs.

Objective: To estimate the magnitude and antimicrobial susceptibility profile of *Salmonella* recovered from export abattoirs located in East Shewa, Ethiopia.

Methods: A cross-sectional study was conducted from January to October 2020. In the study, 345 samples were collected from five export abattoirs using a systematic random sampling method. There were 150 carcass swabs (100 from goats and 50 from sheep), 60 goat skin swabs, 60 knife swabs, and 75 human stools. The isolates were identified and characterized using standard bacteriological procedures and confirmed using *Salmonella* genus-specific primer by polymerase chain reaction. Isolates were subjected to antimicrobial susceptibility to 14 antibiotics using the Kirby–Bauer disk diffusion method, and the results were assessed by using Clinical and Laboratory Standards Institute 2018.

Results: Of the 345 samples, 21 (6.08%; 95% CI 4.9–11.2%) were positive for *Salmonella*. The specific prevalence of *Salmonella* in carcass, skin, and knife swabs were 10 (6.67%; 95% CI 3.5–11.19%), 7 (11.67%; 95% CI 5.70–23.00%), and 4 (6.67%; 95% CI 2.50–16.64%), respectively. There was no statistically significant difference in the occurrence of *Salmonella* among export abattoirs and types of samples (P>0.05). In the current study, *Salmonella* was not isolated from sheep carcass and human stool samples. Among the 21 *Salmonella* isolates, 7 (33.3%) were resistant to at least 1 of the 14 antimicrobial agents tested and 2 (9.04%) of isolates were resistant to two antibiotics, tetracycline, and streptomycin. All isolates were susceptible to kanamycin, chloramphenicol, cephalothin, gentamycin, and ceftriaxone.

Conclusion: *Salmonella* was detected in carcass, skin, and knife samples from export abattoirs, which can have serious public health consequences. Some commonly used drugs in veterinary medicine have developed antimicrobial resistance. Therefore, sufficient sanitation at abattoirs, appropriate cooking of carcasses, and rational drug use is strongly advised. Further in-depth study such as serotyping and antimicrobial-resistant gene identification is recommended.

Keywords: antimicrobial susceptibility, carcass, export abattoirs, prevalence, *Salmonella*

Introduction

Salmonella is a foodborne pathogen that motives morbidity and mortality worldwide.[1] Non-typhoidal *Salmonella* (NTS) *enterica* subsp. *enterica* is responsible for causing significant numbers of foodborne diseases in many countries.[1] *Salmonella* is a ubiquitous pathogen disseminated to distinct animals and the environment.[2,3] Salmonellosis is one of the predominant foodborne zoonotic and animal husbandry trouble for the duration of the world.[4] Globally, about 93.8 million cases of illnesses and 155,000 deaths are associated with gastroenteritis due to *Salmonella* species every year.[5]

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The World Health Organization (WHO) has measured the global burden of foodborne diseases and estimated that NTS *enterica* accounted for more than 78 million cases of foodborne illnesses worldwide with about 59,000 deaths in 2010.[6] NTS species is one of the most major causes of foodborne disorder and manifested with the aid of diarrhea, bacteremia, and focal suppurative infection.[7] Africa was said to have the best burden of foodborne diseases per capita, with a median of 2455 foodborne disability-adjusted life years per 100,000 inhabitants.[8] The manner of getting rid of the gastrointestinal tract during slaughtering of animals is considered one of the most essential sources of the carcass, and organ contamination with *Salmonella* at abattoirs.[9] *Salmonella* contamination in the beef chain can occur at several stages along the food supply chain including production, processing, distribution, retailing, preparing, and handling by the consumer.[9]

Food contaminated with drug-resistant microorganisms is a most vital hazard to public fitness as antibiotic resistance can switch to other bacteria.[10] The emergence and spread of antimicrobial-resistant *Salmonella* originating from food animals or retail meats have grown to be a serious health hazard worldwide, especially in growing countries.[11] The emergence of antibiotic-resistant foodborne pathogens has raised the concern of the public as these pathogens are more virulent, causing an increase in the mortality rate of infected patients.[12]

Drug resistance occurs as a result of unmonitored use of antibiotics in farms for prophylaxis or as growth promoters.[13] This can also signify public health danger by switching of resistant *Salmonella* strains to humans through consumption of contaminated food and food products. However, the sources and transmission routes of *Salmonella* in growing countries are poorly understood due to the lack of coordinated-country-wide epidemiological surveillance systems.[14]

Ethiopia's export abattoirs are private, state-of-the-art Halal-certified slaughterhouses with livestock reception pens, automatic and semiautomatic mechanical slaughter and processing equipment, chilling rooms, air-conditioned deboning facilities, packaging equipment, freezing facilities, and rendering and effluent treatments. These export abattoirs are licensed by independent Islamic affairs Councils accredited by high-level international bodies that evaluated and provided abattoir standards.[15]

In Ethiopia, the prevalence of NTS from cattle, carcasses, and hide of slaughtered bovine ranges from 2.75% to 31%, and the incidence of foodborne *Salmonella* infections has expanded dramatically during the past few years. Studies conducted in different parts of the country have demonstrated the presence of *Salmonella* in human beings,[16,17] and indifferent food animals and food products.[16] Several factors including under- and malnutrition, HIV-AIDS, the unhygienic living circumstances, and the close relations between humans and animals may substantially contribute to the occurrence of Salmonellosis.[16,17]

Assessing the *Salmonella* in food items through isolation and identification of *Salmonella* has greater public health importance. Adequately managed and enforced Good Manufacturing Practices and food safety monitoring and surveillance are important components of a modern food supply chain that play a critical role in the control of foodborne pathogens.[8] However, food systems in Africa are frequently uncoordinated and poorly regulated, resulting in compromised food safety and protection of public health from foodborne illness.[8]

In Ethiopia, different studies have been conducted to analyze the prevalence of *Salmonella* and antimicrobial susceptibility profiles both in veterinary and public health setups.[18,19] However, a well-organized epidemiological investigation on magnitude and antimicrobial susceptibility profile of *Salmonella* from an animal carcass, hide, knife, and human stool is lacking, specifically from export abattoirs. Hence, this study was performed to estimate the magnitude and antimicrobial susceptibility profiles of *Salmonella* in selected export abattoirs in Ethiopia.

Materials and Methods

Study Areas and Periods

In Ethiopia, export abattoirs including Luna, Organic, Halal, Abyssinia and Aljunia were available in different sites of the country. The study was conducted in three selected areas, namely Modjo, Bishoftu, and Dukem, which are located in the East Shewa Zone of the Oromia region (Figure 1). These areas were selected based on the availability of standardized carcass export abattoirs. The study was carried out from January to October 2020. These export abattoirs work based on rules and regulations of the Ministry of Agriculture. Generally, five export abattoirs are found in Modjo, three in

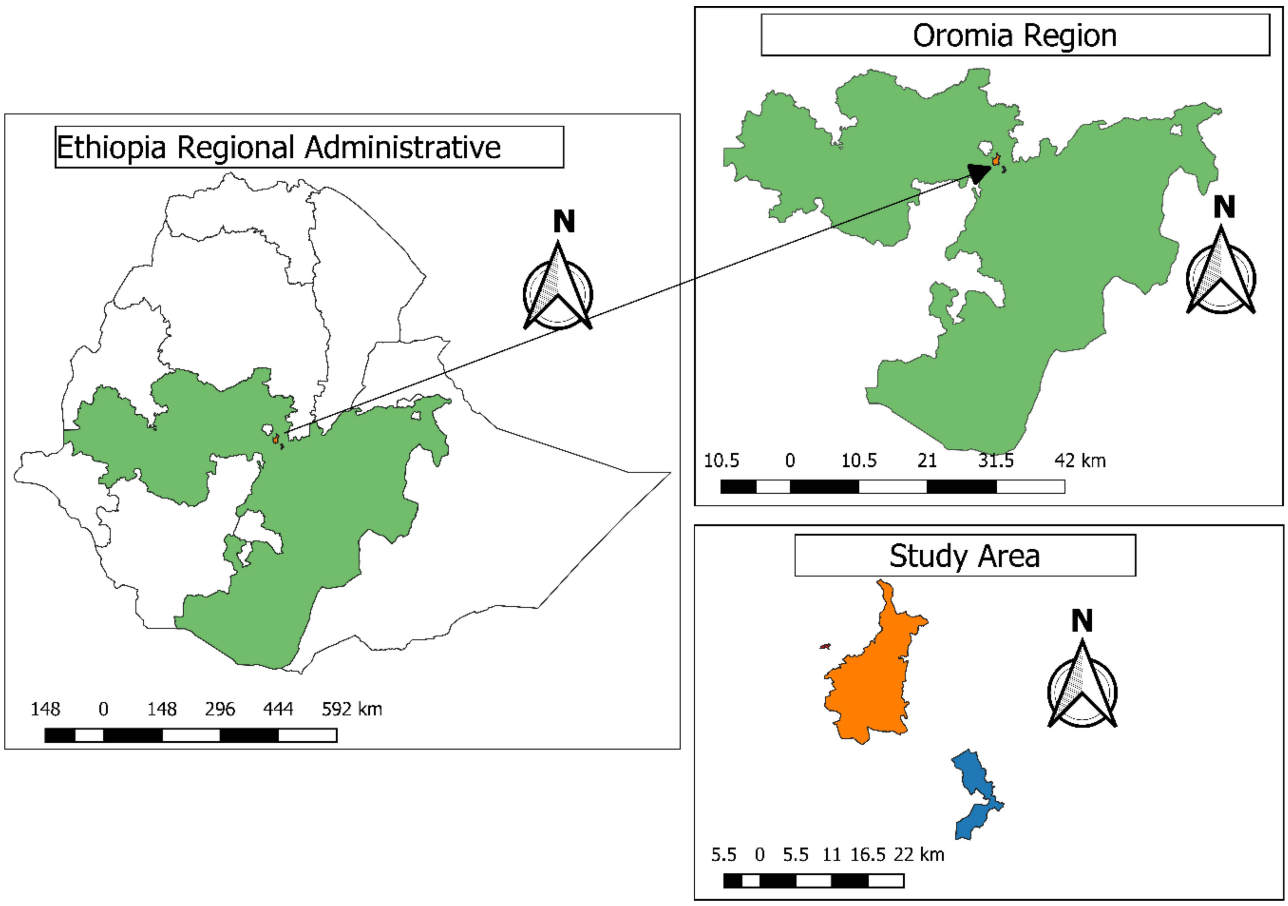

Figure 1 Geographical location of the study areas.
Notes: Oromia, Ethiopia regiona Admin, Modjo Town, Bishoftu Town, Dukem Town.

Bishoftu, and one in Dukem. Three abattoirs were selected from Modjo, and one selected from Bishoftu and Dukem. The total number of workers involved in these export abattoirs was 215. The carcass contamination was reduced by performing the criteria of Hazard Analysis Critical Control Points.

Study Design

A cross-sectional study design was conducted to estimate the magnitude and antimicrobial susceptibility profile of *Salmonella* recovered from export abattoirs located in East Shewa, Ethiopia.

Population

Source of population

The source population included goats, sheep, knives, and personnel working in export abattoirs.

Study Population

The study population included apparently healthy, young, male goats, and sheep that were brought for slaughtering, knives, and personnel working in export abattoirs.

Eligibility Criteria

Inclusion Criteria

- Animals that were brought for slaughtering and that did not receive antibiotics treatment.

- A carcass handler with no clinical symptoms of infection and who did not receive antibiotics treatment were included for the human subject part study.

Exclusion Criteria

- Sick animals that were already being treated with antibiotics and people with clinical symptoms (eg, headache, sneezing, and discomfort).

Operational Definition

Multidrug-Resistance

Resistant to three or greater antimicrobial classes.[12]

Export Abattoir

This is a slaughter facility in respect of which a registration certificate has been issued for the purpose of exporting meat and meat products.

Sample Size Determination

The sample size was calculated by applying the single population proportion estimation formula with a 95% confidence level and with a margin error of 5% desired precision.

The sample size was calculated based on 5.7% and 3.57% expected prevalence of bovine and ovine samples respectively from a previous study done in Addis Abeba Abattoirs Enterprise, Ethiopia.[2]

The sample size was calculated using the following equation:

$$N = \frac{Z^{2*}\,Pexp(1-P)}{d^2}$$

where N = required sample size, Z = standard normal deviation (1.96) at 95% confidence level, Pexp = expected prevalence, and d = desired absolute solution (0.05).

Accordingly, the calculated sample size was 150 considering a 10% non-response rate. However, a total of 345 samples (150 carcass swabs [100 from goats and 50 from sheep], 60 goats skin swabs, 60 knife swabs, and 75 human stool samples) were collected for detection of *Salmonella*. Based on the annual slaughtering capacity of sheep and goats, as well as the number of personnel working in each abattoir, the calculated sample size was proportionally distributed to the five export abattoirs.

Sampling Techniques

To recruit the study participants, a systemic random sampling method was used to enroll eligible study participants. The numbers of study participants to be enrolled from each selected export abattoir were determined by proportionality (based on animal and human study participant load).

Data Collection Procedure

Socio-Demographic Characteristics

For the human sample, after obtaining written consent from all study participants, a semi-structured questionnaire was used to collect socio-demographic characteristics.

Sample Collection and Transportation

Carcass Swabs

Samples were collected from the carcass (n=150, 100 from goats, and 50 from sheep). Each carcass used to be sampled from four regions: neck, abdomen, thorax, and breast. Sampling areas were delineated via sterile aluminum foil templates (10×10 cm) resulting from a total area of 400 cm^2. A sterile cotton-tipped swab (2×3 cm) was first soaked in 9 mL of buffered peptone water (BPW) (Oxoid, England) and rubbed over delineated area horizontally and vertically.[2] Two sterile cotton-tipped swabs were used to acquire sampling areas.

Skin Swabs

Samples were collected from external goat's skin surfaces (n=60). Four regions were selected to collect the skin swabs: abdomen, thorax, neck, and breast. A sterile aluminum foil template (10×10 cm) resulted in a total area of 400 cm^2 placed in these regions. A sterile cotton-tipped swab with wooden shaft was first soaked in 9 mL of sterile BPW and rubbed over delineated area horizontally and vertically.[9] Two sterile cotton-tipped swabs were used to collect the consecutive regions.

Knife Swabs

Samples from the knives (n=60) were collected aseptically using sterile cotton swabs. It was done by rubbing both sides of the knife using a pre-soaked swab.[11] Swab samples were collected from the animals selected for slaughtering.

Stool Samples

After proper instruction, each study participant was informed to bring freshly voided stool in a clean, dry, and leak-proof disposable stool cup, and a total of 75 stool specimens were collected.

Upon completion of all swabbing processes, the wooden shaft was broken off and the cotton swab was left interior the test tubes containing 9 mL of sterilized BPW. The swab samples within tubes were shaken for 30 seconds for uniform distribution of microorganisms earlier than transportation. All samples were labeled, positioned in separate plastic bags, transferred into a sterile icebox, and transported to Medical Microbiology Laboratory, Aklilu Lemma Institute of Pathobiology (ALIPB) within 3–4 hours for the isolation of *Salmonella*.

Microbiological Analysis

Isolation and Identification of *Salmonella*

Isolation and identification of *Salmonella* have been performed at the Department of the Medical Microbiology Laboratory of ALIPB. *Salmonella* isolation and identification had been carried out in line with International Organization for Standardization (ISO), ISO 6579:2002/Amd 1:2007, and global *Salmonella* surveillance and laboratory support of the World Health Organization: Laboratory Protocols (Identification of *Salmonella*).[20,21]

Pre-Enrichment in Nonselective Liquid Medium

The swab samples were soaked in 9 mL of BPW. This pre-enriched sample incubated for 18–24 hours at 37°C for recovery and proliferation of cell would possibly be injured during processing or to make a number of the target organism grow to detectable level.[4]

Enrichment Selective Liquid Media

Enrichment selective broths, namely Rappaport Vassiliadis Soya (RVS) (Oxoid, England CM950-500G) for all samples except stools and Selenite F for a stool sample (Oxoid, England, CM651-500G), were used to inhibit nontargeted microorganisms like gram-positive bacteria and coliforms and approve fast multiplication of *Salmonella*. After pre-enrichment in 0.1 mL of culture from BPW were transferred aseptically into 10 mL of RVS Broth and incubated for 18–24 hours at 41.5°C. For a stool sample, 0.1 mL of cultures from BPW was transferred to 10 mL of Selenite F Broth, homogenized, and incubated for 18–24 hours at 37°C.[19]

Plating Out and Identification

A loopful of 100 µm used to be taken from RVS and Selenite F Broths, streaked into the xylose lysine deoxycholate (XLD) agar (Oxoid, England, CM0469-500 G) plates, and incubated for 18–24 hours at 37°C. Suspected *Salmonella* isolates were subcultured on nutrient agar (Oxoid, England, CM0003-500G) and incubated for 18–24 hours at 37°C. The isolates from the subculture were stored in the refrigerator at 4°C for the biochemical test, molecular testing, and antimicrobial susceptibility test.[4]

Biochemical Characterization

Suspected *Salmonella* colonies from nutrient agar (Oxoid, England CM0003-500G) were picked up and its biochemical characteristics were determined using triple sugar iron agar (Oxoid, England CM277-500G), lysine iron agar (Oxoid,

Table 1 Primers Used to Detect *Salmonella*

Genome	Primers	Sequences (5'–3')
Histidine transport operon gene	Forward Reverse	ACTGGCGTTATCCCTTTCTCTGGTA ATGTTGTCCTGCCCCTGGTAAGAGA

England CM0381-500G), Simmon's citrate agar (Himedia, India CM0129-500G), urea slant (Himedia, India M111A-500G), and sulfide indole motility agar (Oxoid, England S12-500G).[3]

Molecular Techniques

Polymerase chain reaction (PCR) was used to confirm the identification made by phenotypic tests.

DNA Extraction

Bacterial colonies confirmed as *Salmonella* with the aid of biochemical tests were cultured overnight on XLD agar. Then DNA extraction was carried out using the boiling method. The set of primer targeted conserved regions of *Salmonella* forward and reverse were used.[13]

Molecular Confirmation by Using a PCR

All isolates that showed specific biochemical characteristics of *Salmonella* were further confirmed via the use of a genus-specific PCT.[1] It is based on the amplification of a 496-base pair (bp) segment of histidine transport operon gene (Table 1), which is noticeably conserved among species of *Salmonella*. Reference strain of *S. typhimurium* (ATCC 14028) was used as positive control during PCR. PCR amplification was once run in reaction mixtures (20 µL) with master mix (10 µL), forward (0.50 µL) and reverse (0.50 µL) primer, nuclease-free water (8.0 µL), and DNA template (1.0 µL). Amplification was carried out in a thermocycler with temperature profiles of 2 minutes at 94°C for initial denaturation observed through 35 cycles of at 94°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72 °C for 1 minute with remaining extension step at 72°C for 5 minutes.[1]

Agarose Gel Electrophoresis and Visualization of PCR Products

PCR products were electrophoresed using 2 grams agarose powder (Rugby, UK) in 100 mL of 1× TAE buffer (Bio Concept, Switzerland). A quantity of 2 µL of ethidium bromide was mixed with the gel before pouring it into the casting tray. A 100-bp DNA ladder was used as a molecular dimension marker to estimate the size of the products. A band of 496 bp was regarded as superb for *Salmonella*. Gel electrophoresis used to be carried out at 120 volts for 60 minutes considered beneath under an ultraviolet trans-illuminator.[13]

Antimicrobial Susceptibility Test

The isolates confirmed by PCR had been subjected to antibiotic susceptibility test using Kirby–Bauer disk diffusion techniques,[22] on Mueller–Hinton agar (MHA; Oxoid, England), and results interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2018).[23] Antibiotics have been selected based on the routine prescription for human and animal use and their availability on the market of the country. From each PCR confirmed isolate, three to four colonies grown on nutrient agar were transferred to a tube containing 3 mL of nutrient broth (Oxoid, England). The broth culture was incubated for 18–24 hours at 37°C untill its turbidity was adjusted to 0.5 McFarland standards. The suspension was once inoculated onto MHA plates using sterile cotton swabs. The plates have been uniformly inoculated via rubbing toward the whole agar surface and rotating the plates thrice at about 90° degrees. The plates were held at room temperature for 15 minutes to allow drying. Antibiotic-impregnated disks were applied to the floor of the inoculated plate using sterile forceps and incubated aerobically for 24 hours at 37°C. *Salmonella* isolates examined for the antibiotics include kanamycin (30 µg, K), ciprofloxacin (5 µg, CIP), chloramphenicol (30 µg, C), cephalothin (30 µg, CEP), tetracycline (30 µg, TE), ampicillin (10 µg, AMP), nalidixic acid (30 µg, NA), streptomycin (10 µg, S), gentamicin (10 µg, GEN), ceftriaxone (30 µg, CRO), amikacin (10 µg, AN), neomycin (30 µg, N), amoxicillin+clavunic acid (20/10 µg, AMC), and sulfamethoxazole+trimethoprim (1.25/23.75 µg, SXT). Following the incubation of the plates for 24

hours at 37°C, the diameter of the inhibition zone was measured to the nearest millimeter using a digital caliper and interpreted as sensitive (S), intermediate (I), or resistant (R) in accordance with CLSI 2018.[23]

Quality Controls

Standard operating procedures of the laboratory ensured the reliability and validity of test results. A batch of the media was incubated for 24 hours at 37°C, and the media were checked for pH, sterility, ability, and support growth before use. In addition to these, visually the media were checked for depth, smoothness, hemolysis, excessive bubbles, contamination, check for cracked or damaged plates, and frozen or melted agar before use. The media performances were checked with a known positive control standard American Type Culture Collection (ATCC) reference: *S. typhimurium ATCC* 14028 as positive control and *E. coli* ATCC 25922 as negative control.

Data Analysis

Data were entered and analyzed using STATA version 14. Fisher's exact test was used to investigate significant differences among abattoirs and kinds of samples. P-value <0.05 was considered as indicative of a statistical significance difference.

Results

Socio-Demographic Characteristics of Human Study Participants in Selected Export Abattoirs

A total of 75 carcass handlers were enrolled in this study with a response rate of 100%. The sex distributions were 55 men and 20 women with an age range of 22–50 years with a mean age of 32 years (± 8.61 SD). Educational backgrounds were as follows: 3 (5%) primary school, 65 (86.6%) secondary school, and 7 (9.33%) diploma. Most of the respondents were married (50 (67%)). All participants were trained about food hygiene and wearing personnel protective equipment. Among carcass handlers, 65 (86.6%) have certificates in food safety. All participants undertook periodical medical check-ups and washed their hands with soap and water after using the toilet or touching any material (Table 2).

Prevalence of *Salmonella*

During the study period, a total of 345 samples were collected from five export abattoirs. Thirty-six samples showed suspected *Salmonella* colonies on XLD agar and only 21 (6.08%; 95% CI 4.9–11.2%) isolates showed typical biochemical properties of *Salmonella*. These 21 isolates were further confirmed by PCR amplification (Figure 2). *Salmonella* was not isolated from all human stool and sheep carcass swabs. The highest proportion of positivity was detected from goat skin swabs 7/60 (11.67%) followed by goat carcass swabs 10/150 (6.67%), and knife swabs 4/60 (6.67%) (Table 3).

Antimicrobial Susceptibility Testing of *Salmonella* Isolates

Among the 21 molecular confirmed *Salmonella* isolates, all isolates were susceptible to kanamycin, chloramphenicol, cephalothin, gentamycin, and ceftriaxone. A total of 21 (100%) and 1 (4.76%) isolates were intermediately resistant to neomycin, and streptomycin respectively (Table 4).

Overall, 7 (33.33%) of the isolates were resistant to at least 1 of the tested 14 antimicrobial agents. Among the 21 isolates, only 2 (9.04%) were resistant to tetracycline and streptomycin (Table 5).

Discussion

Foodborne gastroenteritis caused by NTS represents a major public health problem worldwide. As *Salmonella* is transmitted through contaminated food or water, its presence in food animals and animal products has relevant public health implications. Thus, monitoring food safety is a key point in preventing and controlling the spread of *Salmonella*, as well as in providing healthier food products.[3] In this study, the overall prevalence of *Salmonella* was 21 (6.08%) with 95% CI 4.90–11.2%.. From this, 10 (6.67%), 7 (11.67%), and 4 (6.67%) were from goat carcass swabs, goat skin swabs,

Table 2 Socio-Demographic Characteristics of Human Study Participants in Selected Export Abattoirs, East Shewa, Ethiopia, from January to October 2020

Variables	Category	Frequency (N=75)	Percentages (%)
Age, years	22–35	25	33.0
	36–50	50	67.0
Sex	Male	55	73.3
	Female	20	26.6
Marital status	Married	50	67.0
	Single	25	33.0
Education	Primary	3	5.0
	Secondary	65	86.6
	Diploma	7	9.33
Residency	Modjo	45	70.0
	Bishoftu	15	15.0
	Dukem	15	15.0
Having certificate of food safety	Yes	65	86.6
	No	10	13.4
Periodical medical check-up	Yes	75	100
	No	-	-
Consumption of raw meat	Yes	1	5.0
	No	74	95
Food hygiene training	Yes	75	100
	No	-	-
Using personnel protective equipment	Yes	75	100
	No	-	-
Work experience years	5–8	62	85.0
	>9	13	15.0
Hand washing by soap and water after using toilet or touching any material	Yes	75	100
	No	-	-

and knife swabs, respectively. The prevalence of *Salmonella* among different samples in this study showed no statistically significant association (P>0.05).

The finding of the current study should be comparable with a study reported in dairy cattle in central Ethiopia (7%),[24] Colorado State University veterinary teaching hospitals (5.9%),[25] and on pork and goat carcass in the Bahamas (5.9%)[26] On the contrary, our finding is lower than the study reported on exotic chickens in Debre Zeit and Modjo, Ethiopia (14.6%)[27]; ground beef at a retail store in Jalisco State, Mexico (56.7%)[28]; from milk and meat in Bangladesh (60%),[29] Kwata slaughterhouse Awka, Anambra State (33.5%)[16]; from abattoir and environment in Nigeria (92.31%)[30]; and raw beef in Wolaita Sodo municipal abattoir, Southern Ethiopia (12.5%).[31] However, the current result is higher than the study conducted on slaughtered cattle in Addis Ababa, Ethiopia (3.7%)[3]; slaughter sheep in Turkey (0.7%)[32]; food handlers at the University of Gondar, Ethiopia, that suggested (3.1%)[33]; from animal-origin food items in Gondar, Ethiopia (5.5%)[34]; and slaughtered bovine and ovine in Addis Ababa Abattoirs Enterprise (4.64%).[2] *Salmonella* was not detected from sheep carcass swabs, which is comparable with the study conducted on slaughter sheep carcass swab in Turkey.[32] The authors would like to factor out that the prevalence may be underestimated because the sample in the current study was a swab rather than meat, and the protocol used to be not the parallel ISO method, which is more sensitive to detect specific serovars. The discrepancies between our study and others could be associated with the degree of exposure of animals to stress factors like transportation and starvation, climatic conditions, management practices, age-groups, species of animals, hygienic conditions adopted kinds of abattoirs and facilities, food coping with and geographical difference.

In the current study, the proportion of *Salmonella* was isolated from skin swabs (11.66%), carcass swabs (6.66%), and knife swabs (6.66%). This result is relatively lower in proportion study conducted to the cattle slaughtered in South

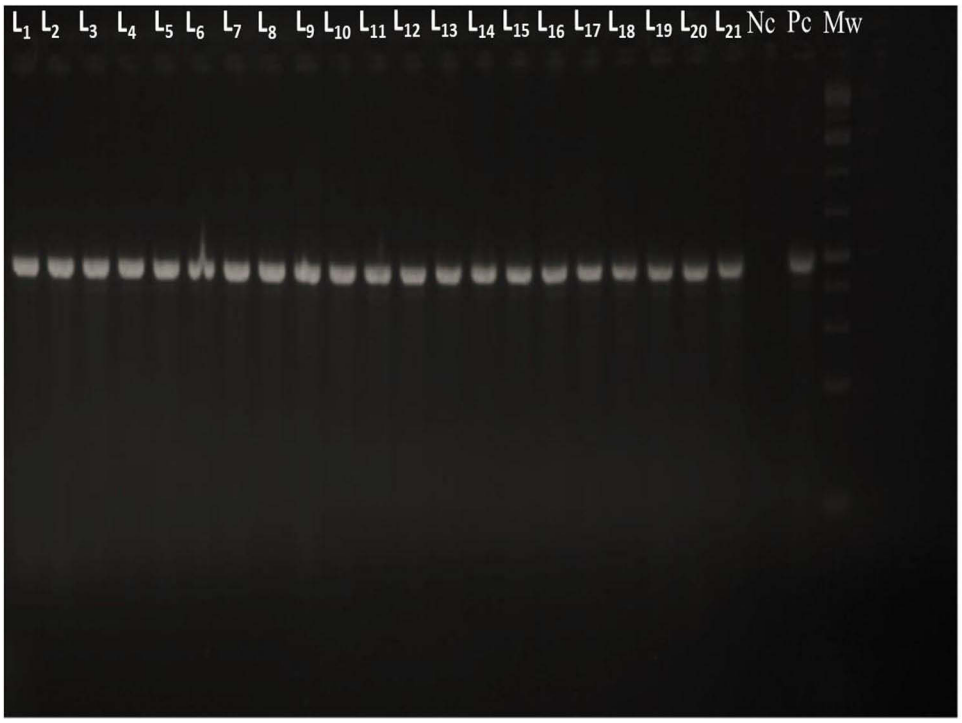

Figure 2 Agarose gel electrophoresis result of PCR product. Legend. Electrophoresis of PCR amplification after serial dilution of *Salmonella* sample in a 0.5% agarose gel stained with ethidium bromide. Agarose gel electrophoresis of *Salmonella* PCR products at 496 bp (16SrRNA gene) amplification of target region of histidine transport operon.

Abbreviations: Mw, molecular weight of marker: (Kb plus DNA ladder), Lane 1–21: number of PCR positives; NC, negative control; PC, positive control (*S. Typhimurium*: ATCC 14028).

Africa for *Salmonella* isolation suggested carcass swabs (30%), skin swabs (59.7%),[4] and knife swabs (16.7%) selected from dairy farms, abattoir, and humans at Asella Town, Ethiopia.[9] However, it is higher than the study conducted on carcass swabs on animal sources in South Africa which reported (1.8%),[35] knife swabs (2.5%) from abattoir and environment in Nigeria,[30] and (1.6%) skin swabs of dairy cattle slaughterhouse in Northern Italy,[36] and (4.5%) Kwata slaughterhouse, Awka, Anambra State.[16] This difference may be attributed to hygienic status, management systems, and cross-contamination among materials used in slaughtering procedures.

Animals that entered abattoirs have been particularly dirty, which contributed to the spread and cross-contamination of skin with the *Salmonella* pathogen. The proportion of carcass contamination recorded in this study, as well as the potential sources of contamination, are diverse. Contact between aprons and then the carcass is unavoidable in many areas and may also result in the carcass-to-carcass transfer of *Salmonella*.[37] The presence of the *Salmonella* execrations in batches of animals in transit and passing through the lairage should result in contamination of the skin.[37] Moreover, it indicated that the exterior surface of the animals serves as a source of the illness for the underlying sterile carcass floor in

Table 3 Detection Rate of *Salmonella* on Different Samples in Selected Export Abattoirs, East Shewa, Ethiopia, from January to October 2020

Sample Types	No. of Samples	No. of Positives	(95% CI)	Exact Test	P-value
Goat carcass swabs	100	10(6.67%)	(3.5, 11.19)		
Knife swabs	60	4(6.67%)	(2.5, 16.64)		
Goat skin swabs	60	7(11.67%)	(5.7, 23)		
Human stool samples	75	0	0(0, 0)	0.304	0.188
Sheep carcass swabs	50	0	0(0, 0)		
Total	345	21(100%)	(4.9, 11.2)		

Table 4 Antibiotic Susceptibility Profiles of *Salmonella* Isolates (n=21) in Selected Export Abattoirs, East Shewa, Ethiopia, from January to October 2020

Antimicrobials	Antibiotic Susceptibility Profiles			
Kanamycin (K)	30 μg	**S (%)** 21(100%)	**I (%)** 0(0%)	**R (%)** 0(0)
Ciprofloxacin (CIP)	5 μg	20(95.24%)	0(0%)	1(4.76%)
Chloramphenicol (C)	30 μg	21(100%)	0(0%)	0(0%)
Cephalothin (CEP)	30 μg	21(100%)	0(0%)	0(0%)
Tetracycline (TE)	30 μg	19(90.47%)	0(0%)	2(9.52%)
Ampicillin (AM)	10 μg	20(95.24%)	0(0%)	1(4.76%)
Nalidixic acid (NA)	30 μg	20(95.24%)	0(0%)	1(4.76%)
Streptomycin (S)	10 μg	18(85.72%)	1(4.76%)	2(9.52%)
Gentamycin (GEN)	10 μg	21(100%)	0(0%)	0(0%)
Ceftriaxone (CRO)	30 μg	21(100%)	0(0%)	0(0%)
Amikacin (AN)	10 μg	20(95.2%)	0(0%)	1(4.76%)
Neomycin (N)	10 μg	0(0%)	20(95.2%)	1(4.76%)
Amoxacillin+Clavulanic acid (AMP)	20/10 μg	20(95.2%)	0(0%)	1(4.76%)
Sulfamethoxazole+ Trimethoprim (SXT)	1.25/23.75 μg	20(95.2%)	0(0%)	1(4.76%)

Abbreviations: S, susceptible; I, intermediate; R, resistant.

Table 5 Drug-Resistance Patterns of *Salmonella* Isolates from Studied Ethiopian Export Abattoirs from January to October 2020

No. of Drugs	Antibiotics	No. of Resistant Isolates	Isolate Origin
One	CIP, AM, NA, AN, N, AMP, and SXT	7(33.33%)	CS
Two	TE (2) and S (2)	2(9.04%)	SS

Abbreviations: CIP, ciprofloxacin; AM, ampicillin; NA, nalidic acid; AN, amikacin; N, neomycin; AMP, amoxacillin+clavulanic acid; SXT, sulfamethoxazole+trimethoprim; TE, tetracycline; S, streptomycin; CS, carcass swab; SS, skin swab.

the course of the dehiding process.[38] It has been indicated that manual operation of all the processing steps in the slaughtering of the animals in the abattoirs, as a substitute than the use of semiautomatic or automatic system in operation will increase the chances of contamination of edible organs and spreading of *Salmonella* in abattoirs' environment.[39] Occasionally, when transferring carcass from one area to another on the floor; there is close contact between the different carcass, and this may also result in the transfer of S*almonella* carcass-to-carcass as well as leftovers anal surfaces feces to the carcass. All these elements may also play a large role in the occurrence of *Salmonella in* chosen export abattoirs in East Shewa, Ethiopia.

In this study, we also assessed the antimicrobial susceptibility of *Salmonella* isolates. The result of the in vitro antibiotics' sensitivity test to *Salmonella* isolates showed distinct degrees of sensitivity toward tested antibiotics ranging from 0% to 100%. The highest susceptibility (100%) was determined toward kanamycin, chloramphenicol, cephalothin, gentamycin, and ceftriaxone while some resistance to tetracycline and streptomycin were seen. Tetracycline and streptomycin have shown greater resistance rates, which could be attributed to the fact that they are among the most commonly used antimicrobials for treating several infectious diseases in livestock. The isolates were susceptible to ceftriaxone and chloramphenicol, which is in settlement with the preceding of cattle slaughtered in Addis Abeba,[3] and dairy cattle in central Ethiopia.[24] In addition, isolates in the current study were susceptible to gentamycin and ceftriaxone, which is in line with preceding studies from food handlers at the University of Gondar, Ethiopia.[33]

Among the 21 *Salmonella* isolates, 7 (33.3%) were resistant to at least one antimicrobial agent and 2 (9.04%) of *Salmonella* isolates were resistant to tetracycline and streptomycin. This conforms with the study conducted in Ecuador that reported resistance rate for other antibiotics ranged from 11.1% up to 33.3%.[40] Antibiotic resistance is the evolutionary response by bacteria to strong selective pressure that results from exposure to antibiotics.[13] This could be an indicator of the acquisition of the resistance genes for those drugs due to the indiscriminate use of drugs at subtherapeutic doses in feed additives to promote growth creating an on-farm selection of antimicrobial-resistant strains.

Hence, continued misuse of antimicrobials may exert stress on resistant bacteria, favoring their emergence and spread. The low prevalence of tetracycline resistance discovered in this study was recorded. This is due to the fact that younger animals are preferred for slaughter, particularly in export abattoirs. When an animal is young, it has a lower threat of acquiring an antimicrobial throughout its continue to be in the community. Antimicrobial resistance is a global public health problem.[4] Resistance to antimicrobials ought to be due to three basic mechanisms: 1) modification of the antibiotic by decreasing absorption or increasing efflux of the antibiotic by using their enzymes, 2) change in the target site of the antibiotic, and 3) acquisition of the ability to break or modify the antibiotic.[41] Several lines of evidence demonstrate that the use of antimicrobial agents in food animals contributes to the emergence and dissemination of antimicrobial resistance in foodborne *Salmonella*.[4] Multidrug resistance has not been recorded in this study. In this study, among the tested antibiotics, kanamycin, ceftriaxone, chloramphenicol cephalothin, and gentamicin were found to be the most effective drugs to inhibit the in vitro growth of these isolates. Thus, these drugs could be used for empirical therapy in the areas where culture facility is no longer available.

Limitation of the Study

In this study, isolates were not serotyped or molecularly characterized due to financial constraints. Selection bias can also be viewed as a constraint.

Conclusion and Recommendation

Investigating the incidence and antimicrobial susceptibility of *Salmonella* from an animal carcass, skin, knife swabs, and humans in export abattoirs is of paramount significance to techniques of minimizing the possible transmission of *Salmonella* between people and animals. Moreover, it is vital in combating the emergence of antibiotic-resistant of *Salmonella*. The information gathered in this cross-sectional study, collectively with different comparable studies, is important to acquire the importance of studying *Salmonella* in export abattoirs. In general, from this study, it can be concluded that the prevalence of *Salmonella* is 21 (6.08%), which appears to be high. This result is significantly high to be a potential source of foodborne salmonellosis. Thus, bacteriological evaluation of *Salmonella* pathogen from export abattoirs was crucial to improving the surveillance system and hygienic standards.

Among the tested antimicrobials, kanamycin, ceftriaxone, chloramphenicol, cephalothin, and gentamicin were 100% susceptible. Antimicrobial treatment techniques need to be based on bacteriological culture followed by antimicrobial susceptibility tests.

Abbreviations

ALIPB, Aklilu Lemma Institute of Pathobiology; BPW, buffered peptone water; CLSI, Clinical and Laboratory Standards Institute; PCR, polymerase chain reaction; RVS Broth, Rappaport Vassiliadis Soya Broth; SPHMMC, Saint Paul's Hospital Millennium Medical College; WHO, World Health Organization; XLD, xylose lysine deoxycholate.

Data Sharing Statement

Data is available upon request from the corresponding author.

Ethical Approval and Consent to Participate

Ethical approval was obtained from St. Paul's Hospital Millennium Medical College (SPHMMC) (Pm23/423) Institutional Review Board (IRB). Written informed consent was obtained from the study participant before the initiation of data collection and it was performed in accordance with the Declaration of Helsinki, and abattoir owners were informed and aware of the purpose of the study. The personal results of any investigation remained confidential. All recognized cases of *Salmonella* in export abattoirs were referred to attending veterinary supervisors. The best practice of veterinary care was taken in sampling part of the animals.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that there is no conflicts of interest regarding the publication of this paper.

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