

Detection of *Listeria* species, factors associated, and antibiogram of *Listeria monocytogenes* in beef at abattoirs, butchers, and restaurants of Ambo and Holeta Towns, Ethiopia

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Abstract

Background

Listeriosis is one of the globally distributed foodborne diseases with the highest fatality rate. Few studies were done on the occurrence of *Listeria* species from meat at abattoirs, butchers, and restaurants in Ethiopia, and there has been no study conducted at Ambo and Holeta town. The objectives of this study were to isolate and identify *Listeria* species, assess factors for contamination of meat, and antibiogram of *Listeria monocytogenes* along the meat chain in Ambo and Holeta towns, Central Ethiopia.

Methods

450 meat samples were collected from abattoirs (n = 150), butchers (n = 150) and restaurants (n = 150) for isolation and identification of *Listeria* species using primary culture and biochemical tests. A questionnaire survey and observational checklist were made to assess the potential risk factors for the occurrence of *Listeria* species such as factors related to socio-demographic characteristics, knowledge on hygiene and practice of food handlers. Pearson's Chi-square and logistic regression analyses were used to assess factors contributing for contamination of meat with *Listeria* species. Kirby Bauer disc diffusion technique was applied to determine the antimicrobial susceptibility profile of *Listeria monocytogenes* isolates.

Results

The overall occurrence of *Listeria* species in both Ambo and Holeta towns was 28.44% (128/450; 95% confidence interval [CI]: 24.32–32.86%). The occurrence of *L. monocytogenes* was 4.4% (20/450; 95% CI: 2.74–6.78%), *L. ivanovii* 2.2% (10/450; 95% CI: 1.07–4.04%), *L. seeligeri* 1.78% (8/450; 95% CI: 0.8–3.47%), *L. welshimeri* 3.77% (17/450; 95% CI: 2.22–5.98%), *L. innocua* 6.22% (28/450; 95% CI: 4.17–8.87%) and *L. grayi* 10.22% (46/450; 95% CI: 7.58–13.39%). The probability of contamination of meat in butchers and restaurants by the *Listeria* species were comparatively higher in high altitude (Holeta) than medium altitudes (Ambo) [OR = 4.91; 95% CI: 2.65–9.07%; p < 0.001], in dry than wet season [OR = 8.78; 95% CI: 2.66–28.99%; p < 0.001] and in butchers and restaurant where the employees work ≥ 9 hours per day than those working ≤ 8 hours per day (OR = 3.57; 95% CI: 1.74–7.36%; p < 0.001). Of the 20 *Listeria monocytogenes* isolates, 16 (80%) were resistant to oxacillin; 14 (70%) were resistant to amikacin and nalidixic acid; 12 (60%) were resistant to chloramphenicol and 11 (55%) were resistant to tetracycline. The *L. monocytogenes* isolates were 95%, 90% and 85% susceptible to amoxicillin, vancomycin, and clindamycin, respectively. All (100%) *Listeria monocytogenes* isolates were resistant for two or more drugs. Nineteen (95%) *L. monocytogenes* isolates were multidrug-resistant. One isolate (5%) had developed resistance to 10 classes of antimicrobial drugs.

Conclusions

Listeria species are widespread in the study areas. The study towns, season and working hours per day are independent predictors of *Listeria* species isolation. Multidrug resistance among *L. monocytogenes* is common. Therefore, regular training for meat handlers, prudent use of drugs, and further serological and molecular studies on *Listeria* species are important.

Introduction

Foodborne bacterial infections describes the adverse health effect associated with eating of contaminated foods with pathogenic bacteria, including those originated from meat and that result either in morbidity or mortality worldwide in general and in developing countries in particular [1]. Meat is the most valuable food of animal origin and its chemical composition makes it one of the most vulnerable vehicles of infections agents [2]. Even though the association of the raw meat consumption habits with health impact over several years has little attention, consumption of raw meat in Ethiopia is deep-rooted tradition [3–5]. Consumption of raw meat contaminated with pathogenic *Listeria* species causes foodborne listeriosis [3, 4, 6]. Foodborne listeriosis is one of the important diseases affecting human health globally related to the increasing global trade and travel [4, 7, 8]. Thus, even though foodborne listeriosis may be comparatively rare, it causes severe and life-threatening infection in immunocompromised groups such as HIV patients, pregnant women, neonates and elderly [9].

Based on the phenotypic and genotypic characteristic similarities and differences, *Listeria* species are grouped in to '*Listeria sensu lato*' and '*Listeria sensu strictu*' [10, 11]. *Listeria sensu strictu* is composed of *L. monocytogenes*, *L. innocua*, *L. seelgerii*, *L. welshimeri* and *L. marthi*. All *Listeria sensu strictu* are catalase-positive, motile at least at 30°C and grow below 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* [12]. *Listeria monocytogenes* causes foodborne disease and consequently, a serious health problem, because of severe symptoms and high mortality. Nowadays, other species of *Listeria* are also known to produce a disease in humans and animals [13].

Risk factors of food contamination are defined to be all the factors necessary for food contamination / foodborne outbreaks, infections [14]. Contamination of meat by microorganisms occurs in abattoir during slaughtering and spread from the intestinal tract and the exterior part of animals. Moreover, it can be contaminated in retailer shop and kitchen from air, workers, knives, cloths, carts, and refrigerators [15]. 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 [7, 16, 17]. Sociodemographic factors, worker's food safety and hygiene information, knowledge on food safety and hygiene, food safety principles and practices, food source and others are

few of the food contamination predictors [14]. The increased use of antimicrobial agents in food animal production and human 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 significant factor for the emergence of multi drugs resistant strains [4, 8]. Meat is a major source of transmission of antimicrobial resistant microorganisms to humans [18]. As a result, control and treatment of listeriosis is difficult and very hazardous without antimicrobial resistance interventions [19]

There have been few studies conducted on the occurrence of *Listeria* species in food samples including meat sources like poultry, mutton, pork, seafood and other foods of animal origins in Ethiopia [3, 4, 7, 20, 21]. However, the occurrence, risk factors and antibiogram of *Listeria* species along the consecutive meat chain from abattoirs, butchers and restaurants have not been studied so far. 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 an 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 isolate and identify *Listeria* specie, assess factors contributing for contamination of beef and determine the antibiogram of *Listeria monocytogenes* isolated from abattoir, butchers and restaurant in Ambo and Holeta towns, central Ethiopia.

Materials And Methods

Study area

The study was conducted in Ambo and Holeta towns found in 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 (masl). 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 [22]. There are 46 legal butchers with their annexed restaurants and one municipality abattoir in Ambo town.

Holeta is located in Finfine special zone, 44 km West of Addis Ababa with 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 [22]. 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. Cattle slaughtered in Ambo and Holeta abattoirs and beef sold in butcher shops and restaurants of Ambo and Holeta towns are the study population.

Study Design

The study was conducted between October 2017 to April 2018 in abattoirs, butchers and restaurants of Ambo and Holeta towns.

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 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% [4] by the following formula [23].

$$n = \frac{1.96^2 \times P_{exp}(1 - P_{exp})}{d^2}$$

Where n = sample size

P_{exp} = Expected prevalence

d = Desired absolute precision

Thus, a sample size of 147 was calculated. Thus a total of 450 meat samples consisting of 150 from abattoirs, 150 from butchers and 150 from restaurants were sampled. For the 20 butchers of Holeta town, 87 samples were assigned (every 29 samples from abattoir, butchers, and restaurants), while the corresponding values for one abattoir, butchers and restaurants (each 46) in Ambo town was 363 (121 samples from each establishment).

Sample collection

Systematic random sampling method was used to select the animals from study population. Following slaughtering, meat samples were collected from three spots of the beef chain (abattoir, butcher, and restaurant) from the same animal. 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, i.e. butcher shops. On the third sampling spot, 250 g raw meat 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 sega*- special meat cut served raw) was purchased. All samples were collected aseptically, and the samples were 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), to prevent spilling and cross-contamination and immediately transported to the Ambo University Veterinary Microbiology Laboratory in a cooler icebox with ice packs and processed within 4 hrs.

Isolation and identification of *Listeria* species

As per the recommendation of ISO [24], 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 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 of *Listeria* species, 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. Subsequently, the cultures showing typical tumbling motility were considered as "presumptive" *Listeria* isolates, which were in turn subjected to detailed biochemical tests viz.; CAMP test, methyl red, Voges-Proskauer, and sugar fermentation tests with xylose, rhamnose, and mannitol for identification of *Listeria* to species level.

Questionnaire Survey

A semi-structured pretested questionnaire survey and observational checklist were used on abattoir personnel, butcher shop workers and restaurant chefs and waiters to assess their hygienic practice in processing and handling beef and beef products along the chain. The independent factors investigated along with their categories include: sex (male vs. female), marital status (single vs. married), the residence of origin (rural vs. urban), educational status (uneducated, primary school, secondary school, and tertiary), time on work per day (≤ 8 hours vs. ≥ 9 hours), information on food hygiene, training on food safety, use of refrigeration, presence of insects and presence of rodents (all yes or. no), sanitation of

butchers and restaurants, hygiene of the slicing materials, hygiene of the cutting boards, hygiene of the weighing balance and food handling surfaces (all categorized as poor, fair, good, and very good).

Antimicrobial Susceptibility Test

The *L. monocytogenes* isolates were subjected to antimicrobial susceptibility test against 16 commercially available antimicrobial disks selected based on common usage. The antimicrobial disc selected were amikacin (30 µg), amoxicillin (10 µg), ampicillin (10 µg), chloramphenicol (30 µg), cefotaxime (30 µg), gentamicin (10 µg), tetracycline (30 µg), oxacillin (1 µg), vancomycin (10 µg), cotrimoxazole (25 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), penicillin (6 µg), and trimethoprim-sulfamethoxazole (23.75 µg). Antimicrobial susceptibility test was conducted using the disk diffusion method following guidelines established by the Clinical and Laboratory Standards Institute CLSI [25]. Two to three pure fresh colonies of the isolates from TSA YE were used to prepare cell suspension in to the Muller Hilton 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 Muller Hinton agar (HiMedia, India). According to the standard procedure CLSI [25], the disks 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 disks was measured with a ruler against black background and compared with standards given by CLSI [25]. *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 utilized to summarize the occurrence, socio demographic characteristics of the respondents and antimicrobial susceptibility data using percentages. Univariable and multivariable logistic regression analyses with their Odd Ratio (OR) and 95% confidence Intervals (95% CI) were used to assess the factors associated with contamination of beef by *Listeria* species. During analysis, for all of the risk factors, the first category of the independent variables (with the lowest percentage) was considered as a reference category. Non-collinear variables were selected with the help of a multicollinearity matrix. Elimination of collinear variable with the poor biological background to explain possible contamination of raw beef by *Listeria* species was used to handle collinear variables. 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 95% confidence interval (CI) was used in all cases and the results were considered significant at $p < 0.05$.

Results

Isolation and identification of *Listeria* species

Out of the total 450 meat samples examined, 128 (28.44%; 95% CI: 24.32–32.86%) were positive for *Listeria* species. The occurrence of *Listeria* species in Ambo town (20.66%) was much lower when compared to Holeta town (60.92%). The highest rate of occurrence of *Listeria* species was recorded in restaurants (30.00%), followed by butchers (29.33%) and then abattoir (26.00%). The occurrence of *Listeria* species in abattoir and restaurant samples of Holeta town was relatively higher than abattoir and restaurant samples of Ambo town (Table 1).

Table 1

The occurrence of *Listeria* species isolated from cattle raw meat from abattoirs, butchers and restaurants of Ambo and Holeta towns, Central Ethiopia.

Study towns	Establishment	Tested	<i>Listeria</i> species		
			No. positive	% occurrence	95% CI [Lower-Upper]
Ambo	Abattoir	121	25	20.66	13.84–28.97
	Butchers	121	22	18.18	11.76–26.22
	Restaurants	121	28	23.14	15.96–31.68
	Sub total	363	75	20.66	16.61–25.20
Holeta	Abattoir	29	14	48.28	29.45–67.47
	Butchers	29	22	75.86	56.46–89.70
	Restaurants	29	17	58.62	38.94 – 76.48
	Sub total	87	53	60.92	49.87–71.21
Subtotal	Abattoirs	150	39	26.00	19.19–33.79
	Butchers	150	44	29.33	22.19–37.31
	Restaurants	150	45	30.00	22.80–38.01
Total		450	128	28.44	24.32–32.86
% = percent, No. = number, CI = Confidence Interval					

Out of the total 128 *Listeria* species isolated, a high contamination rate of *Listeria grayi* and low contamination rate of *Listeria seeligeri* were recorded. The overall occurrence of *L. monocytogenes* was 4.44% (Table 2). The occurrence of *L. monocytogenes* in abattoirs, butchers, and restaurants raw meat samples of cattle were 33%, 1.56%, and 1.56%, respectively.

Table 2
Listeria species identified from cattle raw meat in Ambo and Holeta towns, Oromia, Central Ethiopia (n = 450).

<i>Listeria</i> species	No of positive	Identified <i>Listeria</i> species	
		%	95% CI [Lower-Upper]
<i>Listeria monocytogenes</i>	20	4.44	2.74–6.78
<i>Listeria ivanovii</i>	10	2.22	1.07–4.04
<i>Listeria seeligeri</i>	8	1.78	0.80–3.47
<i>Listeria welshimeri</i>	17	3.78	2.22–5.98
<i>Listeria innocua</i>	28	6.22	4.17–8.87
<i>Listeria grayi</i>	46	10.22	7.58–13.39
Total	128	28.44	24.32–32.86

Risk Factors

Socio-demographic characteristics, knowledge, and practice on food safety and hygiene

All respondents from abattoirs and butchers were male while all respondents from restaurants were females. The majority of the respondents from abattoirs (57.58%) were within the age group of 18–24 years while that of butchers (58.62%) and restaurants (52.41%) were ≥ 35 years and 25–34 years, respectively. The majority of the respondents attended primary education and had less than one year of work experience. Most of the workers in butchers and restaurants work ≥ 9 hours per day (Table 3).

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 in years	18–24	19	57.58	19	13.10	5	3.45
	25–34	11	33.33	41	28.28	76	52.41
	≥ 35	3	9.09	85	58.62	64	44.4
Marital status	Single	14	42.42	55	37.93	77	53.1
	Married	19	57.58	90	62.07	68	46.9
Residence of origin	Rural	26	78.79	54	37.24	82	56.55
	Urban	7	21.21	91	67.76	63	43.45
Religion	Protestant and others	12	36.06	12	8.18	53	36.55
	Orthodox	21	63.64	133	91.72	92	63.45
Education	Illiterate	3	9.09	5	3.45	18	12.41
	Primary school	17	51.52	80	55.17	98	67.59
	Secondary school	6	18.18	14	9.66	-	-
	Tertiary	7	21.21	46	31.72	29	20
Work experience in years	< 1	-	-	93	64.14	73	50.34
	1–3	-	-	19	13.10	47	32.41
	≥ 4	-	-	33	22.76	25	17.24
Working time per day	≤ 8 hours	33	100	33	22.76	46	31.72
	≥ 9 hours	-	-	112	77.24	99	68.28
FrEq. = Frequency.							

The vast majority of respondents had information/knowledge on food hygiene and safety, often without practicing it but had no training on food safety (Table 4).

Table 4

Knowledge and practice on food safety and hygiene of workers of abattoirs, butchers, and restaurants of Ambo and Holeta towns.

Factors	Categories	Abattoirs Workers		Butcher Workers		Restaurants Workers	
		Freq.	%	Freq.	%	Freq.	%
Information on food hygiene & safety	No	3	0.09	34	23.45	20	13.7
	Yes	30	90.91	111	76.55	125	86.21
Training on food safety	Yes	5	15.15	16	11.03	4	2.76
	No	28	84.85	129	88.97	141	97.24
Knowledge of food safety and hygiene	Yes	22	66.67	103	71.03	76	52.41
	No	11	33.33	42	28.97	69	47.59
The practice of food hygiene	No	18	54.55	74	51.03	48	33.1
	Yes	15	45.45	71	48.97	97	66.9
Use of refrigeration	No	33	100	11	7.59	15	10.34
	Yes	-	-	134	92.41	130	89.66
Presence of insects	No	33	100	41	28.28	27	18.67
	Yes			104	71.72	118	61.38
Presence of rodents	Yes	-	-	21	14.48	56	38.62
	No	33	100	124	85.52	89	61.38

Chi-square Analysis

Results of Chi-square analysis showed significant association between cattle raw meat contamination by *Listeria* species and study towns (Chi-square [X^2] = 28.5162, $P = < 0.001$), season ($X^2 = 17.4298$, $P = < 0.001$), number of hours worked per day ($X^2 = 4.4096$, $P = < 0.001$), information on food hygiene and safety ($X^2 = 2.4979$, $P = 0.036$), hygiene of the cutting boards ($X^2 = 12.0199$, $P = 0.007$) and use of refrigerator ($X^2 = 3.9451$, $P = 0.047$). All the other investigated factors were not significantly associated with the isolation rate of *Listeria* species (data not shown) ($P > 0.05$).

Logistic Regression Analyses

Univariable logistic regression analysis showed that the raw cattle meat contamination rate by *Listeria* species was significantly different ($p < 0.001$) between study towns, seasons and working times per day.

Accordingly the risk of cattle raw meat contamination in Holeta town was 4.91 times higher than in Ambo town (OR = 4.91, 95% CI: 2.65–9.07; $p < 0.001$). The risk of *Listeria* species contamination in dry season was 8.78 times higher when compared to the risk of contamination in wet season (OR = 8.78, 95% CI: 2.66–28.99; $P < 0.001$). Similarly, the risk of cattle raw meat contamination was 3.57 times higher in butchers and restaurants where the working hours per day was ≥ 9 hours as compared to butchers and restaurants where the working hours was ≤ 8 per day (OR = 3.57, 95% CI: 1.74–7.36; $p < 0.001$). Univariable logistic regression analysis also showed that the occurrence of *Listeria* species in meat was significantly associated with the hygiene of cutting boards ($p < 0.05$). All the other factors investigated did not show significant association with the risk of cattle raw meat contamination ($p > 0.05$) (Table 5).

Table 5

Univariable logistic regression analysis of factors for contributing for isolation of *Listeria* species from cattle raw meat in butchers and restaurants of Ambo and Holeta towns, central Ethiopia

Factor	Category	No. tested	No. positive (%)	OR (95% CI)	p-value
Source	Butchers	145	40 (27.59)	1.0	-
	Restaurants	145	42 (28.97)	1.07 (0.64–1.78)	0.794
Study town	Ambo	234	50 (21.37)	1.0	-
	Holeta	58	32 (57.14)	4.91 (2.65–9.07)	≤ 0.001
Season	Wet	55	3 (5.45)	1.0	-
	Dry	235	79 (33.62)	8.78 (2.66–28.99)	≤ 0.001
Sex of food handlers	Female	145	40 (27.59)	1.0	-
	Male	145	42 (28.97)	1.07 (0.64–1.78)	0.794
Age of food handlers in years	≥ 35	24	5 (20.83)	1.0	-
	18–24	117	29 (24.79)	1.25 (0.43–3.65)	0.681
	25–34	149	48 (32.2)	1.81 (0.64–5.13)	0.267
Marital status	Single	132	32 (24.24)	1.0	-
	Married	158	50 (31.65)	1.45 (0.86–2.43)	0.164
Residence of origin	Rural	136	34 (25)	1.0	-
	Urban	154	48 (31.17)	1.36 (0.81–2.28)	0.245
Religion	Protestant and others	65	17 (26.15)	1.0	-
	Orthodox	225	65 (28.89)	1.15 (0.61–2.14)	0.666
Education	illiterate	234	50 (21.37)	1.0	-
	Primary school	58	32 (57.14)	1.02 (0.38–2.73)	0.974
	Secondary school	14	4 (28.57)	1.13 (0.26–5.01)	0.869

Factor	Category	No. tested	No. positive (%)	OR (95% CI)	p-value
	Tertiary	75	25 (33.33)	1.42 (0.50-4,04)	0.514
Work experience in years	1-3	166	46 (27.71)	1.0	
	< 1	66	19 (28.79)	1.05 (0.56-1.98)	0.869
	≥ 4	58	17(29.31)	1.08 (0.56-2.09)	0.816
Working time per day	≤ 8 hours	79	10 (12.66)	1.0	0.001
	≥ 9 hours	211	72 (34.12)	3.57 (1.74-7.36)	

Table 5
Continued...

Factor	Category	No. tested	No. positive (%)	OR (95% CI)	p-value
Information on food hygiene & safety	No	54	9(16..67)	1.0	-
	Yes	236	73 (30.93)	2.24 (1.04–4.82)	0.039
Training on food safety	Yes	120	4 (20.00)	1.0	-
	No	270	78 (28.89)	1.63 (0.53–5.01)	0.398
Knowledge of food hygiene and safety	Yes	179	48 (26.82)	1.0	-
	No	111	34 (30.63)	1.21 (0.72–2.03)	0.483
The practice of food hygiene and safety	Yes	122	33(27.05)	1.0	-
	No	168	49 (29.17)	1.11 (0.66–1.87)	0.693
Site of Butchers and Restaurants	Near asphalt	216	61 (28.24)	1.0	-
	Gravel road	74	21 (28.38)	1.01 (0.56–1.81)	0.982
Sanitation of Butchers and Restaurants	Very good	16	1 (6.25)	1.0	-
	Good	30	30 (29.13)	6.16 (0.78–48.77)	0.085
	Fair	45	45 (29.22)	6.95 (0.79–48.29)	0.082
	Poor	6	6 (35.29)	8.18 (0.86–78.05)	0.068
Hygiene of the Slicing materials	Very good	16	1 (6.25)	1.0	-
	Good	100	26 (26.0)	5.27 (0.66–41.89)	0.116
	Poor	13	41 (30.77)	6.95 (0.89–54.09)	0.064
	Fair	161	51 (31.68)	6.67 (0.64–69.34)	0.112
Hygiene of the Cutting boards	Poor	23	1 (4.35)	1.0	-

13527(27.41)

OR = Odd Ratio, CI = Confidence Interval, No. = number, prev. = prevalence

Factor	Category	No. tested	No. positive (%)	OR (95% CI)	p-value
	Very good	108	36 (33.33)	1.47 (0.08–25.32)	0.792
	Fair	16	1 (6.25)	9.56 (1.25–73.18)	0.030
	Good	142	43 (30.28)	11.00 (1.43–84.90)	0.021
Hygiene of the Weighing balance	Poor	19	2 (10.53)	1.0	-
	Very good	30	6 (20.00)	1.51 (0.57–3.99)	0.405
	Good	135	27 (27.41)	2.14 (0.81–5.71)	0.127
	Fair	106	37 (34.91)	2.13 (0.38–11.83)	0.389
Hygiene of the Food handling surfaces	Very good	20	2 (10.00)	1.0	-
	Poor	19	3 (15.79)	1.69 (0.25–11.42)	0.592
	Fair	135	40 (29.63)	3.79 (0.84–17.10)	0.083
	Good	116	37 (31.90)	4.22 (0.93–10.12)	0.062
Use of refrigeration	No	26	3 (11.54)	1.0	
	Yes	264	79 (29.97)	3.27 (0.96–11.22)	0.059
Presence of insects	No	68	18 (26.47)	1.0	
	Yes	222	64 (28.83)	1.13 (0.61–2.08)	0.706
Presence of rodents	Yes	77	21 (27.27)	1.0	
	No	213	61 (28.64)	1.07 (0.60–1.92)	0.820
OR = Odd Ratio, CI = Confidence Interval, No. = number, prev. = prevalence					

The following variables were included in the multivariable logistic regression analysis model: study towns, season, marital status, time on work per day, information on food hygiene and safety, hygiene of food handling surfaces and use of refrigeration. All independent variables are non-collinear with each other as evidenced from the multicollinearity matrix except hygiene of the slicing material vs. sanitation

of butchers and restaurants [$r = 0.76$], hygiene of weighing balance vs. hygiene of the slicing material [$r = 0.56$] and food handling surfaces vs. hygiene of cutting boards [$r = 0.63$]. These collinear variables were not entered into the multivariable model, although hygiene of the slicing material vs. sanitation of butchers and restaurants has more biological ground to explain raw meat contamination by the *Listeria* species. Multivariable logistic regression analysis revealed that study town and season are independent predictors of contamination of meat by *Listeria* species ($p < 0.05$). Information on food hygiene and safety ($p = 0.054$), and the number of hours worked per day ($p = 0.079$), though not significantly associated with the prevalence of *Listeria* species, they are close for association (Table 6).

Table 6

Multivariable logistic regression analysis of predictors of *Listeria* species isolation from cattle raw meat of butchers and restaurants in Ambo and Holeta towns, Central Ethiopia.

Risk factors	Category	OR (95% CI)	p-value
Study town	Ambo	1.0	-
	Holeta	3.80 (1.78–8.13)	≤ 0.001
Season	Wet	1.0	-
	Dry	5.41 (1.56–18.78)	0.008
Marital status	Single	1.0	-
	Married	1.49 (0.81–2.74)	0.195
Working time per day	≤ 8 hours	1.0	-
	≥ 9 hours	2.07 (0.92–4.63)	0.079
Information on food hygiene & safety	No	1.0	-
	Yes	2.25 (0.99–5.12)	0.054
Hygiene of the Food handling surfaces	Very good	1.0	-
	Poor	1.56 (0.20–12.11)	0.668
	Fair	1.76 (0.34–9.01)	0.497
	Good	1.31 (0.25–6.92)	0.747
Use of refrigeration	No	1.0	-
	Yes	3.03 (0.78–11.87)	0.111
Assessment of model fitness to the observed data indicated that observed and predicted values were not significantly different, i.e. the data fitted the model well (Hosmer-Lame show $X^2 = 0.76$, $p = 0.6843$). The reliability of the fitted model has been further evaluated using the ROC curve (ROC = 0.7296). The 95% CI of best-fitting variables into the model with $p \leq 0.05$ are presented in Table 7.			

Table 7

Best fitting model for prediction of *Listeria* species isolation from cattle raw meat of butchers and restaurants in Ambo and Holeta towns, Central Ethiopia.

Risk factors	Category	OR (95% CI)	P-value
Study town	Ambo	1.0	-
	Holeta	3.47 (1.84–6.54)	≤ 0.001
Season	Wet	1.0	-
	Dry	5.48 (1.62–18.56)	0.006
Working time per day	≤ 8 hours	1.0	-
	≥ 9 hours	2.32 (1.08–4.96)	0.030

OR = Odd Ratio, CI = Confidence Interval, With HL $X^2 = 0.76$, P -value = 0.6843 ROC = 0.7296.

Antimicrobial Susceptibility

Out of the 20 *L. monocytogenes* isolates subjected against 16 commercial antimicrobial discs. 16 isolates (80%) were resistant to oxacillin; 14 (70%) were resistant to amikacin and nalidixic acid; 12 (60%) were resistant to chloramphenicol, 11 (55%) were resistant to tetracycline. The *L. monocytogenes* isolates were 95%, 90% and 85% susceptible to amoxicillin, vancomycin, and clindamycin, respectively (Table 8).

Table 8

Results of the antibiogram of *L. monocytogenes* isolates (n = 20) from raw cattle meat samples of the abattoirs, butchers, and restaurants

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

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 antimicrobial (multidrug resistance- MDR). One isolate (5%) had developed resistance to ten classes of antimicrobials. The most common MDR pattern was observed against amikacin, ceftoxamine, nalidixic acid and Oxacillin. Multidrug resistance of *L. monocytogenes* isolates, number of isolates and percentages resistance as shown below (Table 9).

Table 9
Antimicrobial resistance patterns of *Listeria monocytogenes* (n = 20)

Antimicrobials resistance pattern	No of resistant isolates	%
OXA-AMK	1	5
AMP-CIP-CTR	1	5
OXA-AMP-TET-CHL	1	5
OXA-NAL-CLI-CTR	1	5
XST-PEN-AMP CIP-ERY	1	5
XST-GEN-AMK-NAL-ERY	1	5
OXA-NAL-CIP-ERY-CTR	1	5
OXA-AMK-CTR-TET-CHL	1	5
XST-GEN-AMK-NAL-CTR	1	5
XST-OXA-AMK-CTR-CHL	1	5
OXA-AMK-NAL-CIP-CLI-CTR	1	5
OXA-AMK-NAL-CTR-TET-CHL	1	5
OXA-GEN-AMK-NAL-CTR-TET-CHL	1	5
OXA-AMK-NAL-CIP-CTR-TET-CHL	1	5
OXA-PEN-AMK-NAL-CTR-TET-CHL	1	5
OXA-PEN-GEN-AMK-NAL-CTR-TET-CHL	1	5
XST-OXA-GEN-NAL-ERY-CTR-TET-CHL	1	5
OXA-PEN-GEN-AMK-NAL-ERY-CTR-TET-CHL	1	5
XST-OXA-NIT-AMP-AMK-NAL-VAN-TET-CHL	1	5
OXA-PEN-AMX-AMP-GEN-AMK-NAL-CIP-ERY-CLI-VAN-CTR-TET-CHL	1	5
	20	100
AMK-Amikacin, AMP-Ampicillin, AMX-Amoxycillin, 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,		

Discussion

Occurrence of *Listeria* species in raw beef from abattoir, butcher and restaurants

In the present study, *Listeria* species was isolated from 28.44% of raw beef samples from abattoir, butcher, and restaurants which agrees with the reports from Addis Ababa (27.5%) [3]. Other studies have reported significantly higher isolation rates of *Listeria* species in raw meat such as 95% in Brazil [26], 81.5% in Turkey [27], 58% in Nigeria [28], 54.1% in Turkey [29], 51.3% in Ethiopia [21], and 50% in Jordan [30].

The 4.44% isolation rate of *L. monocytogenes* in this study was comparable with the 4.1% from goat meat in Ethiopia [7] and 4.1% in raw beef in Ethiopia [3]. However, the current finding was higher when compared to the 1.29% isolation rate from raw cow and goat meats [31], 1.6% from minced beef [20] and 2.5% from bovine carcasses in Poland [32]. The present finding of *L. monocytogenes* (4.4%) was lower when compared to the 15.6% [33] in Nordic countries, 15.4% in Bangkok [34], 25.5% in Turkey [27]. The relatively low prevalence of *L. monocytogenes* in present study might be attributed to the difference in the study season, geographic conditions, and sample size [35, 36].

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 [37]. The isolation rate of other *Listeria* species in the present study ranged from 1.78% – 10.22%. The 2.22% 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, Mohammed and Abdalla [38] 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 [4]. The 3.77% isolation rate of the *L. welshimeri* in this study is in agreement with the 2.75% [16], and 4% [4] reported previously. On the other hand, the 1.78% isolation rate of *L. seeligeri* in the present study disagrees with the 27% isolation rate of *L. seeligeri* by Al-Nabulsi *et al* [16] but it is in line with the 1% and 2% isolation rate of *L. seeligeri* previously reported by [38] and [4], respectively. The lower occurrence 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 differences in the bacteriological detection methods [4, 38, 39].

Most research reports indicate that *L. innocua* is the most common species isolated from different meat samples. For example, in Ethiopia, 83% [3] and 19% of *L. innocua* [4] have been reported. But, in this study, *L. grayi* (10.22%) and *L. innocua* (6.22%) 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 rate of *L. grayi* (10.22%) and *L. innocua* (6.22%) isolation in the current study is in line with the results of Fissiha [40] in Ethiopia. The abundance of *L. grayi* and *L. innocua* in the present study might be related to the species entrance to the processing environments via as a part of intestinal microbial flora, improper hygienic practices during processing and food handling [41, 42].

Risk Factors Assessment

The occurrence of *Listeria* species in cattle raw meat was nearly 9 times higher in the dry season when compared to the wet season. The significantly high rate isolation of *Listeria* species in cattle raw meat during 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 [43]. 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 [44]. In addition, the shortage of water during dry season might impair 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, the significantly high occurrence of *Listeria* species in butchers and restaurants where the employees work ≥ 9 hrs as compared to those working ≤ 8 hrs might be related to the change in the hygienic behavior of works as they get exhausted/tired. This is the first report indicating that employees working ≥ 9 hrs per day in handling beef are important source of beef contamination in the study areas.

In the present study, the place where the study was carried out was the predictor of *Listeria* species isolation ($p \leq 0.001$). The significantly high recovery rate of *Listeria* species from Holeta (57.14%) as compared to Ambo (21.37%) town might be related to the cool temperate climate of Holeta town favorable for the survival and multiplication of the organisms.

This study showed significant association between the risk of cattle raw meat contamination by *Listeria* species and information on food hygiene and safety. This finding is inconsistent with the reports of E De Boeck *et al* [45] who suggested a significant contribution of the lack of knowledge on food hygiene and practice for high risk of contamination. In contrary to this, the results of the present study showed that less adoption of information on food hygiene and practice as a habit or culture every time during working hours by employees or workers. Thus, it may contribute to the high risk of cattle raw meat contamination by *Listeria* species. Moreover, the absence of law about HACCP (Hazard Analysis Critical Control Point) programs, its implementation, and the widespread inadequate hygienic practices in Ethiopia coupled with only having knowledge on food hygiene and safety without its practice or implementation might be regarded as a contributing factor for meat contamination.

Univariable logistic regression analysis also identified the hygiene of cutting boards as a risk of cattle raw meat contamination by *Listeria* species. This is in line with the findings of previous workers [46–48]. The types or quality, poor hygiene and absence of frequent sanitation of the cutting boards and use of unclean water for washing cutting boards might be linked to a higher chance of cutting boards being contaminated with *Listeria* species [48].

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 [32] and [49]. While, the 70% resistance to amikacin is in contrary to the 100% susceptibility reported by Indrawattana [34]. The resistance against nalidixic acid and tetracycline in this study are in accord with the reports of Maktabi *et al* [35].

The 30% resistance to ciprofloxacin of this study is lower compared to the 44.4% [50] and 56% resistance [51]. The 30% resistance to erythromycin and trimethoprim-sulfamethoxazole disagrees with 87.5% [51] and 69.4% [50] resistance. Although 35% resistance to trimethoprim-sulfamethoxazole and gentamicin in the present study agrees with the 24% [35] and 36.1% [50] resistance, it contradicts with the 87.5% trimethoprim-sulfamethoxazole and 72.21% gentamicin resistance [51]. The 15% resistance to clindamycin is in line with the 12% resistance reported by Maktabi *et al* [35]. However, the 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 [4]. The low level of resistance (5–15%) to amoxicillin, ceftoxamine, vancomycin and clindamycin in this study might be due to absence of usage of these drugs in veterinary medicine in Ethiopia which plausibly suggests that these antimicrobials remain an alternative regimen against the organisms [4, 52]. While the relatively high resistance, ($\geq 30\%$) observed in erythromycin, chloramphenicol, trimethoprim-sulfamethoxazole, oxacillin, tetracycline, gentamycin, 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 antibiogram for this organism.

The present rate of MDR *L. monocytogenes* (95%) is higher when compared to the 72.3% MDR identified from raw foods [53]. In agreement with the present study Odu and Okonko [8] 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 [4]. The MDR to *L. monocytogenes* may occur due to plasmid or chromosomal genes transfer and mutation events in chromosomal genes from other *Listeria* species and Gram-positive bacteria, which may be found in foods [53, 54].

The high percentage of *L. monocytogenes* isolates resistant to the relatively cheaper and commonly available antimicrobials is worrisome as it might lead to the use of mandatory and more expensive drugs [4]. This is a problem for listeriosis high-risk groups in developing countries like Ethiopia because of 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 should be considered as a microbiological hazard for people since the consumption of raw or undercooked meat in the study area 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 works 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 was not included in the logistic regression analysis due to the small number of workers (n = 33) who responded to our questions and the difficulty of linking responses of workers to a particular sample result as all worker handle the carcasses. This makes comparison of the results from abattoirs with that of butchers and restaurants hard.

Conclusions

The study confirmed that contamination of raw meat sold for human consumption by *Listeria* species is widespread in the area. Six *Listeria* species (*Listeria monocytogenes*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria welshimeri*, *Listeria innocua* and *Listeria grayi*) were identified. There overall occurrence of *Listeria* species was medium while the isolation rate of *L. monocytogenes* was relatively low. The study towns, season and working hours per day are independent predictors of *Listeria* species isolation. The high antimicrobials resistance and multidrug resistance among *L. monocytogenes* isolates are 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, regular training should be given for worker 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.

Abbreviations

AIDS: acquired immune deficiency syndrome; CAMP: Christie, Atkins, and Munch-Peterson test; CDC: Center for disease control and prevention; CLSI: Clinical and laboratory standards institute; EC: European commission; ECDC: European Centre for Diseases Prevention and Control; ERS: Economic Research Service; EU: European Union, HACCP: Hazard Analysis and Critical Points; ISO: International Organization for Standardization; LEB: Listeria Enrichment Broth; MDR: Multi Drug-Resistant; OR: Odds Ratio; OXA: Oxford Agar; RTE: Ready-to-eat; TSYEA: Trypticase–soy yeast extract agar; UNEP: United Nations Environmental Protection.

Declarations

Availability of data and materials

The raw datasets used during the current study can be obtained upon the reasonable request of the corresponding author. The questionnaire used during the current study is available as Additional file 1.

Ethics approval and consent to participate

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.

Consent for publication

Not applicable

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Authors' contributions

EZG conceived the idea, designed the project, supervised the work, analyzed and interpreted the data and drafted and approved the final manuscript for publication. GA performed the laboratory tests, participated in drafting the article and in approval of the final version for publication. BMB, EJS and HAA designed the project, supervised the work, revised the manuscript and approved the final article for publication. LMM and NDT participated in data collection, field and laboratory supervision, gave comments on the manuscript and approved the final version for publication.

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Declaration of interests

The authors declare that they have no conflicts of interest.

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