ORIGINAL RESEARCH

Listeria Species Occurrence and Associated Factors and Antibiogram of *Listeria monocytogenes* in Beef at Abattoirs, Butchers, and Restaurants in Ambo and Holeta in Ethiopia

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Purpose: Listeriosis is one of the globally distributed foodborne diseases with the highest fatality rate. The objectives of this study were to isolate and identify *Listeria* species, assess factors for contamination of beef, and antibiogram of *Listeria monocytogenes* in Ambo and Holeta towns, Central Ethiopia.

Materials and Methods: A total of 450 meat samples were collected from abattoirs (n=150), butchers (n=150), and restaurants (n=150) for isolation and identification of *Listeria* species. Logistic regression analysis was used to assess the association between the occurrence of *Listeria* species in meat and potential risk factors. The antimicrobial susceptibility test was done using the Kirby Bauer test.

Results: The overall occurrence of *Listeria* species in Ambo and Holeta towns was 28.4% (128/450; 95% confidence interval [CI]: 24.3–32.9%). The isolation rate of *Listeria monocytogenes* was 4.4%, *Listeria ivanovii* 2.2%, *Listeria seeligeri* 1.8%, *Listeria welshimeri* 3.8%, *Listeria innocua* 6.2%, and *Listeria grayi* 10.2%. The probability of contamination of meat in butchers and restaurants was higher in Holeta than Ambo [OR=3.4; 95%; p=0.001], in dry than wet season [OR=5.2; p=0.009], and where the hygiene of cutting boards was poor (OR=7.7; p=0.008). Of the 20 *Listeria monocytogenes* isolates, 80%, 70%, 60%, and 55% were resistant to oxacillin, amikacin, and nalidixic acid, chloramphenicol, and tetracycline, respectively. The *Listeria monocytogenes* isolates were 95%, 90%, and 85% susceptible to amoxicillin, vancomycin, and clindamycin, respectively. About 95% of *Listeria monocytogenes* isolates were multidrug-resistant. One isolate (5%) had developed resistance to 10 classes of antimicrobial drugs.

Conclusion: *Listeria* species are widespread and study towns, season, and hygiene of cutting boards are independent predictors of isolation of *Listeria* species. Multidrug resistance among *Listeria monocytogenes* was very high. Therefore, adequate cooking of meat, regular training of beef handlers, prudent use of drugs, and further molecular studies on *Listeria* species are important.

Keywords: antimicrobial susceptibility, beef, *Listeria* species, *Listeria monocytogenes*, multidrug resistance, risk factors, Ethiopia

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Introduction

Consumption of raw meat contaminated with pathogenic *Listeria* species causes foodborne listeriosis.[1–3] Foodborne listeriosis is one of the important diseases affecting human health globally related to increasing global trade and travel.[2,4,5]

Foodborne listeriosis is a sporadic disease epidemiologically associated with the ingestion of contaminated food products.[6] Listeriosis causes a spectrum of diseases ranging from milk and self-limiting diseases in healthy people to severe, systemic, and life-threatening infections in susceptible populations such as HIV patients, pregnant women, neonates, people with malignancy, and the elderly.[6,7]

Based on the phenotypic and genotypic characteristic similarities and differences, *Listeria* species are grouped into "Listeria sensu lato" and "Listeria sensu strictu".[8,9] *Listeria* sensu strictu is composed of *L. monocytogenes, L. innocua, L. seelgerii, L. welshimeri*, and *L. marthii*. All *Listeria* sensu strictu are catalase-positive, motile at least at 30°C, and grow below or at 4°C and gram-positive. *Listeria* sensu lato is composed of 11 species comprising *L. grayi* as well as *L. fleischmannii, L. floridensis, L. aquatica, L. newyorkensis, L. cornellensis, L. rocourtiae, L. weihenstephanensis, L. grandensis, L. riparia*, and *L. booriae*.[10] *Listeria* spp are abundant in nature and can survive diverse types of environmental stresses such as pH, temperature, and salt. *Listeria monocytogenes* is the main causes of human listeriosis, which is a serious health problem,[11] because of severe symptoms and high mortality approximately 20%. Nowadays, other species of *Listeria* are also known to produce a disease in humans and animals.[6,12]

Risk factors for food contamination are identified as all factors essential for food contamination/emergence of foodborne infections.[13] Contamination of meat by microorganisms occurs in an abattoir during slaughtering and spread from the intestinal tract and the exterior part of animals. Besides, air, workers, knives, clothes, carts, and refrigerators[14] may also contaminate meat. Poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources, improper storage, poor personal hygiene during preparation, extended shelf-lives refrigeration, inadequate cooling and reheating create a favorable condition for the spread of foodborne etiologic agents.[4,15,16] Socio-demographic factors, worker's food safety, and hygiene information, knowledge on food safety and hygiene, food safety principles and practices, food source, and others are a few of the food contamination predictors.[13]

The increased use of antimicrobial agents in food animal production and humans is a significant factor in the emergence of antimicrobial-resistant bacteria. The repetitive use of antimicrobials in food animal production for treatment and as growth promotion is a significant factor for the emergence of multi drugs resistant strains.[2,5] Meat is a major source of transmission of antimicrobial-resistant microorganisms to humans.[17] As a result, control and treatment of listeriosis are difficult and very hazardous without antimicrobial resistance interventions.[18]

There have been few studies conducted on the occurrence of *Listeria* species in food samples and meat sources including poultry, mutton, pork, seafood, and other foods of animal origins in Ethiopia.[1,2,4,19,20] However, the occurrence, risk factors, and antibiogram of *Listeria* species in meat from abattoirs, butchers, and restaurants have not been sufficiently studied. Careful investigation of raw meat samples collected from the three spots of the beef chain (abattoir, butcher, and restaurant) will help to identify the weak links contributing to the contamination. This helps considerably for subsequent interventions in attempts to protect consumers from foodborne illnesses and to reduce economic losses due to food spoilage. Therefore, the objectives of the present study were to generate epidemiological data on occurrence and risk factors of *Listeria* species as well as the antibiogram of *Listeria monocytogenes*, isolated from raw beef in abattoirs, butchers, and restaurant of Ambo and Holeta towns of central Ethiopia.

Materials and Methods
Study Area
The research was performed in Ambo and Holeta towns of the Oromia region, central Ethiopia. Ambo town is the administrative center of West Shoa Zone located 114 km West of Addis Ababa at the latitude of 8°59′N 37°51′E and longitude of 8.983°N 37.85°E. The elevation of Ambo town ranges from 1900 to 2275 meters above sea level (m.a.s.l.). Its temperature ranges from 19°C to 29°C with an average annual temperature of 22°C and an average annual rainfall of about 900 mm. The town has a total human population of 74,843 out of which 39,192 are males, and 35,651 are females.[21] There are 46 legal butchers with their annexed restaurants and one municipality abattoir in Ambo town.

Holeta is located in Finfine special zone, 44 Kms West of Addis Ababa with a latitude and longitude of 9°3′N38° 30′E/9.050′N38.500°E. Its elevation is 2400 masl. It receives 1144 mm annual average precipitation, with an average minimum and maximum temperature of 6°C and 22°C, respectively. The total human population of Holeta town is 25,593, of whom 12,605 are men and 12,988

women.[21] There are 20 legal butchers with annexed restaurants and one municipality abattoir in Holeta town.

Study Population

All abattoirs, butchers, restaurants in Ambo and Holeta towns constituted the targets of this study. Carcasses of cattle slaughtered in Ambo and Holeta abattoirs are the study subjects.

Study Design

A cross-sectional study was conducted between October 2017 to April 2018 in abattoirs, butchers, and restaurants of Ambo and Holeta towns, to determine the occurrence of the *Listeria* species, potential risk factors for meat contamination, and antimicrobial susceptibility profiles of the *Listeria monocytogenes* from raw beef sampled from three points (abattoir, butcher, and restaurant).

First, the list of all the current legal butchers, restaurants, and abattoirs registered in Ambo and Holeta towns was collected from the towns' municipality (sampling frame) and then the two abattoirs (one from each town) and those butchers with an annexed restaurant for handling meat were purposively identified.

Sample Size Determination

The required sample size was determined using a 95% confidence limit and a 7% sampling error and an expected prevalence of 25% by Thrusfield[22] formula. As a result, a sample size of 147 was calculated. However, 450 samples of meat, consisting of 150 from abattoirs, 150 from butchers, and 150 from restaurants, were sampled. Samples were obtained from all the 20 and 46 butchers in Holeta and Ambo towns, respectively. Eighty-seven samples from the town of Holeta (29 per establishment) and 363 samples from the town of Ambo (121 per establishment) were allocated.

Sample Collection

A systematic random sampling method was used to select animals from the study population. Following slaughtering, meat samples were collected from three spots, ie, Abattoir, butcher, and restaurant. First composite meat samples of about 250 gm were collected from slaughtered cattle from four sites (ramp, flank, brisket, and neck) just after the stage of evisceration. The same procedure was followed on the second sampling spot, ie, butcher house. On the third sampling spot, 250 g raw beef that is prepared to be served for consumption (*Kitfo*;- a traditional

Ethiopian dish made from minced raw beef, chili, spice, and butter blend or *Tire siga- cubes* of meat cut served raw) was collected. All samples were collected aseptically and labeled with necessary information including the sample code, date of sampling, and sampling place (spot). Samples were kept in separate sterile plastic bags (Seward, England) and immediately transported to the Ambo University Veterinary Microbiology Laboratory in an icebox with ice packs. Samples were processed within 4 hrs of arrival at the laboratory.

During sample collection, demographic data from abattoir personnel, butcher shop workers and restaurant chefs, and waiters who were in charge were collected including the sex, marital status, the residence of origin, educational status, work experience in years, and time on work per day. Besides, an observation was made on the hygiene of the cutting boards, the weighing balance, food handling surfaces, and of butchers and restaurants as a whole. Hygienic variables were categorized as poor if there is gross dirt, undesirable smell, insects and as good if gross dirt, bad smell, and insects are absent.

Isolation and Identification of *Listeria* Species

For the isolation and identification of *Listeria* species, the method recommended by the International Standardization Organization (ISO)[23] was followed. Accordingly, 25 g of sample was homogenized in 225 mL of *Listeria* enrichment broth (LEB) containing supplements (HiMedia, India) using a laboratory blender and incubated at 30°C for 24 hrs. Then after 0.1 mL of mixed inoculum was inoculated into a tube containing 10 mL Modified Fraser broth (MFB) and incubated for 24 hrs at 37°C. Following the enrichment procedure, the inoculum was streaked on Oxford agar (OXA) containing manufacturer's supplements and the plates were incubated at 37°C for 24–48 hrs. After 24 hrs of incubation, the growth of *Listeria* species on the Oxford agar plate was examined for black halo colonies, which remain the same after 48 hrs of incubation but with a sunken center.

The presumptive colonies were picked up and further purified on Tryptone Soya Yeast Extract agar (TSYEA). Subsequently, pinpoint colonies on TSYEA were subjected to identification procedures, which included Gram's staining followed by a microscopic examination, catalase test, and oxidase test. The characteristic Gram-positive, coccobacillus, or short rod-shaped organisms, which were catalase-positive

and oxidase negative, were sub-cultured in Brain heart infusion (BHI) broth at 25°C for 12–18 hrs. Then, the cultures showing typical tumbling motility were considered as "presumptive" *Listeria* isolates, which were in turn subjected to detailed biochemical tests viz.; Christe, Atkins, and Munch-Peterson (CAMP) test, methyl red, Voges-Proskauer, and sugar fermentation tests with xylose, rhamnose, and mannitol for identification of *Listeria* to species level.

Antimicrobial Susceptibility Test

The *L. monocytogenes* isolates were subjected to an antimicrobial susceptibility test against 16 commercially available antimicrobial discs (Oxoid, UK) selected based on common usage. The antimicrobial discs selected were amikacin (30μg), amoxicillin (10μg), ampicillin (10μg), chloramphenicol (30μg), cefotaxime (30μg), gentamicin (10μg), tetracycline (30μg), oxacillin (1μg), vancomycin (10μg), cotrimoxazole (25μg), nalidixic acid (30μg), ciprofloxacin (5μg), erythromycin (15μg), clindamycin (2μg), penicillin (6μg), and trimethoprim-sulfamethoxazole (23.75μg). An antimicrobial susceptibility test was conducted using the disc diffusion method following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI).[24] Two to three pure fresh colonies of the isolates from TSAYE were used to prepare a cell suspension in the Mueller Hinton broth (HiMedia, India) and incubated for 24 hrs at 37°C. Following this, the cell suspension turbidity was attuned to equal 0.5 McFarland Standard. Then, a sterile cotton swab was used to spread the bacterial suspension on the Mueller Hinton agar (HiMedia, India). The discs were firmly placed in the interval of 3 cm spacing from each other onto the medium with sterile forceps and then incubated at 37°C for 24 hrs. Then, the diameter of clear zones around the discs was measured with a ruler against a black background and compared with standards given by CLSI.[24] *L. monocytogenes* ATCC7644, *E. coli* ATCC25922, and *S. aureus* ATCC6538 reference strains were used as quality control.

Data Management and Analysis

The collected data were entered into Microsoft Excel spreadsheets (Microsoft Corporation) and analyzed using STATA version 14.2 software (Stata Crop. College Station, USA). Descriptive statistics was used to summarize the occurrence, socio-demographic characteristics of the respondents, and antimicrobial susceptibility data. Univariable and multivariable logistic regression analyses with their Odds Ratio (OR) and 95% confidence Intervals (95% CI) were used to determine potential risk factors

associated with meat contamination by *Listeria* species. During risk factors analysis, for all of the risk factors, the first category of the independent variables (with the lowest prevalence) was considered as a reference category. Non-collinear variables that possessed a p-value of <0.25 in univariable analysis were entered into the multivariable analysis. The model was constructed by a backward exclusion method. Hosmer-Lemeshow goodness-of-fit test was used to assess the model fitness. The reliability of the fitted model was further evaluated using the receiver operating characteristics curve (ROC). The results were considered significant at p<0.05 at all levels of analysis.

Inclusion and Exclusion Criteria

Meats from cattle slaughtered and inspected in the abattoirs, displayed for sale in butcher shops, and used for meat dish preparation in restaurants were included in the study while the meats of other food animals, cattle meat that is not inspected, and cattle meat that is not utilized for raw meat dish preparation were excluded.

Results
Occurrence and Identification of *Listeria* Species

Out of the total 450 meat samples examined, 28.4% were positive for *Listeria* species. The occurrence of *Listeria* species in Ambo town (20.66%) was much lower when compared to Holeta town (60.9%). The highest *Listeria* species occurrence was recorded in restaurants (30.0%), followed by butchers (29.3%) and then abattoir (26.0%). *Listeria* species occurrence in abattoir and restaurant samples of Holeta town was relatively higher than similar samples of Ambo town (Table 1).

Out of the total 128 *Listeria* species isolated, *Listeria grayi* was the most frequent isolate while *Listeria seeligeri* was the least. The percentage of *L. monocytogenes* in abattoirs, butchers, and restaurants raw meat samples of cattle were 33%, 1.6%, and 1.6%, respectively (Table 2).

Risk Factors
Socio-Demographic Characteristics, Knowledge, and Practice on Food Safety and Hygiene
The socio-demographic characteristics, knowledge, and practice on food safety and hygiene of respondents are depicted in Table 3.

Table 1 The Occurrence of *Listeria* Species in Raw Beef from Abattoirs, Butchers, and Restaurants of Ambo and Holeta Towns of Central Ethiopia

Study Towns	Establishment	Tested	*Listeria* Species		
			No. Pos.	%	95% CI [Lower– Upper]
Ambo	Abattoir	121	25	20.7	13.8–29.0
	Butchers	121	22	18.2	11.8–26.2
	Restaurants	121	28	23.1	16.0–31.7
	Sub total	363	75	20.7	16.6–25.2
Holeta	Abattoir	29	14	48.3	29.5–67.5
	Butchers	29	22	75.9	56.5–89.7
	Restaurants	29	17	58.6	38.9–76.5
	Sub total	87	53	60.9	49.9–71.2
Subtotal	Abattoirs	150	39	26.0	19.2–33.8
	Butchers	150	44	29.3	22.2–37.3
	Restaurants	150	45	30.0	22.8–38.0
Total		450	128	28.4	24.3–32.9

Abbreviations: %, percent; No., number; CI, confidence interval; pos, positive.

Table 2 *Listeria* Species Identified from Raw Beef in Ambo and Holeta Towns, Oromia, Central Ethiopia (n=450)

Listeria Species	No of Positive	Identified *Listeria* Species	
		% Occurrence	95% CI [Lower– Upper]
Listeria monocytogenes	20	4.4	2.7–6.8
Listeria ivanovii	10	2.2	1.1–4.0
Listeria seeligeri	8	1.8	0.8–3.5
Listeria welshimeri	17	3.8	2.2–6.0
Listeria innocua	28	6.2	4.2–8.9
Listeria grayi	46	10.2	7.6–13.4
Total	128	28.4	24.3–32.9

Abbreviations: %, percent; No, number; CI, confidence interval.

Logistic Regression Analyses

Univariable logistic regression analysis showed that the occurrence of *Listeria* species in raw beef was significantly different (p<0.001) between study towns, seasons, information on food hygiene and safety, working times per day, and hygiene of the cutting boards. All the remaining variables did not show a significant association with

the risk of raw beef contamination (p>0.05). The risk of raw beef contamination was significantly higher in Holeta town than in Ambo town, in the dry season than wet season, and from butchers and restaurants in which employees working hours per day was ≥ 9 hours as compared to working hours of ≤8 per day (p<0.001) (Table 4).

Among the studied variables study towns, season, working time per day, hygiene of slicing materials, and hygiene of cutting boards fulfilled the criteria (p<0.25 and (r<0.5) and therefore have been incorporated into the multivariable model. Multivariable logistic regression analysis revealed that study town, season, and hygiene of the cutting boards were independent predictors of contamination of raw beef by *Listeria* species (p<0.05). Hygiene of the slicing materials (p=0.051), though not significantly associated, was close for association (Table 4). The full model had Hosmer-Lemeshow X^2 =4.8, p=0.684, and assessment of model fitness to the observed data indicated that observed and predicted values were not significantly different, ie, the data fitted the model well. Moreover, the sensitivity (32.9%), specificity (92.3%), and receiver operating characteristics (ROC) analysis (0.789) suggest the data fitted the model well. The model had a positive predictive value of 62.8% and a negative predictive value of 77.7%.

Antimicrobial Susceptibility

The results of antimicrobial susceptibility testing of the 20 *L. monocytogenes* isolates is shown in Table 5.

All identified *Listeria monocytogenes* had developed resistance to two or more classes of antimicrobial drugs. Nineteen (95%) *L. monocytogenes* isolates were resistant to three or more classes of antimicrobials (multidrug resistance – MDR). One isolate (5%) had developed resistance to nine classes of antimicrobials. The most common MDR pattern was observed against amikacin, cefotaxime, nalidixic acid, and Oxacillin. The patterns of antimicrobial resistance of *L. monocytogenes* isolates were shown below (Table 6).

Discussion
Occurrence of *Listeria* Species in Raw Beef in Abattoirs, Butchers, and Restaurants

In the present study, an overall occurrence of *Listeria* species (28.4%) from samples of raw beef from abattoirs, butchers, and restaurants was in agreement with the

Table 3 Socio-Demographic Characteristics of Workers of Abattoirs, Butchers, and Restaurants of Ambo and Holeta Towns

Variables	Categories	Abattoirs Workers		Butcher Workers		Restaurants Workers	
		Freq.	%	Freq.	%	Freq.	%
Sex	Male	33	100	145	100	–	–
	Female	–	–	–	–	145	100
Age	18–24	19	57.6	19	13.1	5	3.5
	25–34	11	33.3	41	28.3	76	52.4
	≥35	3	9.1	85	58.6	64	44.4
Education	Illiterate	3	9.1	5	3.5	18	12.4
	Primary school	17	51.5	80	55.2	98	67.6
	Secondary school	6	18.2	14	9.7	–	–
	Tertiary	7	21.2	46	31.7	29	20.0
Work experience in years	≤1	5	15.2	93	64.1	73	50.3
	1–3	14	42.4	19	13.1	47	32.4
	≥4	14	42.4	33	22.8	25	17.2
Working time per day	≤8hours	33	100	33	22.8	46	31.7
	≥9hours	–	–	112	77.2	99	68.3

Abbreviations: Freq, frequency; ≥, greater than or equal to; ≤, less than or equal to; <, less than; %, percent.

reports from Addis Ababa (27.5%).[1] Other studies have reported significantly higher isolation rates of *Listeria* species in raw meat such as 95% in Brazil,[25] 81.5%,[26] and 54.1%[27] in Turkey, 58% in Nigeria,[28] 51.3% in Ethiopia,[20] and 50% in Jordan.[29]

The 4.4% isolation rate of *L. monocytogenes* in this study was comparable with the 4.1% from goat meat in Ethiopia[4] and 4.1% in raw beef in Ethiopia.[1] However, the current finding was higher when compared to 1.29% from raw cow and goat meats,[30] 1.6% from minced beef,[19] and 2.5% from bovine carcasses in Poland[31] and was lower when compared to the 15.6% in Nordic countries,[32] 15.4% in Bangkok,[33] 25.5% in Turkey.[26] The relatively low percentage of *L. monocytogenes* (4.4%) in the present study may be attributed to the difference in study season, geographic conditions, and sample size.[34,35]

Meat can be contaminated by *L. monocytogenes* at abattoirs by cross-contamination during slaughtering, evisceration, and other processing steps. Additionally, contamination and growth of *Listeria* at the next chain (butchers and restaurants) could occur due to poor hygienic handling and processing, refrigeration; and the suitability of the meat pH, water activity, and nutrient content.[36] The isolation rate of other *Listeria* species in the present study ranged from 1.8% - 10.2%. The 2.2% isolation rate of *L. ivanovii* in the present study is by far lower than the 21.9% reported by Al-Nabulsi et al[16] and 19.8% by

Alsheikh et al[37] reported from various types of meat and meat products. However, the isolation rate of *L. ivanovii* in the present study is comparable to 2% reported from raw meat in Ethiopia.[2] The 3.8% isolation rate of the *L. welshimeri* in this study is in agreement with the 2.8% from Jordan,[16] and 4% from Ethiopia[2] reported previously. The occurrence of *L. seeligeri* (1.8%) in the present study disagrees with the 27% reported from Amman, Jordan,[16] however, it is in line with the 1% and 2% prevalence of *L. seeligeri* previously reported by Alsheikh et al[37] and Garedew et al,[2] respectively. The lower detection rate of *L. ivanovii, L. welshimeri*, and *L. seeligeri* in the present study might be linked to the hygienic status of food processing environments and the bacteriological detection method differences.[2,37,38]

Most research reports indicate that *L. innocua* is the most common species isolated from different meat samples. For example, in Ethiopia, 83% and 19% of *L. innocua* have been reported.[1,2] In this study, *L. grayi* (10.22%) and *L. innocua* (6.2%) were more common than the other *Listeria* species. Few reports from meat samples showed that *L. grayi* was the second most abundant *Listeria* species next to *L. ivanovii*.[16] The relatively high percentage of *L. grayi* (10.2%) and *L. innocua* (6.2%) in the current study was in line with the reports of from Ethiopia[39] and might be related to the species entrance to the processing environments via as a part of intestinal

Table 4 Logistic Regression Analysis of Potential Risk Factors for *Listeria* Species Isolated from Raw Beef of Butchers and Restaurants in Ambo and Holeta Towns, Central Ethiopia

Risk Factors	Category	No. Tested	No. Positive (%)	Univariable		Multivariable	
				OR (95% CI)	p-value	OR (95% CI)	p-value
Source	Butchers	145	40 (27.6)	1.0	–		
	Restaurants	145	42 (29.0)	1.1 (0.6–1.8)	0.794		
Study town	Ambo	234	50 (21.4)	1.0	–	1.0	–
	Holeta	58	32 (57.1)	4.9 (2.7–9.1)	≤0.001	3.4 (1.7–7.0)	0.001
Season	Wet	55	3 (5.5)	1.0	–	1.0	–
	Dry	235	79 (33.6)	8.8 (2.7–29.0)	≤0.001	5.2(1.5–17.9)	0.009
Sex of food handlers	Female	145	40 (27.6)	1.0	–		
	Male	145	42 (29.0)	1.1 (0.6–1.8)	0.794		
Age of food handlers in years	≥35	24	5 (20.8)	1.0	–		
	18–24	117	29 (24.8)	1.3 (0.4–3.7)	0.681		
	25–34	149	48 (32.2)	1.8 (0.6–5.1)	0.267		
Education	Illiterate	234	50 (21.4)	1.0	–		
	Primary	58	32 (57.1)	1.0 (0.4–2.7)	0.974		
	Secondary	14	4 (28.6)	1.1 (0.3–5.0)	0.869		
	Tertiary	75	25 (33.3)	1.4 (0.5–4,0)	0.514		
Work experience in years	1–3	166	46 (27.7)	1.0	–		
	<1	66	19 (28.8)	1.1 (0.6–2.0)	0.869		
	≥4	58	17 (29.3)	1.1 (0.6–2.1)	0.816		
Working time per day	≤8hours	79	10 (12.7)	1.0	–		
	≥9 hours	211	72 (34.1)	3.6 (1.7–7.4)	0.001		
Hygiene of the slicing materials	Good	174	27 (31.6)	1.0	–	1.0	–
	Poor	100	26 (26.0)	1.5(0.9–2.6)	0.124	2.0 (1.0–3.9)	0.051
Hygiene of the cutting boards	Good	39	2 (5.1)	1.0	–	1.0	–
	Poor	251	80 (31.9)	8.7(2.0–36.8)	0.003	7.7(1.7–34.3)	0.008

Abbreviations: ≥, greater than or equal to; ≤, less than or equal to; <, less than; %, percent; OR, odds ratio; CI, confidence interval; No., number; prev, prevalence.

microbial flora, improper hygienic practices during processing and food handling.[40,41]

Risk Factors Assessment

The occurrence of *Listeria* species in raw beef was nearly 5.2 times higher in the dry season (p=0.009) when compared to the wet season. The significantly high occurrence of *Listeria* species in cattle beef during the dry season might be due to the large sample size (more than 75% of the meat samples) in the dry season and the environmental stress in cattle during the dry season that led to the shedding of the organisms in feces. It is well known that a dry climate harms the persistence of *Listeria* species.[42] Thus, drying is linked to water activity lowering of meat and meat products. As a consequence, the lowered moisture content of the meat enables the organism to persist

in (resist) high temperature.[43] In addition, the shortage of water during the dry season might impair the hygienic activities of personnel and equipment in the establishments thereby increasing the chance of contamination. The isolation of *Listeria* species from meat in both dry and wet seasons suggests the existence of natural reservoirs of the bacteria in cattle or the environment. On the other hand, univariable logistic regression showed a significantly high prevalence of *Listeria* species in butchers and restaurants where the employees work ≥9 hrs as compared to those working ≤8 hrs. This might be related to the change in the hygienic behavior of works as they get exhausted/tired while working ≥9 hrs/day.

In the current study, the place where the study was carried out was the predictor of *Listeria* species isolation (p=0.001). The significantly high recovery rate of *Listeria*

Table 5 Results of the Antibiogram of *Listeria monocytogenes* Isolates (n=20) from Raw Cattle Meat Samples of the Abattoirs, Butchers, and Restaurants

Antimicrobial Class	Antimicrobial Discs and Concentration	*Listeria monocytogenes* Isolates (n=20)	
		No. of Susceptible (%)	No. of Resistant (%)
Aminoglycosides	Amikacin (30μg) Gentamicin (10μg)	6 (30) 13 (65)	14 (70) 7 (35)
Cephem	Cefotaxime (30μg)	5 (25)	15 (75)
Lincosamide	Clindamycin (2μg)	17 (85)	3 (15)
Macrolide	Erythromycin (15μg)	14 (70)	6 (30)
Phenicol	Chloramphenicol (30μg)	8(40)	12(60)
Nitrofuran	Nitrofurantoin (300μg)	19(95)	1(5)
Quinolones	Ciprofloxacin (5μg) Nalidixic Acid (30μg)	14 (70) 6 (30)	6 (30) 14 (70)
Sulfonamide	Trimethoprim-Sulfamethoxazole (23.75μg)	14 (70)	6 (30)
Tetracycline	Tetracycline (30μg)	9 (45)	11 (55)
β-lactams	Ampicillin (10μg) Amoxicillin (25 μg) Penicillin (10 units) Oxacillin (30 μg)	15 (75) 19 (95) 15 (75) 4 (20)	5 (25) 1 (5) 5 (25) 16 (80)
Glycopeptide	Vancomycin (30 μg)	18 (90)	2 (10)

Abbreviations: No, number; μg, microgram; %, percent.

species from Holeta (57.1%) as compared to Ambo (21.4%) town might be related to the cool temperate of Holeta town favorable for the survival and multiplication of the organisms.

Multivariable logistic regression analysis also identified the hygiene of the cutting boards (p=0.008) as a risk of raw beef contamination by *Listeria* species. This is in line with the findings of previous workers.[44–46] The types or quality of cutting boards and the absence of frequent sanitation of the cutting boards, and the use of unclean water for washing cutting boards might be linked to a higher chance of cutting boards being contaminated with *Listeria* species.[46]

Antimicrobial Susceptibility Profile of the *L. monocytogenes* Isolates

The highest percentage of resistance was noted for oxacillin (75%) followed by 55–70% resistance to amikacin, nalidixic acid, tetracycline, and chloramphenicol. The 75% resistance of oxacillin in the present study is comparable with the 72.2% resistance reported by Wieczorek et al[31] and Gomez et al.[47] While the 70% resistance to

amikacin is contrary to the 100% susceptibility reported by Indrawattana et al.[33] The resistance against nalidixic acid and tetracycline in this study is in accord with the reports of Maktabi et al.[35]

The 30% resistance to ciprofloxacin in this study is lower when compared to the 44.4% and 56% resistance reported from Nigeria.[48,49] Similarly, the 30% resistance to erythromycin and trimethoprim-sulfamethoxazole reported in the current study disagrees with the 87.5%[49] and 69.4%[48] resistance reported previously from Nigeria. Although the resistance to trimethoprim-sulfamethoxazole (30%) and gentamicin (35%) in the present study agrees with the 24%[35] and 36.1%[48] resistance, it contradicts with the 87.5% trimethoprim-sulfamethoxazole and 72.2% gentamicin resistance.[49] The 15% resistance to clindamycin is in line with the 12% resistance reported by Maktabi et al.[35] However, 15% of cefotaxime and 25% penicillin resistance in this study is highly divergent from the 77.5% cefotaxime and 66.7% penicillin resistance reported from Ethiopia.[2] The low level of resistance (5–15%) to amoxicillin, cefotaxime, vancomycin, and clindamycin in this study might be due

Table 6 Antimicrobial Resistance Patterns of *Listeria monocytogenes* (n=20)

Antimicrobials Resistance Pattern	Number of the Resistant Isolates	Percent
OXA-AMK	1	5
AMP-CIP-CTR	2	10
OXA-TET-CHL	1	5
XST-AMK-NAL-ERY	2	10
XST-AMK-NAL-CTR	1	5
OXA-NAL-ERY-CTR	1	5
OXA-AMK-CTR-TET-CHL	1	5
XST-OXA-AMK-CTR-CHL	1	5
OXA-AMK-NAL-CLI-CTR	1	5
OXA-AMK-NAL-TET-CHL	1	5
OXA-AMK-NAL-CTR-TET-CHL	4	20
OXA-AMK-NAL-ERY-CTR-TET-CHL	1	5
XST-OXA-NIT-AMK-NAL-VAN-TET-CHL	1	5
XST-OXA-GEN-NAL-ERY-CTR-TET-CHL	1	5
OXA-AMK-NAL-ERY-CLI-VAN-CTR-TET-CHL	1	5
Total	20	100

Abbreviations: AMK, amikacin; AMP, ampicillin; AMX, amoxicillin; CIP, ciprofloxacin; CHL, chloramphenicol; CLI, clindamycin; CTX, cefotaxime; GEN, gentamicin; ERY, erythromycin; NAL, nalidixic acid; NIT, nitrofurantoin; OXA, oxacillin; PEN, penicillin; TET, tetracycline; VAN, vancomycin; XST, trimethoprim-sulfamethoxazole.

to the absence of usage of these drugs in veterinary medicine in Ethiopia which plausibly suggests that these antimicrobials remain an alternative regimen against the organisms.[2] The relatively high resistance (≥30%) observed in erythromycin, chloramphenicol, trimethoprim-sulfamethoxazole, oxacillin, tetracycline, gentamicin, and trimethoprim-sulfamethoxazole might be related to the more frequent or improper usage, particularly in the public health sectors. Nevertheless, the study warrants frequent surveillance on the change in the pattern of antibiograms for this organism.

The present MDR *L. monocytogenes* isolates (95%) was higher when compared to the 72.3% MDR identified from raw foods.[50] In agreement with the present study, Odu and Okonko[5] also reported *L. monocytogenes* isolates that are 100% MDR. The MDR patterns of *L. monocytogenes* in the study towns might be due to the non-prescribed frequent and non-judicious usage of antimicrobials in livestock and public health sectors in the study towns.[2] The MDR to *L. monocytogenes* may occur due to plasmid or chromosomal gene transfer and mutation events in chromosomal genes from other *Listeria* species and Gram-positive bacteria that may be found in foods.[50,51]

The high rate of antimicrobial-resistant *L. monocytogenes* isolates towards the relatively cheaper and commonly available antimicrobials is worrisome as it might lead to the use of mandatory and more expensive drugs.[2] This is a problem for listeriosis high-risk groups in developing countries like Ethiopia because the high cost of hospitalization and recent drug treatment leads to an economic burden on families and societies.

The isolation of *L. monocytogenes* from raw meat is a microbiological hazard for people since the consumption of raw or undercooked meat in the study areas is a widespread food habit. The present findings have also great implications for the public health in Ethiopia because of the high fatality of listeriosis, the abundance of immunocompromised people, and inadequate hygiene and awareness of the community.

The limitations of this study include the following: Firstly, due to the lack of resources and facilities, serotyping and molecular work on *Listeria* species have not been done. Secondly, antimicrobial susceptibility testing was performed only for *L. monocytogenes*. Thirdly, samples from meat handlers, equipment, and the environment were not taken to see their association. Fourthly, the questionnaire data collected from abattoir workers were not included in the logistic regression analysis due to the small number of workers (n=33) hard to compare with that of butchers and restaurants and the difficulty of linking responses of workers to a particular sample result as all workers handle the carcasses.

Conclusion

The study confirmed that contamination of raw beef sold for human consumption by *Listeria* species is widespread in the study area. Six *Listeria* species (*Listeria monocytogenes*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria welshimeri*, *Listeria innocua*, and *Listeria grayi*) were identified. The overall occurrence of *Listeria* species was

medium while that of *L. monocytogenes* was low. The study towns, season, information on food hygiene and safety, and hygiene of meat cutting boards were independent predictors of *Listeria* species isolation. The high rate of antimicrobial resistance among *L. monocytogenes* isolates is of great public health importance. Amoxicillin, clindamycin, nitrofurantoin, and vancomycin might be good drugs for the treatment of *L. monocytogenes* infections in the study areas. Therefore, adequate heat treatment of beef before consumption, regular training for workers in the establishments, and follow-up on the prudent use of antimicrobial drugs in veterinary and public health sectors are important; and subsequently, further serological and molecular studies on *Listeria* species are proposed.

Data Sharing Statement

All the data supporting our findings were incorporated within the manuscript.

Ethical Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki. All study subjects were informed about the study and written informed consents were obtained from all the owners and workers of the Ambo and Holeta town butchers, restaurants, and abattoirs. Confidentiality was assured by using codes. Ethical clearance was obtained from the Ambo University research and ethical review committee.

Author Contributions

All authors made a significant contribution to the work reported either in the conception of the study, study design, execution, acquisition of data, analysis, and interpretation or in all these areas. Moreover, all authors took part in 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.

Disclosure

The authors report no conflicts of interest in this work.

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