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ORIGINAL ARTICLE

Studies on the Prevalence, Risk Factors, Public Health Implications and Antibiogram of *Listeria monocytogenes* in Sheep Meat Collected from Municipal Abattoir and Butcher Shops in Addis Ababa

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Abstract

Listeria monocytogenes, the chief cause of listeriosis, is one of the important emerging food-borne bacterial zoonotic pathogens of global significance. The present study was undertaken to determine the presence of *L. monocytogenes* in raw meat of market and abattoir. A cross-sectional study was conducted from October 2013 to April 2014 to isolate *L. monocytogenes* from swab samples on sheep meat from abattoir, butcher shops, equipments, and also to determine antibiotic resistance profiles of the isolates. A total of 873 swab samples comprising of 384 from the abattoir and 384 from butcher shops were obtained aseptically using systematic random sampling technique, and 105 swabs were collected from equipments. Questionnaire survey was conducted to assess the hygienic practices of meat production in raw meat of market and abattoir, and possible risk factors regarding the contamination of sheep meat. *Listeria monocytogenes* was isolated and identified using standard bacteriological techniques. Antimicrobial susceptibility test was also conducted on 36 isolates of *L. monocytogenes*. The overall prevalence of *L. monocytogenes* was 4.1%; and the prevalence of 2.1%, 5.5%, and 6.7% was recorded from abattoir, butcher shops, and equipments, respectively. The study also revealed multi-drug resistant isolates in 24/36 (66.7%) of two or more antimicrobials. In addition, the presence of *L. monocytogenes* attributed to unclean working environment and improper handling of meat, till it reaches to the consumer. Preventive measures to avoid the presence of pathogenic *L. monocytogenes* in raw meat and meat products should be undertaken, emphasizing the need for improved hygienic practices during meat production, and also during distribution, and consumption of the final products.

Keywords: Abattoir, Addis Ababa, Antimicrobial susceptibility, Hygienic practice, *Listeria monocytogenes*, Prevalence, Sheep meat.

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1. Introduction

Animals are important as producers of meat, milk, and eggs, which are part of the food chain and provide high value of protein food. They have long played a key role in supplying calories, and protein for human food in virtually all parts of the world, both directly in the form of animal products, and indirectly from the contribution of manure and draught power to crop production and generation of income to enable purchase of food (ESAP, 2001). Animals naturally harbor many food-borne bacteria in their intestines that can cause illness in humans, but often do not cause illness in the animals. During slaughter, meat and poultry carcasses can become contaminated, if they are exposed to small amounts of intestinal contents (Pal, 2015; Pal and Mahendra, 2015).

Food safety has emerged as an important global issue with international trade and public health implications. *Listeria monocytogenes* associated with outbreaks have been reported from a wide variety of foods (Pal, 2013; Pal and Awel, 2014). The bacterium has been isolated from meat, poultry, milk, cheese, and other dairy products, and vegetables (Antunes *et al*., 2002; Kumar, 2011; Khan *et al*., 2013; Pal and Awel, 2014; Pal, 2015). *L. monocytogenes* is a significant pathogen of food safety concern, as it can induce

disease in humans, and can be transferred to food products derived from animals (Hassan *et al*., 2000; Pal, 2013).

Food-borne pathogens are the leading cause of illness and death in many countries of the world costing billions of dollars in medical care and medical and social costs (Pal and Mahendra, 2015). Changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement, and poor hygiene practices are major contributing factors (Nafisa *et al*., 2010; Pal and Mahendra, 2015). The modern food industry aims to decrease the use of preservatives, and to increase shelf lives, so the food safety is widely based on the cold chain. Even though temperatures lower than optimum decrease growth rates in all bacteria, growth inhibition of *L. monocytogenes* is not complete until temperature is below 0°C (Markkula, 2013).

Food safety is one of the leading issues for the agricultural industry, for both livestock and producers of the property that are influencing consumer demand for meat products, and therefore, microbiological safety is considered as very important factor. Listeriosis is one of the important emerging food-borne bacterial zoonotic diseases of worldwide distribution (Pal, 2007; Gebretsadik *et al*., 2011; Kumar, 2011; Pal and Mahendra, 2015). It affects mainly the infants, pregnant women, elderly, and immunocompromised individuals (Pal, 2015). It is associated with the highest case fatality rate of 30% approximately, unlike infection with other common food-borne pathogens (Khan *et al*., 2013).

Information on the occurrence and distribution of *L. monocytogenes* and other *Listeria* species is very limited both in the veterinary and public health sectors in Ethiopia (Gebretsadik *et al*., 2011). In developing countries, there have been very few or no reports on *L. monocytogenes.* This might be true because no one has given it due attention or were unaware of its occurrence (Molla *et al.*, 2004). The present study was contemplated with the objectives to determine the prevalence of *Listeria monocytogenes* in meat of sheep, to conduct the antibiotic susceptibility of the isolates of *L. monocytogenes* from sheep meat, and to elucidate the risk factors, and the public health implications of *L. monocytogenes* in butchers and meat handlers.

2. Materials and Methods

2.1 Study Area

The study was carried out in Addis Ababa Central Ethiopia. Addis Ababa is the capital city of Federal Democratic Republic of Ethiopia, and it has an area of 51,000 hectare in the central highlands with an average altitude of 2000-2560 meters above sea level. The area is characterized by bimodal rainfall with an average of 1100 mm, the highest percentage of rain falls during the long rainy season from June to September. The short rainy season is from February to April. Addis Ababa has an estimated human population of 3.15 million (CSA, 2007).

2.2 Study Abattoir and Origin of Samples

Addis Ababa Abattoir Enterprise was established before 65 years ago, and is located at the heart of Addis Ababa. The abattoir has different components such as slaughter hall, chilling room, detention meat room, condemned meat room, hide and skin room, veterinary office, water supply (cold and warm), electric generator, vehicles and incinerator. The abattoir has six separate slaughter halls, three for bovine, two each for ovine and caprine, and one for swine. The abattoir is a high output abattoir in the country providing 50% of the daily beef requirements of the city's residents. Most of the cattle slaughtered at the abattoir are adult males of local Zebu, through lesser numbers of crossbred males, calves as well as culled dairy cows. Species of animals slaughtered included bovine, ovine, caprine, and swine (CSA, 2007). In the abattoir, regular meat inspection is being conducted by meat inspector as well as veterinarians from Ministry of Agriculture. The abattoir has both clean and dirty areas, so that after skinning and evisceration, carcass follows the clean lines until inspection and transporting while those offal, skin etc, to dirty areas as by product preparation, like pet animal feeds, hide, and skin for sale as well as, those unfit ones and condemned organs to incinerators for burning (CSA, 2007).

The swab samples were collected aseptically from sheep meat from Addis Ababa Abattoir Enterprise and butcher shops located in the city. In addition, swabs were also collected from equipments like knives, cutting tables, and hooks.

2.3 Study Population and Sample Size Determination

The study population represented sheep meat and equipments such as knives, cutting tables, and hooks. The approximate sample size required was determined from expected prevalence of 50% with defined precision of 5% and level of confidence of 95% (Thrusfield, 2005).

$$n = \frac{1.96^2 \, Pexp \, (1-Pexp)}{d^2}$$

Where, n = required sample size
Pexp = expected prevalence
d = desired absolute precision

Therefore, by using estimated prevalence of 50% in raw meat of sheep and taking a confidence interval of 95% and 5% absolute precision, the minimum sample size required for this study was 768 sheep meat swab samples. A total of 873 samples comprising of 384 sheep meat swabs from the butcher shops, 384 sheep meat swab from the Addis Ababa Abattoir Enterprise were used for the study. In addition, 105 swabs were also obtained from equipments like knives, cutting tables and hooks (Table 1).

Table 1: Distribution of the type and number of samples collected

Type of sample	Number of samples
Sheep meat swabs	768
Knives	40
Cutting tables	45
Hooks	20
Total	873

2.4 Study Design

A cross-sectional study was conducted to determine the prevalence of *L. monocytogenes* and antibiotic susceptibility test from September 2013 to May 2014 in sheep meat slaughtered at Addis Ababa Abattoir Enterprise, and meat presented for sale in different butcher houses in the city. Each sample was brought at the Food Microbiology Laboratory of Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. On each sampling day, all the required samples (sheep meat swab sample, knives, cutting tables, and hooks) were taken for isolation of the bacterium.

2.5 Sampling Techniques

In Addis Ababa Municipal Abattoir, the maximum numbers of animals are slaughtered on Wednesday and Friday, and mainly during holidays. Carcasses were examined just after evisceration before washing. The meat was swabbed without distinction of race, sex or age at Addis Ababa Abattoir Enterprise, and different butcher shops during several visits. The carcasses were chosen in a systematic random sampling method and examined just after the stage of evisceration. And for the butcher shops, convincing sampling type was performed, and the samples were taken only from the sites where they were sold to the consumers.

All samples were collected aseptically using disposable gloves to avoid contamination, and the samples were labeled with necessary information including the date of sampling, sample code and sample type. The selected meat was swabbed aseptically using the method described in ISO11290-1 (1996) by placing sterile template (10 x 10 cm) on specific sites of a carcass. A sterile cotton tipped swab (2x3 cm) fitted with shaft, was first soaked in an approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England) rubbed first horizontally, and then vertically several times on the carcasses. The abdomen (flank), thorax (lateral), crutch, and breast (lateral), which were sites with the highest rate of contamination, was chosen for sampling. On completion of the rubbing process, the cotton swab was left in the test tube. Finally, the carcass swabs taken was kept in a transport medium (buffered peptone water), and transported to the Food Microbiology of Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia for microbiological analysis. Up on arrival, the samples were stored in refrigerator at 4°C.

2.6 Isolation and Identification of *Listeria monocytogenes*

The techniques recommended by the French Association for Standardization (AFNOR,1993) and International Standards Organization (ISO 11290-1, 1996) were employed for the isolation and identification of *L. monocytogenes*.

2.6.1 Primary Selective Enrichment

Each sample kept in buffered peptone water was mixed thoroughly to ensure the homogeneity of its contents, and about 0.1 ml was obtained aseptically into 10 ml of prepared *Listeria* Enrichment Broth (LEB) followed by mixing, and the sample was kept in the incubator and incubated at 30^{o}C for 48 hrs.

2.6.2 Secondary Selective Enrichment

The secondary selective enrichment medium with full concentration of selective agents was employed. From the pre-enrichment culture (Listeria Enrichment Broth), after being well mixed 0.1 ml was transferred into 10 ml of Half Fraser broth, and was incubated at 35°C for 24 hours.

2.6.3 Isolation and Identification

From Half Fraser Broth showing black color, a loopful of the culture was streaked onto PALCAM agar plates and OXA agar plates and incubated at 37^{o}C for 24 to 48 hours. Identification of *Listeria* species on PALCAM agar plates was based on aesculin hydrolysis and mannitol fermentation. All *Listeria* species hydrolysed aesculin as evidenced by a blackening of the medium. Mannitol fermentation was demonstrated by a color change in the colony and/or surrounding medium from red or gray to yellow due to the production of acidic end products. The selectivity of the PALCAM medium is achieved through the

presence of lithium chloride, polymixin B sulphate, and acriflavine hydrochloride present in the medium base and ceftazidime provided by PALCAM antimicrobial supplement. These agents effectively suppress the growth of most commonly occurring non-*Listeria* species of bacteria present in food samples. On PALCAM agar, typical colonies were grey-green with a black sunken center and a black halo, and on Oxford agar, colonies appeared brown black or greenish black with a depressed center and a surrounding black halo.

2.6.4 Confirmation

Colonies suspected to be *Listeria* was transferred onto Tryptose yeast extract agar plate (TSYEA), and incubated at 37^{o}C for 18 to 24 hours. The colonies assumed to be *Listeria* were characterized by using Gram's staining, characteristics of hemolysis, carbohydrate utilization, and CAMP (Christie Atkins Munch Peterson) test following standard methods (AFNOR, 1993; ISO 11290-1, 1996).

The CAMP test was undertaken using *Staphylococcus aureus* (CIP: Collection of Institute of Pasteur, 5710). It was streaked vertically in a single line across a sheep blood agar plate and *Listeria* isolates horizontally to *S. aureus* streak. The plates were incubated at 37^{o}C for 18 to 24 hours. An enhanced zone of beta hemolysis between the test strain and culture of *S. aureus* was considered a positive reaction (ISO 11290-1, 1996). *L. monocytogenes* showed an enhanced zone of hemolysis, forming a narrow head towards the culture of *S. aureus*. For the carbohydrate utilization test, single isolated colony from TSYEA was transferred into test tubes containing xylose, rhamnose, and mannitol, and incubated at 37^{o}C for up to 5 days. Positive reactions were indicated by yellow color (acid formation).

2.7 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed for *L. monocytogenes* and other *Listeria* isolates by using Muller Hinton Agar. The common conventional antimicrobial drugs such as amoxyclav, chloramphenicol, ampicillin, streptomycin, tetracycline, vancomycin, ciprofluxcin, gentamicin, sulfamethtrimethoprin, penicillin, co-trimexazole, oxacillin, and clindamycin were tested. The method applied for antimicrobial testing was agar plate antibiotic disk diffusion method, using Kirby-Bauer technique by 0.5 McFarland Standard (Mac Gowan *et al.,* 1990; Antunes *et al*., 2002; Hansen *et al*., 2005). Two pure colonies of the isolate was taken from the tryptone yeast extract agar and suspended in Muller Hinton Broth (MHB) and then, incubated at 37^{o}C for 1-2 hrs. The suspension was checked for the development of slight turbidity. It was inoculated, by dipping a sterile cotton swab into it and wiping on the Muller Hinton agar, according to the standard procedure (NCCLS), and then the antimicrobial discs was firmly placed on it, and the plates were incubated at 37^{o}C for 24 hrs. The results were interpreted in accordance with the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2004). The resistance profile of the strains was reported according to the abbreviation for the antibiotics to which they showed resistance.

2.8 Questionnaire Survey

Questionnaire survey was conducted to the meat value chains in the study sites, and a detailed and organized questionnaire format was designed. A structured questionnaire were prepared and pre-tested; and 50 butchers and 50 abattoir workers were surveyed. The questions and answers were written in English.

2.9 Data Management and Analysis

The data obtained through questionnaire survey, and laboratory results of the samples were entered into databases using Microsoft Excel computer program and analyzed using SPSS version 20 statistical computer software programs. Descriptive statistics were used to describe the nature and the characteristics of the data. Comparisons between the prevalence of groups were analysed by using Chi-square (χ^2) test. For all tests, p-value less than 0.05 were considered to be significant.

3. Results

3.1 Prevalence of *Listeria monocytogenes* in Abattoir and Butcher Shops

From a total of 873 samples examined, the overall prevalence of *L. monocytogenes* was recorded 4.1% (Fig 2). The prevalence rate of *L. monocytogenes* varied between sample sources. Out of each 384 samples collected from the abattoir and butcher shops, the prevalence of *L. monocytogenes* were 2.1%, and 5.5%, respectively. The result was higher in butcher shops than abattoir, and there was significant difference in the prevalence of *L. monocytogenes* from these sources of samples ($p<0.05$) (Table 3). Out of 105 equipment samples obtained from both abattoir and butcher shops, the prevalence of *L. monocytogenes* was 6.7%. There was no significant difference in the prevalence of *L. monocytogenes* both in case of abattoir and butcher shops ($p>0.05$).

An overall prevalence (4.1%) of *L. monocytogenes* was demonstrated in different sample sources when they were analyzed together (Table 2). Cutting table was found to have the highest prevalence

Table 2: Overall prevalence of *Listeria monocytogenes* from different source of samples

Sample type	No. Examined	Prevalence (%)	95 % CI
Abattoir	384	8 (2.1) a	0.1-4.1
Butcher	384	21(5.5) ab	3.5-7.5
Cutting table	45	4 (8.9) ab	3.1-14.7
Hook	20	(0.0) b	-
Knife	40	3 (7.5) a	1.3-13.7
Total	873	36 (4.1)	-

*^{ab} *Proportions (%) with similar letters are not statistically significant (with p-value = 0.05), CI= Confidence interval; %= Percent of prevalence*

Table 3: Prevalence of *Listeria monocytogenes* from different sources of samples

Source of sample	No. of examined	Prevalence (%)	OR	CI of OR	χ^2	p-value
Abattoir	384	8 (2.1)	1	-	-	-
Butcher	384	21 (5.5)	2.7	1.2-6.2	6.1	0.02
Total	768	29 (3.8)	-	-	-	-

OR= odds ratio; CI= confidence interval; χ^2= Chi square

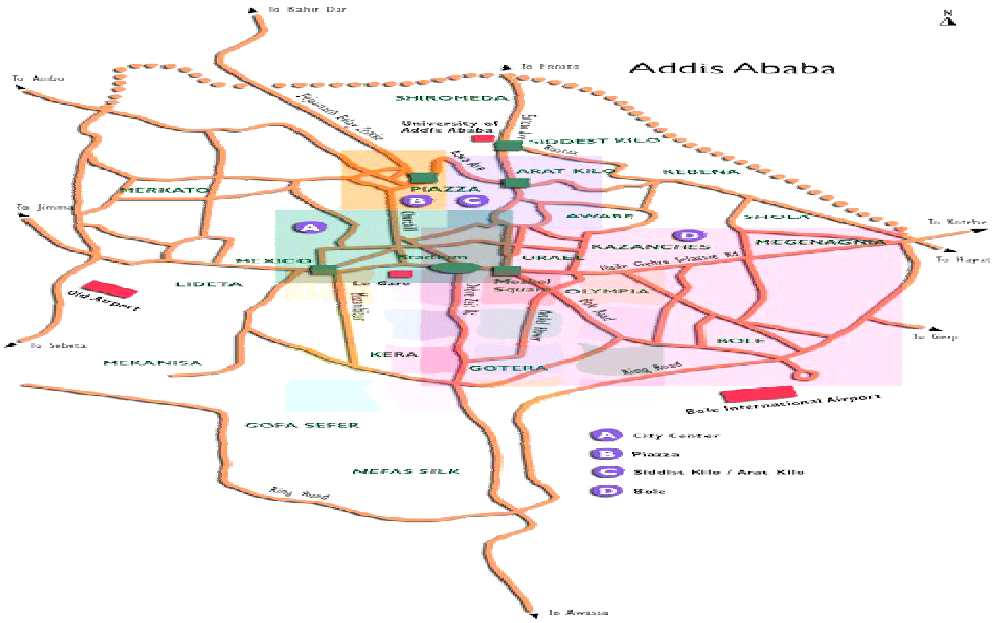

Fig 1: Map of Addis Ababa, the capital of Ethiopia

(8.9%) followed by knife (7.5%) (Fig 2). The least prevalence was found in hook (0.0%) that had statistically significant difference comparing with the others items. Though there was difference in the prevalence among the others samples (abattoir, butcher, cutting table, and knife), it was not statistically significant.

3.2 Prevalence of *Listeria monocytogenes* in Abattoir and Butcher shops

Out of 768 swab samples examined during the study period, 29 (3.8%) i.e. 8 (2.1%) from abattoir and 21 (5.5%) from butchers were positive for *L. monocytogenes*. The total prevalence of *L. monocytogenes* from abattoir and butcher shops was 3.8% (N=768). The prevalence in butcher was higher with statically significant difference (P=0.02). The prevalence of the bacterium in butcher houses was almost three times (OR= 2.7, CI= 1.2-6.2) higher than the causative agent identified from abattoir. It is also indicated in the figure below (Table 3, Fig 3).

3.3 Contamination Rate of *Listeria monocytogenes* in Equipments

The contamination rate of *L. monocytogenes* in meat surface contact materials (hook, knife and cutting table) is presented in Table 4. Although there was not even one sample positive for hooks (Fig 3), there was no statistically significant difference among hook, knife, and cutting table (P= 0.2).

3.4 Antimicrobial Susceptibility

A total of 36 isolates of *L. monocytogenes* were tested for antimicrobial susceptibility. All isolates of *L. monocytogenes* were susceptible to amoxyclav. Out of 36 isolates, 28 (77.8%) were resistant to tetracycline, and 9 (25%) were resistant to penicillin. Four (11.1%) were equally resistant to streptomycin and ampicillin, 7 (19.4%) were equally resistant to sulfamethoprim and oxacillin, 2 (5.6%) were equally resistant to clindamycin and vancomycin. Interestingly, 34 (94.1%) isolates of *L. monocytogenes* were equally susceptible to vancomycin, and co-trimexasole. The details of susceptibility pattern of the isolates are presented in Table 5. The study also revealed multi-drug resistance isolates in 10/36 (22.7%), 13/36 (36.1%), and 24/36 (66.7%) for one, two, and two or more drug antimicrobials, respectively.

3.5 Findings of Questionnaire Survey

3.5.1 Findings of Questionnaire Survey in Abattoir

A total of 50 respondents were surveyed from the abattoir. About 60% of the abattoir workers had completed high school level. Out of 50 respondents, all (100%) had taken a lesson on personal hygiene. About 56% and 36% of respondents washed their hands once, and twice per day during the course of working time, respectively. Interestingly, 74% of the respondents reported the use of detergent. Most of the respondents (92%) washed their hands after the use of toilet. All (100%) of the respondents cleaned the working surfaces between each process and after the work. About 80% of the respondents washed their working knives after the completion of the work, and the rests washed several times during the course of working time. As on observational assessment, 76% of the closets of butchers were dirty. Almost all of the workers in the working room wore aprons and put a hair covering. Approximately, 78% of them did not wear any jewelry. Regarding the hygienic status of the abattoir, it was in a medium status.

3.5.2 Findings of Questionnaire Survey in Butcher Shops

A total of 50 respondents were surveyed from butcher shops. About 48% of the butchers were in an educational level of elementary, and 38% had completed high school level. Approximately, 50% of the respondents had taken a lesson on personal hygiene. About 46% and 36% of the respondents washed their hands twice and once per day during the course of working time, respectively. It was interesting to note that 86% of the respondents reported to use a detergent. As observed during the current study, about 96% of the respondents washed their hands after toilet.

About 38% of the respondents reported that the cashier was handling the money. The majority (62%) of the respondents handled the money by themselves. Most of the butchers (72%) cleaned the working surfaces, and washing of knives after work was performed by 7.8% of the butchers. As on observational assessment, 60% of the closet of the butchers was dirty. Most of them (74%) did not wear a hair covering. Wearing of jewelry was observed in 38% of the butchers. About the hygienic status of the butcher shops, 50%, 30% and 20% had poor, moderate, and good status, respectively.

4. Discussion

Listeria species are ubiquitous in nature, and have been isolated from wide environmental sources (Liu, 2008; Raorane *et al*., 2014; Pal, 2015). The organism possesses ability to survive in harsh conditions and therefore, can persist in the environment. Because of such persistence, *Listeria* species can easily enter in the food chain. Of the known *Listeria* species, *L. monocytogenes* is pathogenic to humans and animals (Pal, 2007; Raorane *et al*., 2014). The organism is sensitive to the antibiotics such as ampicillin, erythromycin, and penicillin but showed resistance to cephalosporines, fluroquinolones, and tetracycline (Pal, 2015).

Raw meat and other raw food products commonly found in the retail environment may be contaminated with pathogens, including *L. monocytogenes*. Retail environments are much more open with many people coming and going. These open retail environments may allow for the introduction of *L. monocytogenes* at various points and times of the -

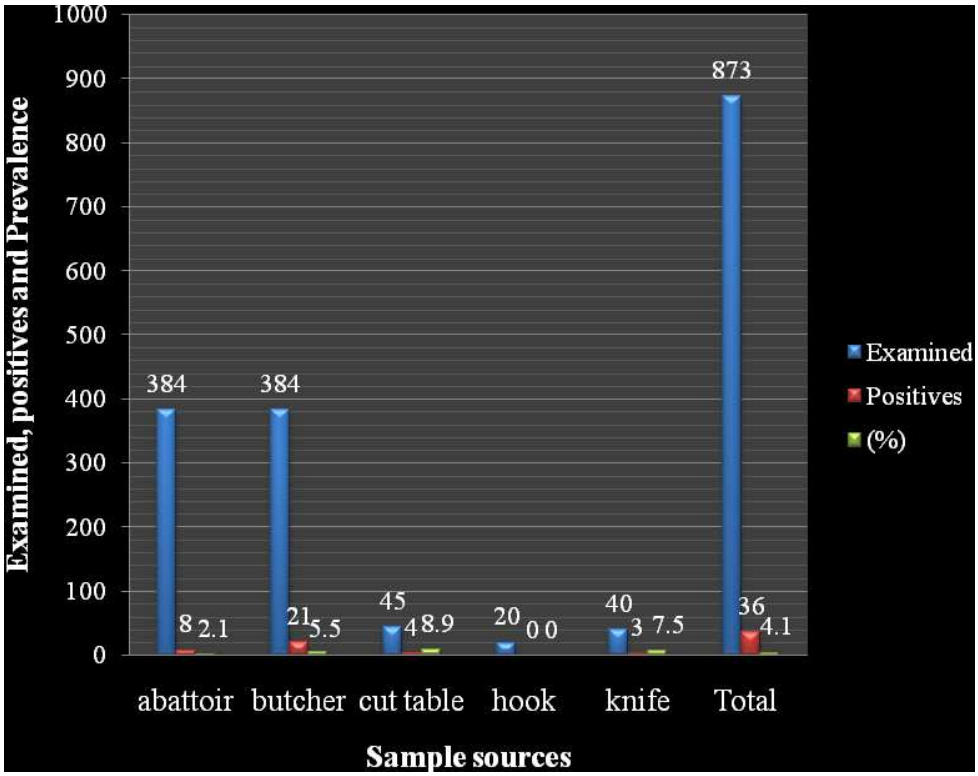

Fig 2: Overall prevalence of *Listeria monocytogenes*

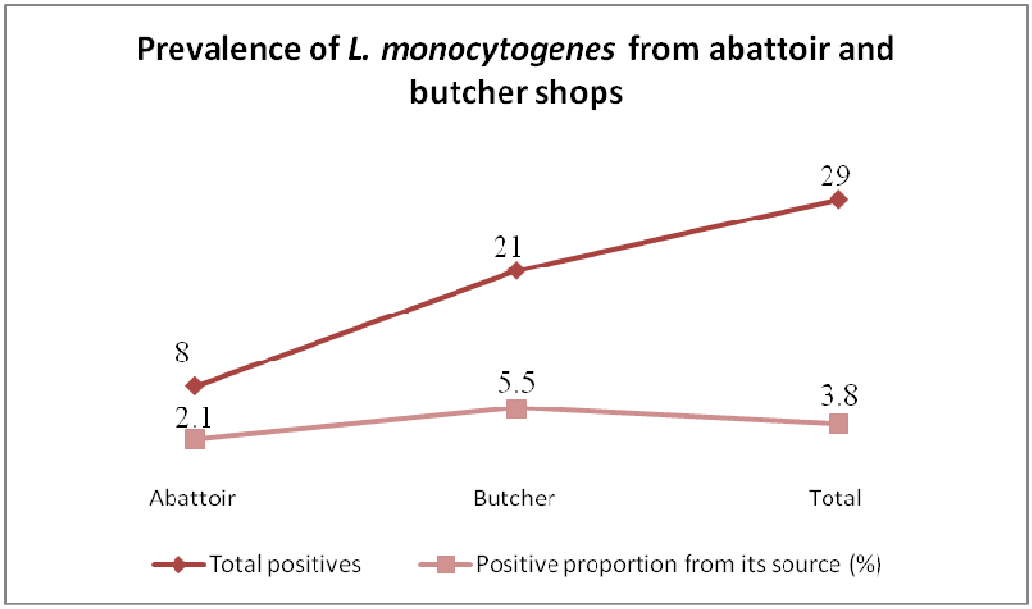

Fig 3: Proportion of positive prevalence in abattoir and butcher shops

Table 4: Prevalence of *Listeria monocytogenes* in meat contact surface materials

Source of sample	No. of examined	Total positive	Prevalence (%)	χ^2	p-value
Hook	20	0	0.00	-	-
Knife	40	3	3 (7.5)	3.1	0.2
Cutting table	45	4	4 (8.9)	-	-
Total	105	7	7 (6.7)	-	-

Table 5: Susceptibility of *Listeria monocytogenes* isolates to different antimicrobials

Antimicrobial	***Listeria monocytogenes***		
	S	**R**	**I**
	N (%)	N (%)	N (%)
Ampicillin	32 (88.9)	4 (11.1)	0
Chloramphenicol	32 (88.9)	3 (8.3)	1 (2.8)
Ciprofloxacin	28 (77.8)	7 (19.4)	1 (2.8)
Penicillin	24 (66.7)	9 (25)	3 (8.3)
Tetracycline	04 (11.1)	28 (77.8)	4 (11.1)
Vancomycin	34 (94.4)	2 (5.6)	0
Co-trimexazole	34 (94.4)	2 (5.6)	0
Streptomycin	30 (83.3)	4 (11.1)	2 (5.6)
Gentamycin	35 (97.2)	1 (2.8)	0
Amoxyclav	36 (100)	0	0
Clindamycin	29 (80.5)	2 (5.6)	5 (13.9)
Sulfamethrimethoprim	24 (66.7)	7 (19.4)	5 (13.9)
Oxacillin	28 (77.8)	7 (19.4)	1 (2.8)

S= Susceptible; R= Resistant; I= Intermediate

day, potentially making control of *L. monocytogenes* in the retailed environment more difficult (Cutter *et al*., 2006). The detection and identification of *Listeria* species have attracted the attention of many researchers. This specific interest is related to the presence of *L. monocytogenes*, one of the most important food-borne pathogens, in the genus *Listeria*. It is often found in various raw foods, such as uncooked meat, and vegetables, as well as in processed foods that become contaminated after processing like soft cheese and cold cuts (Pal, 2015). It is widely diffused in the environment, and this fact can cause the contamination of food during production and distribution (Cocolin *et al*., 2002).

In the present study, *L. monocytogenes* was isolated from 36 of 873 samples, giving a prevalence rate of 4.1%. The specific prevalence of *L. monocytogenes* based on sample sources was found to be statistically significant. The prevalence of *L. monocytogenes* in sheep meat at abattoir and butchers shops was 3.8. Our results seem to be in agreement with the observations of Pociecha and co-workers (1991) and Ankpolat and others (2004) who noted a prevalence of 3.2% and 5% from ovine carcass in New Zealand and Turkey slaughter houses, respectively. Molla *et al.* (2004) have demonstrated a prevalence of 5.1% in raw and ready-to-eat food products. The higher prevalence of 30% and 40% of *L. monocytogenes* was recorded by Mac Gowan *et al.* (1994) and Gilbirt *et al.* (2009), respectively. The prevalence of 4.0% of *L. monocytogenes* from gall bladder of sheep in slaughter house was recorded by Al-Ali *et al*. (2012). Several

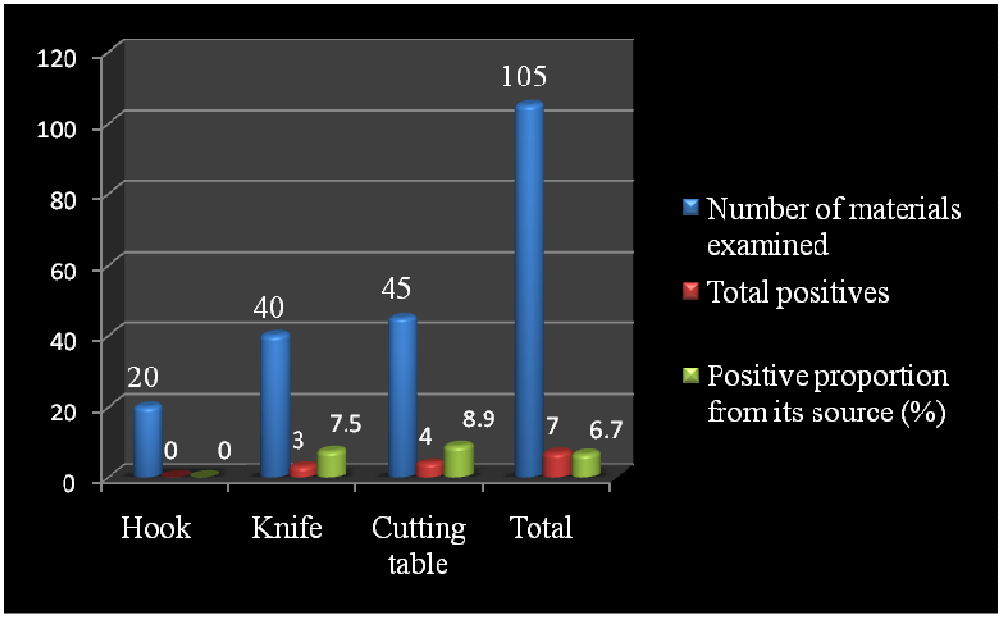

Fig 4: The positive proportion of the surface materials to *Listeria monocytogenes*

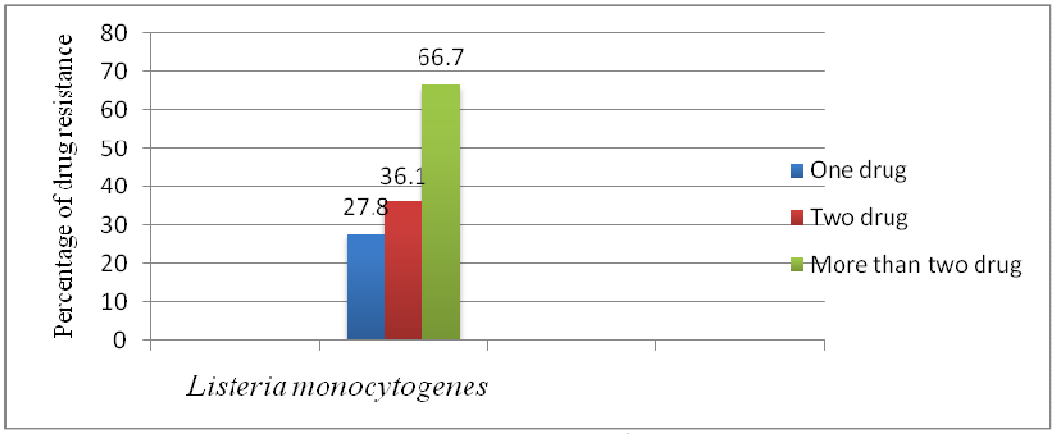

Fig 5: Multidrug resistance in *Listeria monocytogenes* for selected antimicrobial agents

studies confirmed that a prevalence of 4% by Ndahi *et al*. (2013) in ready- to- eat foods, 2.4% by Ennaja *et al*. (2008) from meat and meat products, and 4.7% by Yucel *et al.* (2005) from meat products. On the contrary, there was no isolation of *L. monocytogenes* at abattoir from sheep meat in Germany (Cohen *et al*., 2006). The prevalence of *L. monocytogenes* in meat samples differ from country to country. The reasons of low and high prevalence rates may be attributed to differences in hygienic conditions of slaughter houses, storage, and processing in various countries.

The specific prevalence of *L. monocytogenes* from equipments was found to be statistically not significant. Dirty or contaminated equipments can contaminate the safe food. Improperly cleaned equipment can be a source of *L. monocytogenes* contamination. Based on FDA reports and food-borne outbreak reports provided to the CDC, three risk factors have been identified most frequently as contributing to the contamination, spread and growth of food-borne pathogens, including *L. monocytogenes*, in processing or retail environments. These are cross-contamination, improper cleaning and sanitation, and improper time and temperature control (Cutter *et al*., 2006; Pal and Mahendra, 2015).

In our study, the equipments were identified as the potential source of contamination of meat with a prevalence of 6.7% of *L. monocytogenes*, which was lower than the findings of Lowry and Tiong (1988) who recorded 13% prevalence of *L. monocytogenes* in

Table 6: Summary of observational assessment and knowledge of workers on hygienic practices in abattoir

Abattoir activity	Performance	No.of respondents	Percent
Educational status	1-4	5	10
	5-8	15	30
	9-12	30	60
Lesson on personal hygiene	Yes	50	100
	No		
Time interval of washing hands	Once	28	56
	Twice	18	36
	Other	4	8
Washing of hands	With water only	13	26
	With detergent	37	74
Washing of hands after toilet	Yes	46	92
	No	4	8
Clean and disinfect working surfaces	Before work	0	0
	Between each process	50	100
Washing of knives	After work	40	80
	Between process	10	20
View of closets	Neat	12	24
	Dirty	38	76
Wearing of aprons	Yes	50	100
	No	0	0
Hair	Covered	50	100
	Not covered	0	0
Wearing of jewelry	Worn	11	22
	Not worn	39	78

food contact surfaces. Therefore, control measures to reduce the carriage of the pathogens in ruminants prior to slaughter should be reviewed with reference to the current regulations and guidelines relating to the primary production. A higher prevalence (25.64%) of *L. monocytogenes* in other country was reported by Jankuloski *et al.* (2007). The variation of prevalence in the two study sites may be because of environmental contamination, and poor sanitary conditions while handling of the meat before it reached to the consumer. The contamination occurs in an increasing level along the food value chain starting from slaughtering at the abattoir level, during distribution of the meat, and improper handling of the meat handlers who sold it.

Antibiotic resistant bacteria pose a growing problem of concern, worldwide since the bacteria can be easily circulated in the environment. Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous. A relatively high number of strains are resistance to the antimicrobials commonly used in the therapeutic protocols of many humans and animals infections (Normanno *et al*., 2007). In the current study, a resistance for tetracycline was 77.8%. On the contrary, 0% resistance was reported by Conter *et al*. (2013). Findings of resistant as well as poly-resistant strains of *L. monocytogenes* to antimicrobial drugs were rather sporadic. It is pertinent to mention that such strains isolated from foods, were frequently found resistance to tetracycline and penicillin (Navratilova *et al*., 2004).

In this study, our results regarding tetracycline resistance for *L. monocytogenes* was higher than the previous findings of Gupta and Sharma (2013) who observed 25%. However, in case of amoxyclav, we found 100% isolates of *L. monocytogenes* sensitive. This observation was lower than the results of Gupta and Sharma (2013 who reported 75% sensitivity. Thus, the resistance figures from different countries can considerably vary from very low to very high, probably reflecting the use of antimicrobials in those countries. Except tetracycline and penicillin, most antimicrobial

Table 7: Summary of observational assessment and knowledge of workers on hygienic practice in butcher shops

Questionnaire and observation type	Performance	No. of respondents	Percent
Educational status	1-4	7	14
	5-8	24	48
	9-12	19	38
Lesson on personal hygiene	Yes	25	50
	No	25	50
Time interval of washing hands	Once	18	36
	Twice	23	46
	Other	9	18
Washing of hands	With water only	7	14
	Water and detergent	43	86
Washing of hands after toilet	Yes	48	94
	No	2	6
Handling money	Cashier	19	38
	Butcher	31	62
Cleaning working surfaces	Before work	14	28
	After work	36	72
Washing of knives	After work	39	78
	Between each selling	11	22
View of closets	Neat	20	40
	Dirty	30	60
Hair covering	Covered	13	26
	Not covered	37	74
Wearing of jewelry	Worn	19	38
	Not worn	31	62
Hygienic status of the butcher house	Good	10	20
	Moderate	15	30
	Poor	25	50

drugs tested during this study effectively prevented the growth of *L. monocytogenes*.

The current study revealed that chloramphenicol resistance was found to be 8.3%, which was lower than the findings of Gupta and Sharma (2013) who reported 50%. It is worth mentioning that *L. monocytogenes* isolates examined in this study from all types of samples were resistant to 19.4% and 77.8%. On the contrary, percentages of resistance to ciprofloxacin and tetracycline were 1.8%, and 9%, respectively as reported by Zhang (2005). In the current study, resistance for ampicillin was observed 11.1%. We found multidrug resistance in *L. monocytogenes*. Similarly, workers from other countries also reported multidrug resistance (Zhang, 2005; Gupta and Sharma, 2013).

A larger sample size is needed to determine if there are differences between antimicrobial resistance patterns of the isolates among different sample sources. Considering that *L. monocytogenes* is slowly becoming antibiotic resistant, a continued surveillance of emerging antimicrobial resistance of this pathogen is important for effective treatment (Conter *et al*., 2009). As drug resistance is a growing public health concern, sincere attempts are needed to mitigate this problem.

Contamination of foods by *L. monocytogenes* can occur in all the steps from farm to table, which emphasizes the need to put forward control measures at every step. This can happen at retail level. During processing, the source of contamination can be surfaces, equipment, and workers, and persistence of strains has been detected at retail level. Employees can

contaminate food with *L. monocytogenes* if proper personal hygiene policies are not followed or if employees do not take the proper steps to safely receive, store, prepare, and serve food. They also may be a source for *L. monocytogenes* since some humans are known to carry the pathogen in their gastrointestinal tracts. Poor personal hygiene practices, such as improper hand washing or dirty uniforms, can lead to the contamination of food and equipment with *L. monocytogenes* (Cutter *et al*., 2006).

Proper motivation, education, and training of employees and managers in food industries are vital to keep consumers safe against food-borne diseases (Pal and Mahendra, 2015). In this study, 100% of the respondents from the abattoir had taken a lesson on personal hygiene but from the butcher shops, only 50% of the respondents had taken a lesson on personal hygiene. Therefore, all currently available educational approaches need to be critically evaluated and adopting of employees training to minimize *L. monocytogenes* cross contamination (Crandal *et al*., 2011). Furthermore, 92% of the abattoir workers, and 96% of the butchers washed their hands after the use of toilet. In contrary, few workers did not wash their hands. This may contribute to a low level of contamination of meat. One previous study revealed a prevalence of 16.7% and 27.8% from smoked and cooked meat products and from fermented dry meat products, respectively (Navratilova *et al*., 2004).

The results of this study showed that most of the respondents (74% of abattoir workers and 86% of butchers) used a detergent for washing of hands. Findings of *L. monocytogenes* in swabs from equipments and working surfaces witnessed the fact that contamination of meat and meat products is due to secondary soiling from the environment or equipment of meat-processing plants. Contamination generally increased during cutting, probably as a result of cross contamination. Also, in the retail and food service environment, contamination may be transferred between ready-to-eat products (Lianou and Sofos, 2007). The type of handling that ready-to-eat meat receives may also influence the level of *L. monocytogenes* contamination. In a survey of retail packaged meats, there was a significantly higher prevalence of *L. monocytogenes* reported in products cut into cubes (61.5% out of 13) compared with sliced products (4.6% out of 196) (Angelidis and Koutsoumanis, 2006).

5. Conclusion

The consumption of improper meat is not safe from consumer point of view, as it may lead to the transmission of various diseases. In this study, the results of bacteriological assessment showed that raw meat from market and slaughter houses are a source of *L. monocytogenes*. In addition, the presence of this bacterium may be attributed to the unclean working environment, poor sanitary conditions of persons who are contacting with the meat and the equipments. This may result in low meat quality and might potentially cause food poisoning, especially in susceptible groups, which include pregnant women, children, elderly, and immunosuppressed persons. Due to high risk and public health concern, it may cause a high case fatality rate. The detection of this pathogen in ready to eat processed food makes it unfit for human consumption.

Listeria monocytogenes may not be seen as potential clinical threat in Ethiopia today, with the increasing trend of transnational spread and emerging diseases. The probable risk that it might pose in the years to come cannot be ignored. The present study demonstrated the possible risk of *L. monocytogenes* after consuming meat and ready-to eat food stuffs available in the markets, and also highlighted the need for an effective and efficient storage process to keep such food safe, till they reached the consumers. Numerous risk factors are associated with the contamination and growth of *L. monocytogenes* in abattoir and market places. These factors need to be addressed and considered a serious hazard to identify control measures for an effective prevention and control program of this emerging food-borne pathogen. Further, the sources of infection and modes of transmission should be ascertained. In addition, addressing communication, risk perception and consumer practices to the public are mandatory.

The present study revealed widespread resistance by *L. monocytogenes* to commonly used antimicrobials. In addition, the prevalence of multi-drug resistance of the bacterium is also phenomenon, which gives cause for serious concern. In order to detect early changes in bacteria susceptibilities before a high prevalence of resistance is developed, regular monitoring of antimicrobial resistance to pathogenic bacteria should be practiced. The genetic mechanisms, which mediate antimicrobial resistance in this bacterium, would also need further studies.

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