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OCCURRENCE OF *LISTERIA MONOCYTOGENES* IN READY-TO-EAT FOODS OF ANIMAL ORIGIN AND ITS ANTIBIOTIC SUSCEPTIBILITY PROFILE, BISHOFTU AND DUKEM TOWNS, CENTERAL ETHIOPIA

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ABSTRACT

Listeriosis is one of the most important food-borne bacterial zoonotic diseases worldwide caused by Listeria species, as a result of food and environmental contamination. This disease is becoming an emerging bacterial disease, with low incidence but high case fatality rate. Across-sectional study was performed from November 2016 to April 2017 in Bishoftu and Dukem town of Central Ethiopia. Our study was aimed to determine the occurrence of listeria monocytogenes and its antibiotic susceptibility profile from ready-to-eat foods (raw milk, pasteurized milk, yogurt, cottage cheese, ice cream, raw beef, chicken and fish) of animal origin. A total of 340 food samples were collected randomly and bacterial isolation was performed according to the United States Food and Drug Administration protocol. Of the samples examined, 112 (32.9%) were found to be positive for Listeria species. The percentage of occurrence in each food item was 52.5% in cottage cheese, 50% in raw beef, 33.75% in raw milk, 16% in ice cream, 20% in yogurt, chicken and fish and 8% in pasteurized milk. L. monocytogenes was detected in 5.8% of the samples analyzed. It was isolated mainly from chicken and yogurt 3.3%, cottage cheese 5%, raw milk 8.7%, followed by raw beef 11.2%. In addition other Listeria species were identified as L. grayi 9.7%, L. innocua 5.2%, L. seeligeri 3.5%, L. welshimeri 2.9%, and L. ivanovii 2.6%. It was shown that L. monocytogenes and other Listeria species were found in ready-to-eat foods of animal origin. Antibiotic susceptibility test revealed resistant of 80% for Penicillin, 40% for Nalidixic acid, 15% for Tetracycline and 10% for Ceftriaxone and Ampicillin, while 100% of the isolates were susceptible to sulfamethoxazole, streptomycin, Clindamycin and Oxacillin. In general this study revealed that L. monocytogenes and other listeria species were detected in ready- to-eat food of animal origin in two town of central Ethiopia and the majority of them are susceptible to most antibiotics tested but resistant to penicillin and nalidixic acid.

KEYWORDS: Bishoftu, Dukem, Listeria species, L. monocytogenes Ready-to-eat food of animal origin.

1. INTRODUCTION

Listeriosis is one of the most important emerging food-borne bacterial zoonotic diseases of human being acquired through consumption of contaminated food of animal origin (OIE, 2008). Soil contamination and ingestion of contaminated food are the primary modes of transmission (Fentahun and Fresebehat, 2012). Ingestion of foods like fresh and soft cheese, raw meat, and fish may be the cause of sporadic cases or outbreaks. Animals are exposed by ingestion, inhalation, or direct contact with the Listeria bacterium. In animals, Listeria species is most frequently transmitted by contaminated silage feeding. It is most often found in silage that is poorly fermented with a pH 5.69 (Nightingale *et al.*, 2004). *L. monocytogenes* can be transmitted directly from by handling of aborted fetus and specimens from infected animals (Ramaswamy *et al.*, 2007).

The genus *Listeria* consisted of six species *Listeria monocytogenes, L. ivanovii, L. innocua, L. welshimeri*, *L. seeligeri* and *L. grayi* (OIE, 2014). Of these *L grayi, L. monocytogenes* and *L. ivanovii* are pathogenic (Liu *et al.*, 2006) While, *L monocytogenes* has been known to cause listeriosis in humans and animals (Schukken, 2003). *L. ivanovii*, which causes abortion in animals (Low and Donachie, 1997). Sporadic human infections due to *L. seeligeri* and *L. innocua* have also been reported (Perrin *et al.*, 2003), the rest species reported are not identified properly (Schmitt, 2003).

Listeriosis is ubiquitous in the environment and they are often found in animal products such as raw milk and meat due to unsanitary practices during milking and slaughtering (Schuchat *et al.*, 1991). Listeria species with its unique physiological characteristic can survive in temperatures from -7℃ to 45°C (Gandhi, 2006). All members of the genus *Listeria* are widely distributed in nature and have been isolated from soil, vegetation, sewage, water, and animal feed, fresh and frozen meat including chicken, slaughter house wastes and in the faeces of healthy animals including humans (McLauchlin, 2004).

Listeriosis has so far been reported and known to cause serial deaths in a number of individuals and in different regions, especially in Europe and the USA (Todar's, 2003). However, in most African countries, there are a few reports on Listeria species and listeriosis, when compared to the Europe and USA (Firehiwot, 2007). This is because; the organism has not been given much attention, might be due to lack of adequate facility and life style differences, prevailing poor food handling and sanitation practices. Listeriosis has been treated by various drugs which were effective but reports showed that the trend goes towards resistance (Teuber, 1999). The antibiotic susceptibility test is very important in hospitals to give effective treatment against listeriosis.

Information on the status of food borne Listeriosis and *Listeria species* is limited both in the veterinary and public health sectors in Ethiopia. In addition, the occurrence of the bacteria in foods of animal origin is not yet well documented (Gebretsadik *et al.*, 2011). Previous studies reported the presence of *L. monocytogenes* and other *Listeria* species in food of animal origin in Ethiopia. But our study focused on ready- to- eat food of animal origin mainly from public dinning places, milk collection centers, supermarkets, Butcher house and milk processing companies of Bishoftu and Dukem town.

Hence, the general objective of the present study was to undertake to determine the occurrence of *listeria species* and antibiotic susceptibility profile of *listeria monocytogenes* isolated from ready to-eat foods of animal origin collected from Bishoftu and Dukem town, central Ethiopia.

Therefore the specific objectives of this study were:

- To isolate and identify *Listeria monocytogenes* from ready-to-eat foods of animal origin from Bishoftu and Dukem town public dinning places and processing companies, Ethiopia.
- To conduct antibiotic susceptibility profile test on *Listeria monocytogenes* isolated from ready-to-eat foods of animal origin.

2. MATERIALS AND METHODS

2.1 Study Areas

The study was conducted between November 2016 to April 2017 in two towns of central Ethiopia, namely Bishoftu and Dukem. Bishoftu is located 47 km south east of Addis Ababa at 9°N latitude, 40°E longitude and at an altitude of 1850 meters above sea level. Bishoftu experiences a bimodal pattern of rainfall with the long rainy season extending from June to September and a short rainy season from March to May and dry season from October to February with an average annual rainfall of 800mm. The mean annual minimum and maximum temperature is 12.3 °C and 27.7 °C, respectively, with an overall average of 18.7 °C. The mean relative humidity is 61.3% (CSA, 2006).According to (Geo names, 2014) geographical data base the population of Bishoftu is 104,215.The town is a hub of 8 restaurants, 216 cafeteria, 4 milk processing companies 136-hotel, 150-supermarket and 250- butchers shop where most of them provide ready- to- eat food of animal origin for both the inhabitants - tourists and Addis Ababa market.

Dukem is located 37 km south east of Addis Ababa at 8°48′°N latitude 38°54°E longitude and an elevation of 2100 meters above sea level. Dukem experiences a bimodal pattern of rainfall with the long rainy season extending from June to September and a short rainy season from March to May and dry season from October to February with an average annual rainfall of 800mm. The mean annual minimum and maximum temperature is 25.83 ^{o}C and 11.9 ^{o}C, respectively, with an overall average of 18.86 °C. The mean relative precipitation is 861mm (CSA, 2006).According to (Geo names, 2014) geographical data base the population of Dukem is town estimated to be around 94,000 and the town is a hub of 5-restaurants, 2- milk collection center, 120- butchers, 80-cafeteria and, 42-supermarket where most of them provide ready- to- eat food of animal origin for both the inhabitants, tourists and Addis Ababa market.

2.2 Study Samples and Their Origin

Ready-to-eat food of animal origin (raw beef, raw milk, Pasteurized milk, cottage cheese, yogurt and ice cream) which are frozen or not and raw but frozen fish and chicken samples were used for this study. Because *Listeria monocytogenes* is a food borne pathogen that is widely dispersed in the environment; it can grow at refrigeration temperature and at un favorable conditions of pH (up to pH 4.7) and salt (up to 10%).The origin of these samples were from Restaurants, butcher house, milk collection center, supermarket, cafeteria and small ice cream shops found at Bishoftu and Dukem town.

2.3 Study Population and Sample Size Determination

This study used raw beef, raw milk, Pasteurized milk, chicken, fish, cottage cheese, yogurt and ice cream as a study population. The approximate sample size required was determined, according to (Thrusfeld, 2005) from previous prevalence of by Molla *et al.*, (2004) 32.6% with defined precision of 5% and level of confidence of 95%.

$$N=\frac{1.962\ P\ exp\ (1\text{-}Pexp)}{d2}$$

Where, N = required sample size

P_{exp} = expected prevalence
d = desired absolute precision

Therefore, by using estimated prevalence of 32.6% in ready-to-eat food of animal origin (raw beef, raw milk, Pasteurized milk, chicken, fish , cottage cheese, yogurt and ice cream and taking a confidence interval of 95% and 5% absolute precision, the minimum sample size required for this study was greater or equal 337 total sample. Accordingly from total of 340 samples comprising of raw meat (80), raw milk (80), Pasteurized milk (25) chicken (30), fish (30), cottage cheese (40), yogurt (30) and ice cream (25) (Table 2).

Table 1: Distribution of the type and number of samples included in the study.
[3]

Type of Sample	Proportion of sample
Raw beef	80
Raw Milk	80
Pasteurized Milk	25
Cottage Cheese	40
Yogurt	30
Ice Cream	25
Chicken	30
Fish	30
Total	340

2.4 Study Methodology

2.4.1 Study design

A cross-sectional study design was conducted from November 2016 to April 2017 to determine the occurrence and antibiotic susceptibility profile of *listeria monocytogenes* isolated from ready-to-eat foods of animal origin (raw beef, raw milk, Pasteurized milk, cottage cheese, yogurt and ice cream) which are frozen or not and raw but frozen (fish, chicken) in Bishoftu and Dukem town, central Ethiopia. Microbiological analysis of the sample was performed at Microbiology laboratory of College of Veterinary Medicine and Agriculture, AAU, Bishoftu. Study samples were collected using simple random sampling based on proportional allocation from a complete list of source.

2.4.2 Sampling technique and sample transportation

A total of 340 ready- to-eat-food of animal origin samples (raw beef (80), raw milk (80), Pasteurized milk (25), cottage cheese (40), yogurt (30) and ice cream (25) frozen or not and frozen (fish (30) and chicken (30) of only Ethiopian products were purchased randomly from municipal licensed retail shops, butchers, cafeterias and open markets of Bishoftu and Dukem towns of Central Ethiopia. Samples of 500gram (solid) or 500 ml (liquid) of each food category were purchased and examined according to the procedures described by the United States Food and Drug Administration and Center for Food Safety and Applied Nutrition (USFDA/CFSAN 2003) and (Curtis and Lee 1995) were employed. For the best possible recovery *Listeria* species were pre-enriched, selectively enriched and selectively plated. In transporting the samples an icebox containing ice packs was used and further analysis was done immediately upon arrival or stored at freezing temperature. Frozen samples were thawed at room temperature 4-6 hours before processing. 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 and source.

2.4.3 Sample preparation and processing

Twenty five (25) grams of raw beef, chicken and fish, sample were chopped from each of the purchased 500 g food samples using sterile knife; this was transferred in to sterile plastic bag which contained 225 ml of buffered *Listeria* pre-enrichment broth (Tryptone soya broth, Oxoid Ltd., Hampshire, UK) and homogenized using a laboratory blender (Stomacher 400, Seward, England) at high speed for 2 minutes. Similarly twenty five (25) milliliter of raw milk, Pasteurized milk and yogurt as well as 25gram of cottage cheese and ice cream from each of the purchased 500 gram or milliliter food samples were homogenized thoroughly using sterile spoon and added to sterile beakers separately.

For the Selective Enrichment those samples prepared above were transferred to sterile plastic bag and kept inside the incubator for 4 hours at 30° C initially and then selective agents were added (acriflavin, naladixic acid and the antifungal agent cycloheximide) and incubation was continued for a total of 48 hours at 30° C for subsequent processing according to the procedures described by the United States Food and Drug Administration and Center for Food Safety and Applied Nutrition (USFDA/CFSAN 2003) and (Curtis and Lee 1995). In our case each Raw milk, pasteurized milk, cottage cheese, yogurt and samples of 25 milliliter and raw beef, chicken and fish and ice cream sample of 25 grams which were incubated at 30 ^{O}C for 48 hours in a plastic bag were enriched in 225mL of *Listeria* broth-LEB (Difco Laboratories, Detroit, USA) (1:9 V/V ratio) and after 4 hours of incubation, selective reagents (*Listeria* selective supplement, Difco) were added and the incubation step was carried out at 30°C for 48 hours.

Those samples which were selectively enriched and incubated for 48 hours at 30°C were subjected to Selective Plating. This was done by picking a loop full of colonies showing black color from *Listeria* broth-LEB medium (UVM II, Oxoid Ltd., Hampshire, UK) and streaking onto PALCAM (Merck, Darmstadt, Germany) and OXA(ISO, 1996) agar plates for further incubation 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 was 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 (Polymyxin Acriflavin Lithium Chloride Ceftazidime Aesculin Mannitol) agar provided by PALCAM antimicrobial supplement (acriflavin, nalidixic acid, cyclohexamide).These agents effectively suppress 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 (Falana *et al*., 2003).

2.4.4 Confirmatory Tests

Colonies suspected to be *Listeria species* was transferred on to Tryptose yeast extract agar plate (TSYEA) and incubated at 37^{o}C for 18 to 24 hours. Those assumed *Listeria* colonies were characterized by using primary identification tests (Gram's staining, motility and catalase test) and secondary tests(characteristics of haemolysis, carbohydrate utilization and CAMP (Christie Atkins Munch Peterson).

Primary biochemical/ identification tests

Gram staining was performed by taking listeria suspected colony from the TSAYE (Trypticase Soy Agar with 0.6% Yeast Extract) plate and transferred on a clean microscopic slide. The stain was performed according to the recommended procedure (EC, 1996).The slide was examined under the oil emersion (100x) objective and a characteristic morphology of short rod was found.

Motility test was performed by taking atypical listeria suspected a colony from the TSAYE (Trypticase Soy Agar with 0.6% Yeast Extract) plate and was inoculated into semisolid motility media (EC, 1996). After incubating at 25^{0}C for 2-4hours, the stabs were examined L. species showed umbrella- shaped growth in the subsurface (Quinn, 2004).

Catalase test was performed by taking a typical listeria suspected colonies from the TSAYE (Trypticase Soy Agar with 0.6% Yeast Extract) and were transferred onto a clean microscopic glass slide and one drop of 3% hydrogen peroxide was added and positive reaction were identified due to the formation of bubble what is the scientific terms here (EC, 1996).

Secondary biochemical/ identification tests

Haemolysis test was performed by taking a loop full of a typical listeria suspected colonies from Trypticase Soy agar with 0.6% Yeast Extract (TSAYE) and streaked onto blood agar plates supplemented with 7% defibrinated sheep Blood and incubated for 24 hours at 35°C. After incubation was examined *L. ivanovii* produces wider zone of hemolysis and *L. monocytogenes* and non pathogenic *L. seeligeri* have narrow zone of beta hemolysis in sheep blood agar (Table -3) (Quinn, 2004).

CAMP was undertaken by using *Staphylococcus aureus* (CI NVI: Collection of Institute of National veterinary institute) streaked vertically on sheep blood agar plate and isolated *Listeria* species from TSAYE (Trypticase Soy Agar with 0.6% Yeast Extract) were streaked horizontally without touching the vertical streaks and incubated for 24-48 hours at 35°C *L.monocytogenes* showed an enhanced zone of hemolysis, forming an arrow head towards the *S. aureus* culture.

Carbohydrate utilization test was also performed by taking a loop full of isolated colonies from TSYEA and transferring it into 10 ml of carbohydrate utilization broth containing xylose, rhamnose and mannitol (Scharlau, Barcelona, Spain) in each test tube and incubated at 37^{o}C for up to 5 days. Positive reactions were identified by yellow color (acid formation) formation (Kiiyukia, 2003).

Table 2: Biochemical differentiation and identification of Listeria species.

	Acid produced from			**CAMP test**	
	Xylose	**Rhamnose**	Mannitol	**Hemolysis**	***S. aureu***
L. monocytogenes	-	+	-	+	+
L. innocua	-	v	-	-	-
L. ivanovii	+	-	-	++	-
L. seeligeri	+	-	-	(+)	(+)
L. welshimeri	+	v	-	-	-
L. grayi	-	-	+	-	-

2.5 Antibiotic Susceptibility Profile of Listeria monocytogenes

Disk diffusion susceptibility tests were performed according to Clinical and Laboratory Standards Institute standard reference procedure (CLSI, 2005).Three to five well isolated colonies of *L. monocytogenes* were transferred into 10 ml Tryptic Soy Broth (TSB, Merck), incubated at 37°C for 24 hours, diluted 1:10 (9 ml 0.1% peptone water) (Merck) to a turbidity equivalent to 0.5 McFarland standard (approximately 108 cfu/ml). This entire surface of a dried Mueller-Hinton Agar (MHA, Merck) plate was rubbed by sterile cotton swab containing suspension of broth cultured organism. The MHA plates were held at room temperature under a biological hood for 10 minutes to allow evaporation/adsorption of free surface liquid(Morobe *et al*., 2009).

Antibiotic discs like Tetracycline30µ, Sulfamethoxazole, 25µgAmoxicilline, 25µg, Oxacillin30µ, Penicillin10µg, Ampicilline10µg, Clindamycin10µg, Nalidixicacid3 0µg, Ceftriaxone 30 µg and Streptomycin 25µg (Oxoid) were placed on the surface of each inoculated MHA plate. After incubation at 37°C for 24 hours, the diameter (in of the zone around each disk was measured and interpreted in accordance with the Clinical and Laboratory Standards Institute Standards guidelines (Wikler, 2006) to classify the antibiotic susceptibility profile of each isolate.

2.6 Data Management and Analysis

Data was entered and analyzed using descriptive statistics to describe the frequency of listeria species from different food samples, antimicrobial susceptibility pattern. STATA version 13 was used to compare prevalence of groups, chi-square ($\chi 2$) test, and the odds ratio for samples and sample source in relation to recovery rate was calculated, p-value less than 0.05 were considered to be significant.

3. RESULTS

3.1 Bacterial Isolation

3.1.1 Colony characteristics

Microbiological analysis was performed after selectively plating of the food items which were processed by selective enrichment. After selective plating on *Listeria* broth-LEB medium (UVM II, Oxoid Ltd., Hampshire, UK) characteristic black colored colonies were observed. A loop full of these black colored colonies was further plated on PALCAM (Merck, Darmstadt, Germany) and OXA (ISO, 1996) agar plates. The colony characteristic on PALCAM agar plates was evidenced by a blackening of the medium as a result of aesculin hydrolysis where as 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 (figure 5A indicate blue arrow) .Whereas the colony characteristic on Oxford agar, was examined and the colonies appeared brown black or greenish black with a depressed center and a surrounding black halo (figure5B indicate arrow).

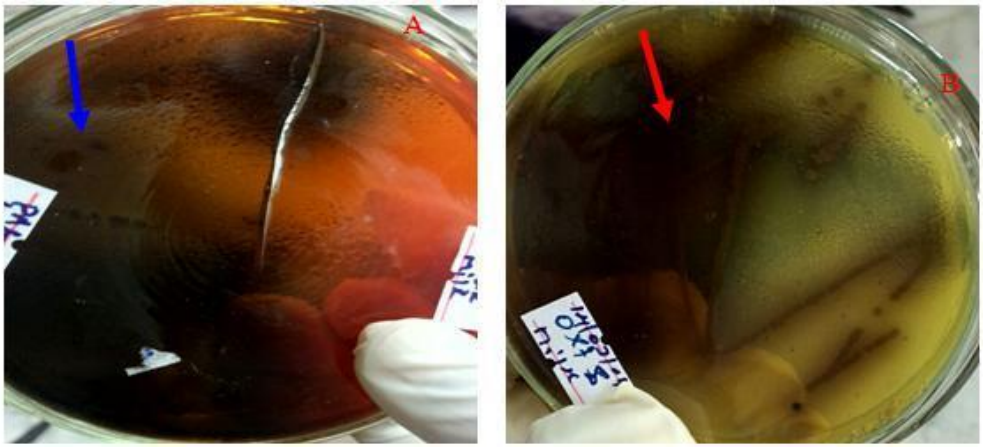

Figure 1: PALCAM (A) and Oxford (B) agar plates were evidenced by a blackening and brown black or greenish black of the medium respectively.

3.1.2 Gram's staining, catalase and motility test

Up on Gram staining all Listeria species were examined and were found to be gram positive with morphology of rod and slim short rod under the microscopic (figure 7A). The Catalase test was also revealed by gas bubbles (Oxygen) formation for all Listeria species (figure7B). The Motility of Listeria species were observed at 20-25°C by forming umbrella growth pattern around the stabs.

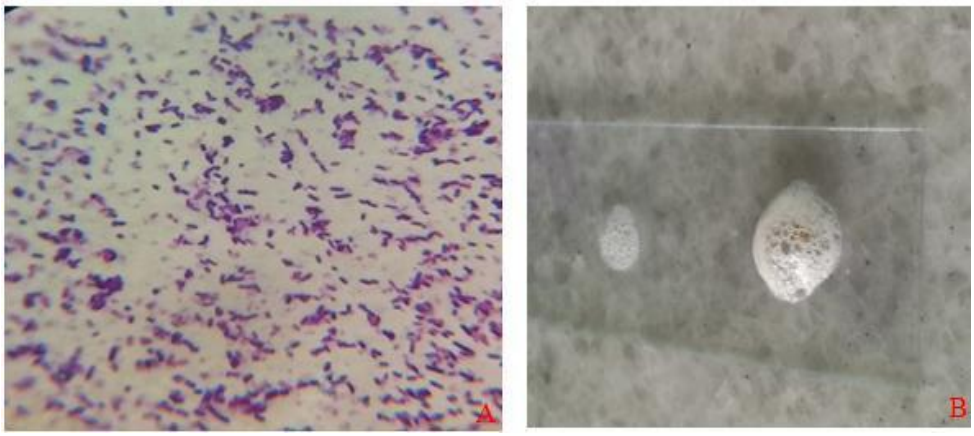

Figure 2: Gram staining (A) and Catalase test (B) result of Listeria species isolated.

3.1.3 Haemolysis

A loop full of black colored colonies from PALCAM were streaked on Blood Agar (Oxoid Ltd., Hampshire, UK) supplemented with 5% sheep blood hemolytic Listeria species showed narrow slight zones of clearing (ß-haemolysis) for *Listeria monocytogenes*, wide clear zones of ß-haemolysis for *L. ivanovii* and no clear zone for *L. innocua* around the stabs(figure 9).

3.1.4 Carbohydrate utilization test

For this purpose colonies suspected of listeria species from TSYEA was transferred into a tube of containing 10 ml of carbohydrate utilization broth supplemented with xylose, rhamnose and mannitol (Scharlau, Barcelona, Spain) and incubated at 37^{o}C 13 for up to 5 days. After that yellow color (acid formation) formation was observed and interpreted as positive reaction (figure 7A&B).

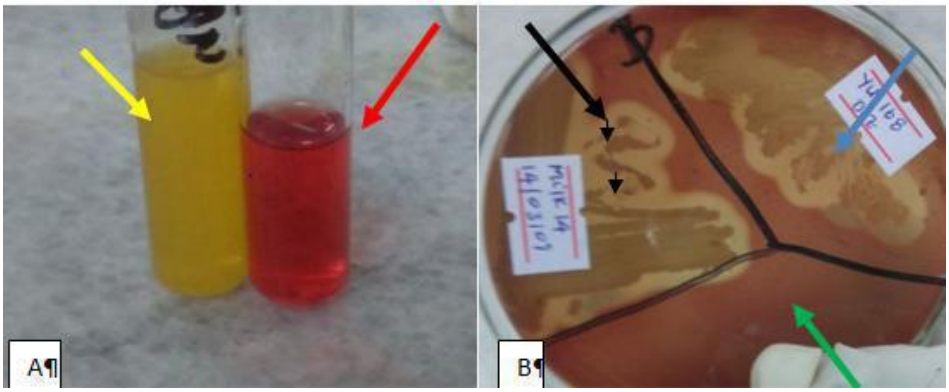

Figure 3: Carbohydrate utilization (xylose, rhamnose and mannitol) A) Positive for yellow color arrow and Negative for red color and fig-B) hemolytic nature of Listeria species on the blood agar plates black color arrow for *L. ivanovii* & blue color arrow for *Listeria monocytogenes* & green color for Negative control.

3.1.5 CAMP test

Colonies suspected of *listeria species* were transferred onto Tryptose yeast extract agar (Tryptone soya broth, Oxoid Ltd., Hampshire, UK) and incubated at 37^{o}C for 18 to 24 hours. This test was carried out using standard strain of *Staphylococcus aureus* (CI NVI: Collection of Institute of National veterinary institute) by streaking vertically on sheep blood agar and streaking test organisms horizontally (perpendicular) to *S. aureus* streak. Test results were read and an enhanced arrow head zone of β hemolysis between the test strain and the culture of *S. aureus* was considered a positive reaction. *L. monocytogenes* showed an enhanced zone hemolysis, forming an arrow head towards the *S. aureus* culture and *L. seeligeri* shows weak hemolysis while other *Listeria* species are not hemolytic.

3.2 Occurrence of Listeria Species from Food item

Out of a total of 340 food samples analyzed, 112 (32.9%) were positive for Listeria species. Listeria species were isolated from Raw beef, fish, chicken, raw milk, cottage cheese, yogurt, ice cream and pasteurized milk samples in different study site. The occurrence of listeria species between the two study sites in different ready-to-eat food of animal origin was also seen. Out of 190 and 150 ready-to-eat food of animal origin collected from Bishoftu and Dukem town 63 and 49 were found to be contaminated by one of listeria species. The level of contamination of food samples by Listeria species varied where cottage cheese (52.5%) takes the largest share of all sample types followed by raw beef (50%) and raw milk (33.75%). Contamination was also found in yogurt, chicken and fish (20%), ice cream (16%) and pasteurized milk (8%) in a decreasing order (figuer-8). Statistical significant association between the food type and listeria species was found raw milk, cottage cheese and raw beef at (X^2= 34.6, P-value= 0.000) significant level. In this study the Odds ratio of contamination of food items by listeria species was seen at 12.71 and 11.5 time higher in Cottage cheese and raw beef as compared to the reference pasteurized milk (Table: 4).

Table 3: Detection of Listeria species in different food item of animal origin.

Type of sample	Number	No positive	Mean	P-value	OR	X^2	P-value
Pasteurized milk	25	2(8%)	0,080	Ref	1,0**	34,6	0000
Cottage cheese	40	21(52.5%)	0,525	0.002	12,71		
Chicken	30	6(20%)	0,200	0.223	2,87		
Fish	30	6(20%)	0,200	0.223	2,87		
Ice cream	25	4(16%)	0,160	0.393	2,19		
Raw beef	80	40(50%)	0,500	0.022	11,50		
Raw Milk	80	27(33.75%)	0,338	0.022	5,86		
Yogurt	30	6(20%)	0,200	0.223	2,87		
Total	**340**	**112(32.9%**					

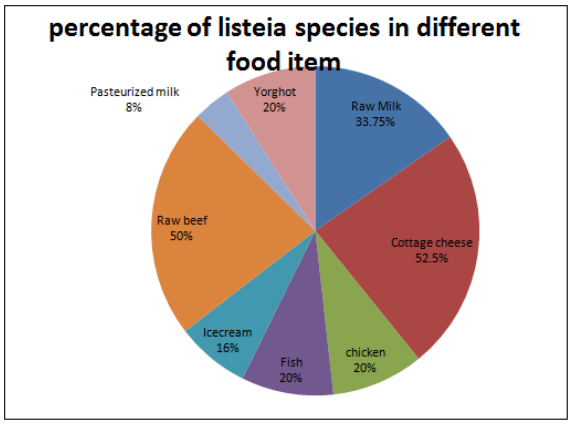

Figure 1: percentage of in different food item of animal origin

The distribution of listeria species isolated from different types of food samples is presented in Table 5. The dominant listeria species isolated in the present study was *Listeria grayi* 33(9.7%). It was frequently detected in cottage cheese 10(25%), raw beef 8(10%) raw milk 6(7.5%), Chicken and fish 3(10%) followed by ice cream 2(8%) and Yogurt 1(3.3%) samples. *L. monocytogenes* was the second most frequently isolated *Listeria* species 20(5.8%). Among the food samples analyzed, the occurrence of *L. monocytogenes* was the highest in raw beef 9(11.2 %), raw milk 7 (8.75%), cottage cheese 2(5%) followed by yogurt and chicken 1(3.3%) food samples. *L. innocua* (5.3%), *L. Seeligeri* 3.5%, *L. welshimeri* and others 2.9% was isolated on different food item.

Table 4: Distribution of Listeria species isolated from various types of food samples.

Listeria species	Raw milk	Pasteurized milk	Yoghurt	Cottage Cheese	Ice cream	Raw beef	Chicken	Fish	Total n=340
L.monocytogenes	7	0	1	2	0	9	1	0	20(5.8%)
L. innocua	4	0	2	1	2	6	0	3	18(5.29%)
L. ivanovii	2	1	1	2	0	3	0	0	9(2.6%)
L. grayi	6	0	1	10	2	8	3	3	33(9.7%)
L. seeligeri	2	1	0	3	0	6	0	0	12(3.5%)
L. welshimeri	3	0	0	0	0	5	2	0	10(2.9%)
Others	3	0	1	3	0	3	0	0	10(2.9%)
Total	27(33.7%)	2(8%)	6(20%)	21(52.5%)	4(16%)	40(50%)	6(20%)	6(20%)	112(32.9%)

3.3 Occurrence of Listeria Species from different sources

The occurrence of *Listeria* species varied between sample sources. Out of 340 types of food samples collected from the cafeterias, restaurants, milk collection centre, milk processing companies, butcher shops, supermarkets, hotels and open mark ate; the highest percentage of occurrence was found from samples collected from butchers shop (61.5%) and open market (53.3%) followed by samples collected from milk collection center and hotels (34%).The lowest percentage of listeria organism isolation was found in sample collected from Cafeteria (11.1%).Statically significant association between sample source and isolation of listeria organism was seen from sample obtained from butcher shop, open market and milk collection center and hotels at $X^{2=}$ 28.9172, $p<0.05$. In this study the Odds ratio of having *Listeria species* among sample source was seen at 12.800 times higher in butcher shop as compared to the reference sample source cafeterias (Table 6).

Table 5: Overall prevalence of Listeria species from different source of samples.

Sample source	Collected sample	Prevalence (%)	OR	P-value
Cafeterias	27	11.1%	1.00**	
Restaurants	45	31.1%	3.613	0.063
Milk collection canter	80	33.7%	4.075	0.032
Milk processing companies	16	18.75%	1.846	0.489
Butchers shop	39	61.5%	12.800	0.000
Supermarkets	52	23%	2.400	0.208
Hotel	62	33.8%	4.098	0.035
Open mark ate	15	53.3%	9.143	0.006
**Reference				

3.4 Antibiotic Susceptibility Test

In the present study a total of 20 isolates of *Listeria monocytogenes* were tested for antibiotic susceptibility. Out of twenty isolates of *L.monocytogens* 3(15%) of them were found to be resistant to tetracycline, 16(80%) to penicillin, 8(40%) Nalidixic acid, 2(10%) Ceftriaxone and Ampicillin; All isolates were susceptible to sulfamethoxazole, streptomycin, Clindamycin and Oxacillin. Intermediate susceptibility of some isolate was found to tetracycline, amoxicillin, penicillin, nalidixic acid and Ceftriaxone. The majority of the isolates were susceptible to the entire antibiotic disc except penicillin

and Ceftriaxone (Table 7).The diameter of zone of inhibition was measured in millimeter (Figure 9). The study also revealed multi-drug resistance isolates in 2/20 (10%), 3/20 (15%) for two and two or more antibiotics respectively.

Table 6: Antibiotic susceptibility profile of isolated *L. monocytogenes* (n=3).

Antibiotics	Resistance	Intermediate	Susceptible
Tetracycline	3(15%)	9(45%)	8(40%)
Sulfamethoxazole	0	0	100%
Amoxicillin	0	3(15)	17(85%)
Oxacillin	0	0	100%
Penicillin	16(80%)	3(15%)	1(5%)
Ampicillin	2(10%)	0	18(90%)
Clindamycin	0	0	100%
Nalidixic acid	8(40%)	2(10%)	10(50%)
Ceftriaxone	2(10%)	16(80%)	2(10%)
Streptomycin	0	0	100%

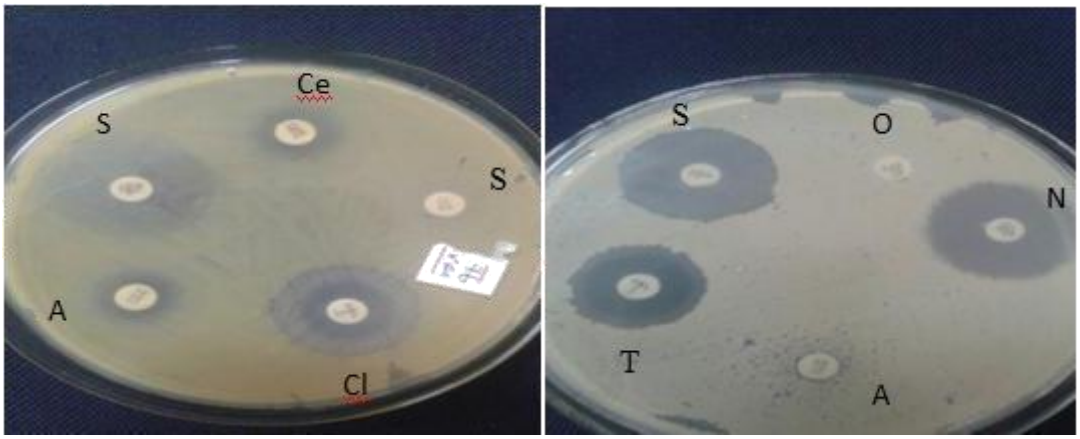

Figure 4: Mueller-Hinton Agar plates showing the effect of the antibiotics of *Listeria monocytogenes.*

S -Streptomycin O- Oxacillin
Ce-Ceftriaxone N- Nalidixic acid
SU-Sulfamethoxazole T- Tetracycline
Cl-Clindamycin A-Amoxicillin

4. DISCUSSION

Food borne pathogens are among the common causes of illness and death as well as public health problem which result in loss labor both in developed and developing countries. WHO estimated that in developed countries, upto70% of cases of diarrheal diseases are associated with the consumption of contaminated food per year .which are common food borne pathogen specifically *listeria* spp., *salmonella* spp., *Escherichia coli, staphylococcus* spp (WHO, 2004)

4.1 Occurrence of Listeria Organism from Ready-To- Eat Food Items and Sources

The overall occurrence of Listeria species in all food samples examined in Bishoftu and Dukem town was 32.9% out of 340 ready-to-eat food samples. From this 5.8% of the food item was found to be contaminated with *listeria monocytogenes.* This suggests the presence of this organism in ready -to-eat foods of animal origin indicating significant public health hazard. Our finding is higher than previous research done in Gonder which was 25 %(Garedew *et al*., 2015) and Addis Ababa 26.6% (Desalegn *et al.,* 2009), this might be due to the difference in the study site, the methodology used and the protocol followed. But our finding agrees with the report of Molla which is 32.6% of listeria species isolation from various food sources (Molla, 2004). With respect to the occurrence of *Listeria monocytogenes* (Molla, 2004; Gebretsadik *et al.,* 2011) reported comparable figure to ours from various food sources.

The highest level of contamination of food samples by Listeria species in our study was found in cottage cheese (52.5%), raw beef (50%) and Raw milk (33.75%).This is in agreement with the findings of (Molla , 2004) who reported about 50.6% of listeria species in raw meat. This is because it is assumed that because evisceration, food handling as well as the ubiquitous nature of the organism can increase the level of contamination (Vitas *et al.*,2004).Our study disagrees with the findings of (Garedew *et al*., 2015) in Gonder town who reported lower contamination level to the mentioned food items. Moscalewski *et al*. (2008) in Brazil also recorded 12.2%

of *Listeria* species from cheese. This might be due to the difference in the study site, the methodology used and the protocol followed.

In addition to the above food items our study also revealed a significant level of contamination in yogurt, chicken and fish (20%) as well as in ice cream (16%).This is comparable to the findings of (Uyttendaele,1999, Vitas,2004 ,Loura, 2005 and Soni, 2008) who reported this organism in different food of animal origin. But it disagrees with the report of Mugampoza *et al.*, (2011) in Uganda who states that the significant level of contamination in these food items.

The presence of these bacteria in pasteurized milk (8%) in our study could be post-processing contamination. Contrary to our finding, a previous study from Ethiopia Garedew *et al.*, (2015), Desalegn *et al.*, (2009) did not isolate any of the Listeria species in pasteurized milk. The possible explanation for this discrepancy could be due to difference in isolation methods and sample sources. But there are possibilities where this organism can enter later in the process as reported (Fleming *et al*., 1985).

This study also tried to see the occurrence of Listeria species with respect to the sample source. We found that the occurrence varied between sample sources where the highest was seen in butchers shops and open market followed by samples collected from milk collection center and hotels. This is in agreement with the finding of Selamawit, (2014) who reported about 5.5 % *L. monocytogenes* which is higher in butcher shops. Contamination generally increased during cutting, probably as a result of cross contamination. Also, in the retail and food service environment, contamination might be transferred between ready-to-eat food products (Lianou and Sofos, 2007).

Species distribution of the organism in the study sites indicated that *L. monocytogenes* (5.8%) and *L. innocua* (5.3%). This is in agreement with the report of other studies indicated 1 to 70% prevalence of *Listeria* species in beef samples (Rocourt, 2001, Dhanashree, 2003). This might be a treat in our situation where the tradition of consuming raw or undercooked animal products aggravates the public health risk associated with *L. monocytogenes* as raw meat slices, ground meat and meat processing environments are known to harbor the organism (Ryu, 1992).

With respect to the sample type the highest level of occurrence of *L. monocytogenes* was seen in raw beef, raw milk, cottage cheese and yogurt and Chicken in a decreasing order. This was comparable with Gebretsadik, (2011) in Addis Ababa who reported *L. monocytogenes* was isolated in 5.4% and this is similar to the previous studies who reported the frequent occurrence of *L.monocytogenes* in different food of animal origin .Research results from China have had reported that, *L. monocytogenes* of 5.79% from China food products (Chao, 2007).Our finding disagrees with Soni *et al*., (Soni, 2008) in Varanasi, India who reported 5.8% of cow milk samples were positive for *L. monocytogenes* and none of the milk products i.e., cheese and ice cream was positive for *L. monocytogenes*. (Ryu, 1992, Dhanashree, 2003) also reported the occurrence of *L. monocytogenes* in India (2.9%) and Japan (6.1%) in contrary to our finding.

In general from this study we found that a significant level of contamination of Ready-to-eat items by *listeria species* and *L.monocytogenes* from different sources found in Bishoftu and Dukem town. This suggests the presence of this organism in ready -to-eat foods of animal origin indicating significant public health hazard and needs strict precaution.

4.2 Antibiotic Susceptibility Profile of *L. Monocytogenes*

Treating human and animal disease is becoming a huge problem because of evolvement of antibiotic resistant bacteria. Higher numbers of isolates are resistance to the antimicrobials commonly used in the therapeutic protocols of many humans and animal infections (Normanno *et al*., 2007). In the present study a higher percentage of resistant isolates were found for penicillin (80%) followed by Nalidixic acid (40%), Tetracyclin (15%), Ceftriaxone and Ampicillin equally (10%). Our finding was in agreement with the result reported by Firehiwot (2007) and Tefera, (2014) in Ethiopia where isolates were resistant to tetracycline, penicillin, rifampcillin and erythromycin. On contrary to our finding Gunes, (2012) in USA reported susceptible isolates to penicillin G, tetracycline, rifampicin, chloramphenicol, vancomycin, gentamicin, Streptomycin, erythromycin and trimethoprim this might be due to the sample origin, source and study site difference.

The majority of the isolates were susceptible to the entire antibiotic discs used in this study except penicillin and Ceftriaxone. The isolates were 100% susceptible to sulfamethoxazole, Oxacillin, Clindamycin and streptomycin. This is in agreement with the report of Firehiwot, 2007; Osaili *et al*., 2012 and Sharma *et al*., 2012 from Ethiopia, Jordan and India where isolates of *Listeria monocytogenes* were susceptible to gentamicin, sulfamethoxazole, Ampicillin and streptomycin. In this study we found that even though most antibiotics tested are effective but there are still resistant isolates found from ready- to- eat food of animal origin which would be a future challenge.

5. CONCLUSION AND RECOMMENDATIONS

This study has established the presence and distribution of *L. monocytogenes* and other *Listeria* species in different types of ready-to-eat foods of animal origin in Bishoftu and Dukem Ethiopia. The presence of Listeria species, particularly *L. Monocytogenes* in Ready-to-eat

food of animal origin and uncooked products could be a potential risk for consumers. The presence of these bacteria might be attributed to the unclean working environment, poor sanitary conditions of persons who are contacting with the milk and milk products, meat, Fish and chicken meat with their equipment used. Even though most antibiotics (Sulfamethoxazole, Streptomycin, Clindamycin and Oxacillin) tested are effective but there are still resistant isolates (Penicillin, Nalidixic acid, Tetracycline, Ampicillin & Ceftriaxone) found from ready-to- eat food of animal origin.

Therefore, based on the above convincing remarks, the following recommendations are forwarded:

- Effective control program should focus on understanding the sources of the pathogen and factors that contribute to the risk of contamination, growth and spread of the pathogen.
- Further study on the detection of this organism using sensitive techniques (molecular) should be done.
- The bacterial load of listeria species from ready-to-eat food of animal origin should be done.
- Awareness should be made in the production of microbiologically safe ready-to- eat food of animal origin based on the implementation and application of general preventative measures, good hygienic practices and good manufacturing practices.
- An extensive survey of the Occurrence of *listeria species* and *L. monocytogenes* in the whole part of Ethiopia should be undertaken.
- Regular monitoring of antibiotic susceptibility profile to *L. monocytogenes* and other pathogenic bacteria should be practiced.

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6. REFERENCE

1. Advisory Committee on the Microbiological Safety of Food (ACMSF), A.d., H.o.c., group on vulnerable groups (2009): Report on the increased incidence of listeriosis in the UK.
2. Anderson, N.W., Buchan, B.W., Riebe, K.M., Parsons, L.N., Gnacinski, S. and Ledeboer, N.A. (2012): Effects of solid-medium type on routine identification of bacterial isolates by use of matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J. Clin. Microbiol*., 50: 1008–1013.
3. Arslan, S. and Özdemir, F. (2008): Prevalence and antimicrobial resistance of *Listeria* spp. in homemade white cheese. *Food Cont*. Issue 19: 360-36.
4. Awaisheh, S.S. (2010): Incidence and contamination level of *Listeria monocytogenes* and other Listeria *spp*. in ready-to-eat meat products in Jordan. *J Food Pro.*, 73: 535-540.
5. BAM (2003): Detection and enumeration of *Listeria monocytogenes* Bacteriological Analytical Manual BAM, Available at http://www.cfsan.fda.gov/_ebam/bam-5.html.Accessed on May, 14: 2009.
6. Bhilegaonkar, K.N., Bachhil, V.N., Kapoor, K.N., Kumar, R.K. and Rathore, R.S. (2001): Effect of NaCl and pH on the growth and survivability of *L. monocytogenes* Indian J. Comp. *Microbiol. Immunol Infect. Dis.*, 22: 122-124.
7. Boerlin, P., Rocourt, J., Grimont, F., Grimont, P.A.D., Jacquet, C. and Piffaretti, J.C. (1992): Listeria *ivanovii subsp. londoniensis* subsp. *Int. J. Syst. Bacteriol*., 42: 69-73.
8. Brosch, R., Chen, J. and Luchansky, J.B. (1994): Pulsed-field fingerprinting of *listeriae* identification of genomic divisions for *Listeria monocytogenes* and their correlation with serovar. *Appl Environ Microbiol* ., 60: 2584–2592.
9. Buckley, M., Reid, A., (2010): Global food safety keeping food safe from farm to table A ReportfromtheAmericanAcademyofMicrobiologyAvailableonlinihttp://academy.asm.org/images/stories/documents/ Global *Food Safety*. *pdf*., pp. 1-41. [1 June 2011].
10. Bunning, V.K., Donnelly, C.W., Peeler, J.T., Briggs, E.H., Bradshaw, J.G., Crawford, R.G., Beliveau, C.M. and Tierney, J.T. (1988): Thermal inactivation of *Listeria monocytogenes* within bovine milk phagocytes. *Appl. Environ Microbiol*., 54: 364-370.
11. Chao, G., and Zhou, X., Jiao, X., Qian, X. X., u. (2007): L. Prevalence and antimicrobial resistance of food borne pathogens isolated from food products in China. Food borne Pathog Dis, 4: 277-84.
12. CLSI (2005): Performance Standards for antimicrobial susceptibility testing; fifteenth informational supplement, M100_S15, Vol 25,

No.1.Clinical and Laboratory Standards Institute Wayne, Pa.
13. Cocolin, L., Stella, S., Nappi, R., Bozzetta, E., Cantoni, C. and Comi, G. (2005): Analysis of PCR-based methods for characterization of *Listeria monocytogenes* strains isolated from different sources. *Int. J. Food Microbiol.*, 103: 167–178.
14. Cossart, P. (2011): Spreading *Listeria monocytogenes* in the body PNAS, 108: 19484 19491.
15. CSA (2006): Central Statistical Authority, agricultural sample survey, Report on livestock 2007/2008. Stastical bulletin 361, Addis Ababa, Ethiopia.
16. Curtis, G.D.W., Lee, W.H. (1995): Culture media and methods for isolation of *Listeria monocytogenes* in foods, *Int J Food Microbiol*., 26: 1-13.
17. Desalegn, M., Bayleyegn, M., Marie-Thérèse T., Josef K.,Goetz H. and Wondwossen, A.(2009):Occurrence and Distribution of *Listeria monocytogenes* and Other *Listeria* Species in Ready-to-Eat and Raw Meat Products Berliner und Münchener Tierärztliche Wochenschrift, 122: 20–24.
18. Dhanashree, B., Otta, S.K., Karunasagar, I., Goebel, W. and Karunasagar, I., (2003): Incidence of *Listeria species* in clinical and food samples in Mangalore, *India*. *Food Microbiol,* 20: 447-453.
19. Doyle, E.M. (1999b): Use of organic acids to control *Listeria* in meat. American meat institute Foundation. VW- Madison. Organic Acids P. 6.
20. EFSA (2011): The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009; European Food Safety Authority/Eu-ropean Centre for Disease Prevention and Control. EFSA J 9: pdf no. 2090. [378 pp.].
21. El-Malek, A.M., Hassan, S.F., Ali, R., Hassane in, M., Abdelazeem and Elsayh, K.I. (2010): Occurrence of *Listeria species* in meat, chicken product and human stools in Assiut city, Egypt with PCR use for rapid identification of *Listeria monocytogenes*, Veterinary World, 3: 353-359.
22. Esteban, J.I. B., Oporto, G., Aduriz, R.A. J. and Hurtado, A. (2009): Faecal shedding and strain diversity of *Listeria monocytogenes* in healthy ruminants and swine in Northern Spain. *J.BMC Vet Res.*, 5: 2.
23. European Commission, Be information (1996): The Certification of the Number Fraction of Negative Capsules and the Mean Number of Colony Forming Particles of *Listeria monocytogenes* in one Capsule Containing Artificially Contaminated Milk Powder.
24. Eyasu, T.(2015): Prevalence of *Listeria monocytogenes* in raw bovine milk and milk products from central highlands of Ethiopia, *Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia*, JIDC/ *Journal Of Infection In Developing Conteries.*
25. Fallah, A.A, Saei-Dehkondi, S.S, Rahnama, M., Tahmas by, H. and Mahzounieh, M. (2012): Prevalence and antimicrobial resistance patterns of *Listeria* species isolated from poultry products marketed in Iran. Food Control, 28: 327-332.
26. Farber, J.M. And Peterkin, P.I. (1991): *Listeria monocytogenes*, a food borne pathogen. Microbiol, 55: 476 – 511.
27. FDA (2012): Bad bug book Food borne pathogenic microorganisms and natural toxins handbook, 2nded, US Food and Drug Administration, Silver Spring, p. 100–104. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm.
28. FDA/CDC (2003b): Reducing the Risk of *Listeria monocytogenes.* Update of the *Listeria* Action Plan.www.foodsafety.gov.
29. FDA/CFSAN (2003a): Detection and Enumeration of *L. monocytogenes* in foods, Bacteriological Analytical Manual Online. http//*www.cfsan.fda.gov*.
30. Fentahun, T. and Fresebehat, A. (2012): A review of Listeriosis in Small Ruminants. *J. Advances in Biological Research*, 6: 202-209.
31. Firehiwot, A. (2007): Prevalence and antimicrobial profile of *Listeria monocytogenes* in retail meat and dairy products in Addis Ababa and its surrounding towns, Ethiopia. MSc thesis, Addis Ababa University, Addis Ababa, Ethiopia.
32. Fleming, D.W., Cochi, S.L., MacDonald, K.L., Brondum, J., Hayes, P.S., Plikaytis, B.D., Holmes, M.B., Audurier, A., Broome, C.V., Reingold, A.L. (1985): Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N Engl J Med.*, 312: 404-407.
33. Franciosa, G., Pourshaban, M., Gianfranceschi, M. and Aureli, P. (1998): Genetic typing of human and food isolates of *Listeria monocytogenes* from episodes of listeriosis. *European Journal of Epidemio*, 14: 205–210.
34. Frye, D., M., Zweig, R., Sturgeon, J., Tormey, M., Le Cavalier, M., Lee, I., Lawani, L. and Mascola, L. (2002): An outbreak of febrile gastroen-teritis associated with delicatessen meat contaminated with *Listeria monocytogenes*. In: *J. Clin Infe Dise*, 35: 943-949.
35. Gandhi, M., Chikindas, M., L. (2006): Listeria A food borne pathogen that knows how to survive, *Int J Food Microbiol.*, 2007 Jan 1; 113: 1-15, *Epub* Sep 28.
36. Garedew, L., Ayele, T., Tigist, B., Seleshe, N., Elias, K., Mebrat, E., Abraham and Tamiru, B. (2015): Prevalence and antimicrobial susceptibility profile of *listeria species* from ready-to-eat foods of animal origin in Gondar Town, Ethiopia, *BMC Microbiology*, 15: 100
37. Gebretsadik, S., Kassa, T., Alemayehu, H., Huruy, K. and Kebede, N. (2011): Isolation and characterization of *Listeria monocytogenes* and other *Listeria* species in foods of animal origin in

Addis Ababa, Ethiopia. *Journal of Infection and Public Health*., 4: 22-9.
38. Gellin, B.G. and Broom, C.V. (2001): *Listeriosis*, Morb. Mort. Wkly Rep, 261: 1313.
39. Geo names, (2014): Geographical data base, the population of Debre Zeyit Ethiopia.
40. Geoffroy, C., Gaillard, J.L., Alouf, J. E. and Berche P. (1987): Purification, characterization and toxicity of the sulfhydryl-activated hemolysin *listeriolysin* O from *Listeria monocytogenes*. *Infect. Immun*., 55: 1641-1646.
41. Gilot, P. and Content, J. (2002): Specific identification of *Listeria welshimeri* and *Listeria monocytogenes* by PCR assays targeting a gene encoding a fibro nectin-binding protein. *J Clin Microbiol*., 40: 698-703.
42. Graves, L., Swaminathan, B., Reeves, M., Hunter, S.B., Weaver, R.E., Plikaytis, B.D. and Schuchat, A. (1994): Comparison of ribotyping and multilocus enzyme electrophoresis for subtyping of *Listeria monocytogenes* isolates. *J Clin Microbiol*, 32: 2936–2943.
43. Guerra, M.M., Bernardo, F. and McLauchlin, J. (2002): Amplified fragment length polymorphism (AFLP) analysis of *Listeria monocytogenes*. Systematic and Applied Microbiol, 25: 456–461.
44. Guillet, C., Join-Lambert, O., Le M.A., Leclercq, A., Mechai, F., Mamzer-Bruneel, M.F., Bielecka, M.K., Scortti, M., Disson, O., Berche, P., Vazquez-Boland, J., Lortholary, O., Lecuit, M. (2010): Human listeriosis caused by *Listeria ivanovii*. *Emerg Infe Dis.*, 16: 136–138.
45. Gunes E. Altuntas (2012): Antibiotic and Bacteriocin Sensitivity of *Listeria monocytogenes* Strains Isolated from Different Foods, http://dx.doi.org/10.4236/fns.2012.33052 Published Online March 2012 (http://www.SciRP.org/journal/fns) USA *Food and Nutrition Sciences*, 2012; 3: 363-368.
46. Gurtler, Joshua (2006): Hot Topics in Food Safety. Pathogens and Antimicrobials– *Listeria* In Session Summaries, Members of the IAFP Student Professional Development Group, Food Pro, 20: 824-845.
47. Hitchins, A.D. (2002): Detection and enumeration of *Listeria monocytogenes* in foods. In: Bacteriological Analytical Manual online. 8th ed.
48. Hubalek, Z. (2003): Emerging human infectious diseases: anthroponoses, zoonoses, and sapronoses. *Emerg Infect Dis*., 9: 403–404.
49. International Organization for Standardization (11290-1, 1996): Microbiology of food and animal feeding stuffs Horizontal method for the detection and enumeration of *Listeria monocytogenes*, ISO, Geneva, Switzerland.
50. ISO (2004): Microbiology of food and animal feeding stuffs Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method, Geneva, Switzerland Pp. 1.
51. James, M., Jay, Martin, J.L. and David, A.G. (2005): Modern Food Microbiology. Springer Science Business Media, USA, Seventh edi, 9: 591-612.
52. Jemmi, T. and Stephan, R. (2006): *Listeria monocytogenes*: food-borne pathogen and hygiene indicator, *Rev., Sci., tech., Off. int., Epiz*, 25: 571-580.
53. Jenkins, E., M., Njoku-Obi1, A. N. and Adams, E., W. (1964): Purification of the soluble hemolysis of *L.monocytogenes*. *J Bacteriol*, 88(2): 418-424.
54. Jennifer, J.L., Mochael, D.P. and Robert, C. G. (1980): *Listeria monocytogenes* and other *Listeria* species in meat and meat products-*A Review. J Food Prot*., 53: 81-91.
55. Johan, V., Neveltlaan, O. and Haag, D. (2004): 2nd Informatory Note on Refrigeration and Food *Listeria Monocytogenes* in Refrigerated Foods, International institute of refrigeration. iifiir@iifiir.org - Web: www.iifiir.org.
56. Kasalica, A., Vuković, V., Vranješ, A. and Memiši, N. (2011): *Listeria monocytogenes* in Milk and Dairy Products*: Biotechnol. in Animal Husbandry*, 27: 1067-1082 40: 1327-1332.
57. Katla, T., Naterstad, K., Vancanneyt, M., Swings, J. and Axelsson, L. (2003): Differences in susceptibility of *Listeria monocytogenes* strains to Sakacin P, Sakacin A, Pediocin PA-1, and Nisin. *Appl Environ Microbiol*., 69: 4431-443.
58. Kerouanton, A., Brisabois, A., Denoyer, E., Dilasser, F., Grout, J., Salvat, G. and Picard, B. (1998): Comparison of five typing methods for the epidemiological study of *Listeria monocytogenes. Int. J. Food Microbiol*., 43: 61–71.
59. Kerr, K.G., Birkenhead, D., Seale, K., Major, J. and Hawkey, P.M. (1993): A research note on, prevalence of *Listeria* species on the hands of food workers. *J Food Prot*, 56: 525-527.
60. Kiiyukia C. (2003): Laboratory Manual of Food Microbiology for Ethiopian Health and Nutrition Research Institute food Microbiology Laboratory, UNIDO Project. Addis Abeba.
61. Kingdom, G. C. and Sword, C. P. (1970): Effects of *Listeria monocytogenes* hemolysin on phagocytic cells and lysosomes. *Infect. Immun*., 1: 35-36.
62. Lahuerta, A., Westrell, T., Takkinen, J., Boelaert, F., Rizzi, V., Helwigh, B., Borck, B., Korsgaard, H., Ammon, A., Mäkela, P. (2011): Zoonoses in the European Union: origin, distribution and dynamics the EFSA-ECDC summary report 2009. Euro Surveill 16: pii 19832.
63. Li, Q., Sherwood, J.S. and Logue, C.M., (2007): Antimicrobial resistance of *Listeria* species.
64. Liu, D. (2006): Identification, sub typing and virulence determination of *Listeria monocytogenes*, an important food borne pathogen: *J Med Microbiol.*, 55: 645-659.
65. Liu, X.M., Chen, Y., Fan, Y.X. and Wang, M.Q. (2006): Food borne diseases occurred in 2003 report of the National Food borne Diseases Surveillance System, China *Wei Sheng Yan Jiu.*, 35: 201–204.

66. Loura, C.A.C., Almeida, R.C.C. and Almeida, P.F. (2005): The incidence and level of *Listeria spp*. and *Listeria monocytogenes* contamination in processed poultry at a poultry processing plant, *J Food Safety*., 25: 19-29.
67. Low, J., C. and Donachie, W. (1997): A review of *Listeria monocytogenes* and listeriosis. *Vet. J*., 153: 9–29.
68. M.A., Wikler, (2006): ―Performance Standards for Antimicrobial Susceptibility Testing, Sixteenth Informational Supplement, M100-S16, *Clinical and Laboratory Standards Institute* (*CLSI*), 26(3): Pennsylvania.
69. MacDonald, P.D., Whitwam, R.E. and Boggs, J.D., (2005): Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese, *Clin. Infect.,* Dis, 40: 677-682.
70. Mahmoodi, M.M., (2010): Occurrence of *Listeria monocytogenes* in raw milk and dairy products in Noorabad, *Iran. J. Anim., Vet, Adv.*, 9: 16 –9.
71. Malik, S.V.S., Barbuddhe, S, B. and Chaudhari, S.P. (2002): Listeric infections in humans and animals in Indian subcontinent: *A review. Trop Anl Hlth Prod*., 34: 359-381.
72. Manzano, M., Cocolin, L., Cantoni, C. and Comi, G. (2000): Temperature gradient gel electrophoresis of the amplified product of a small 16S rRNA gene fragment for the identification of *Listeria* species isolated from food. *J Food Prot.,* 63: 659–661.
73. McLauchlin, J., Mitchell, R.T., Smerdon, W.J, Jewell, K. (2004): *Listeria monocytogenes* and listeriosis: a review of hazard characterization for use in microbiological risk assessment of foods. *Int J Food Micrbiol*., 92: 15-33
74. Molla, B., Yilma, R., Alemayehu, D. (2004): *Listeria monocytogenes* and other *Listeria species* in retail meat and milk products in A.A., Ethiopia. *Ethiop J Health Dev*., 18: 131-212.
75. Morobe, I., C., Obi, C., L., Nyila, M., A., Gashe, B., A. and Matsheka, M., I., (2009): Prevalence, antimicrobial resistance profiles of *Listeria monocytogenes* from various foods in Gaborone, Botswana, *Afri. J. of Biotechnol*., 8: 6383-6387.
76. Morrow, R.A., Harrison, M.A. and Harrison, J.A. (2004): Fate of acid adapted and none adapted E. coli, L. monocytogenes and Salmonella on ground or whole beef jerky. Center for Food Safety, University of Georgia, Griffin, GA (http://www.griffin.uga.edu/cfs).
77. Mugampoza, D., Muyanja, C., Ogwok, P., Serunjogi, M., Nasinyama, G. (2011): Occurrence of *Listeria monocytogenes* in bulked raw milk and traditionally fermented dairy products in Uganda. African *J. Food, Ag, Nut. Dev*, 11: 4610-4622.
78. Muhammed, W., Muleta, D., Deneke, Y., Gashaw, A. and Bitew, M. (2013): Studies on occurrence of *Listeria monocytogenes* and other species in milk and milk products in retail market of Jimma town, Ethiopia *Asian J. Dairy and Food Res.*, 32: 35-39.
79. Mulu, S., (2014): Studies on the prevalence, risk factors, public health implication and antibiogram of *Listeria monocytogenes* in sheep meat collected from municipal abattoir and butcher shops in Addis Ababa.
80. National Committee for Clinical Laboratory Standards, (1988): Performance Standards for Antimicrobial Disc Susceptibility Test. National Committee for Clinical Laboratory Standards; Villanova, PA: Publication M2-T4.
81. Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F., van der Giessen, J., Kruse, H.(2010): Food borne diseases the challenges of 20 years ago still persist while new ones continue to emerge: *Int. J. Food Microbiol*., 139: 3–15.
82. Nightingale, K.K., Schukken, Y.H. and Nightingale, C.R (2004): Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Applied Environmental Microbiology*., 70: 4458-4467.
83. O', Donnell, E.T. (1995): The Incidence of Salmonella and Listeria in raw milk from farm bulk tanks in England and Wales. *Int. J. Dairy Technol.*, 48:125–129. Oevermann, A., Andreas, Z. and Vandevelde, M. (2010): Rhombencephalitis caused by *Listeria monocytogenes* in humans and ruminants: A zoonosis on the rise in disciplinary perspectives on infectious disease, Hind awi Publishing Corporation, 1-22.
84. OIE (2008): *Listeria monocytogenes* OIE terrestrial manual. Pp.12391254. wwwoie.int/eng/normes/manual/A-index htm.
85. OIE (2014): *Listeria monocytogenes.* Version adopted by the World Assembly of Delegates of the OIE in May 2014.Paris.
86. Oliver, S.P., Jayarao, B. M., Almeida R.A. (2005): Food borne Pathogens in Milk and the Dairy Farm Environment: Food Safety and Public Health Implications. *J Food.Pathoges .Dis.*, 2: 115-129.
87. Ooi, S., T. and Lorber, B. (2005): Gastroenteritis due to *Listeria monocytogenes. J. Clin. Infect. Dis.*, Issue.
88. Opinion of the scientific committee on veterinary measures relating to Public Health on *Listeria monocytogenes*, (1999): European commission. Scientific health opinions. http://europa.eu.int.
89. Osaili, T., Al-Nabulsi, A., Taha, M., Al-Holy, M., Alaboudi, A. k., Al-Rousan, W. and Shaker, R. (2012): Occurrence and Antimicrobial Susceptibility of *Listeria monocytogenes* Isolated from Brined White Cheese in Jordan .*J. of Food Science; Issue.*, 44: 86-91.
90. Pal, A., Labuza, T.P. and Diez-Gonzalez, F., (2008): Comparison of primary predictive models to study the growth of *Listeria monocytogenes* at low temperatures in liquid cultures and selection of fastest growing ribotypes in meat and turkey product slurries. *J. Food Microb.*, 25: 460–470.

91. Pal, M., (2007): Zoonoses Second edition Satyam Publishers, Jaipur, India. Pp.118-119.Pal M., Bekele T. and Feleke A. (2012 a): Public health significance of pasteurized milk. Beverage Food World, 39: 55-56.
92. Palumbo, J. D., Borucki, M. K., Mandrell, R. E. and Gorski, L., (2003): Serotyping of *Listeria monocytogenes* by enzyme-linked immunosorbent assay and identification of mixed- serotype cultures by colony immunoblotting. *J Clin Microbiol.*, 41: 564–571.
93. Parihar, V. S., (2004): Zoonotic Aspects of *Listeria monocytogenes*, Master of Science Programme in Veterinary Medicine for International Students Faculty of Veterinary Medicine and Animal Science Swedish University of Agricultural Sciences, 10: 9-10.
94. Peeler, J. T. and Bunning, V. (1994): Kelly Hazard assessment of *L. monocytogenes* in the processing of Bovine milk. *J Food Prot*., 57: 689-697.
95. Perrin, M., Bemer, M., Delemare, C. (2003): *Fatal Case of Listeria innocua Bacteremia. J Clin Microbiol*., 41: 5308–5309.
96. Quinn, P. J., Carter, M. E., Markey, B. K. and Carter, G. R. (2004): Clinical Veterinary Microbiology. Elsevier Limited, Philadelphia, USA, Pp. 331-344.
97. Radostits, O.M., Gay, C.C., Hinche lift, K.W., and Constabel, P.D. (2007): Veterinary Medicine, A text book of the disease of cattle, sheep, pig, goat and horses.10th ed., Elsevier, London, Pp. 1007-1040.
98. Rahimi, E., M., Ameri & H., Momtaz (2010): Prevalence and antimicrobial resistance of *Listeria* species isolated from milk and dairy products in Iran. *Food Control*., 21: 1448–1452.
99. Ramaswamy, V., Cresence, V.M., Rejith,a J.S., Lekshmi, M.U., Dharsana, K.S., Prasad, S.P, and Vijila, H.M. (2007): *Listeria* Review of Epidemiology and Pathogenesis. *Journal of Microbiology, Immunology and Infection*., 40: 4-13.
100. Richard, L., Laurie, C. and Judy, D., (2008): The Food Safety Hazard Guide Book. Food Safety Information, London, 7: 47-49.
101. Rocourt, J. and Cossart, P. (2001): *Listeria monocytogenes.* In: Doyle MP, Beuchat LR and Montville TJ (eds), Food microbiology fundamentals and frontiers. ASM Press, Washington D.C. U.S.A, p_p 337-351.
102. Rocourt, J., Buchrieser, C. (2007): The genus *Listeria* and *Listeria monocytogenes* ,Phylogenetic position, taxonomy, and identification. Ch 1 In: Ryser ET, Marth EH (eds) Listeria, listeriosis and food safety. 3rd ed, CRC Press Taylor and Francis Group, Boca Raton, Pp. 1–20.
103. Rodas-Sua´rez, O. R., Flores-Pedroche, J.F., Betancourt-Rule, J. M. Quin, ones-Ramı´rez, E.I. and Va´zquez-Salinas, C. (2006): Occurrence and Antibiotic Sensitivity of Listeria.
104. Rohrbach, Barton, W., Draughon, Ann, F., Michael, Davidson, P., Stephen and Oliver, P. (1992): Prevalence of *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia entercolitica* a *Salmonella* in Bulk Tank milk: Risk factors and Risk of human exposure. *J Food Prot*., 55: 93-97.
105. Ryser, E.T., Marth, E.H. (2007): *Listeria*, *Listeriosis*, and Food Safety, Taylor and Francis, Boca Raton, FL.
106. Ryu, C.H., Igimi, S., Inoue, S. and Kumagai, S., (1992): The incidence of *Listeria* species in retail foods in Japan. *Int J Food Microbiol*., 16: 157-160.
107. Saito, A., Sawada, T., Ueda, F. and Hondo, R. (1998): Classification of *Listeria monocytogenes* by PCR-restriction enzyme analysis in the two genes of *hly*A and *iap*. New Microbiol, 21: 87–92.
108. Schuchat, A., Swaminathan, B. and Broome, C.V. (1991): Epidemiology of human listeriosis. *J Cli, Microbiol,* 4: 169–183.
109. Schukken, Y.H., Grohn, Y.T. and Wiedemann, M. (2003): Epidemiology of listeriosis. In: Torrence ME and Issacson RE (Eds), Microbial food safety in animal agriculture Current topics. Iowa State Press, Iowa, USA, pp 221-232.
110. Seeliger, H. P. R. and Hohne, K., (1979): Serotyping of *Listeria monocytogenes* and related species. Methods Microbiol, 13: 31–49.
111. Selamawit, M. (2014): studies on the prevalence, risk factors, public health implication and antibiogram of *listeria monocytogenes* in sheep meat collected from municipal abattoir and butcher shops in Addis Ababa. Msc. Thesis, Addis Ababa University Department of Microbiology, Immunology and public health Faculty of Medicine, Addis Ababa, Ethiopia, Pp.1-98.
112. Sharma, D., Kuma, P., Saharan, B. and Malik, A. (2012): Isolation, Identification and Antibiotic Susceptibility Profiling of Antimicrobial Resistant *Listeria monocytogenes* from Dairy Milk, *Internat. J. of Microbial Resource Techno.*, 1: 1-41.
113. Siegman-Igra, Y., Levin, R. and Weinberger, M. (2002): *Listeria monocytogenes:* infection in Israel and review of cases worldwide. *Emerg, Infect. Dis*., 8: 305-310.
114. Skovgaard, N. and Morgen, C.A., (1988): Detection of *Listeria* spp. in faeces from animals, in feeds, and in raw foods of animal origin, *Int J Food Microbiol*., 6: 229-242.
115. Slifko, T.R., Smith, H.V. and Rose, J.B. (2000): Emerging parasite zoonoses associated with water and food. *Intl. J. Parasitol*., 30: 1379–1393.
116. Soni, D.K., Singh, R.K., Singh, D.V. and Dubey, S.K. (2013): Characterization of *Listeria monocytogenes* isolated from Ganges water, human clinical and milk samples at Varanasi, India. *Appl. Environ. Microbiol.,* 68: 6273-6282.
117. Soni, D.K., Singh, R.K., Singh, D.V. and Dubey, S.K., (2008): Characterization of *Listeria monocytogenes* isolated from Ganges water, human clinical and milk samples of Varanasi, India, Infection, Genetics and Evolution, 14: 83-91.

118. Stephan, R., Cernela, N., Ziegler, D., Pflüger, V., Tonolla, M., Ravasi, D., Fredriksson-Ahomaa, M. and Hächler, H., (2011): Rapid species specific identification and subtyping of Yersinia enterocolitica by MALDI-TOF mass spectrometry. *J. Microbiol*., 87: 150–153.
119. Stuart, M.R. and Pease, P.E., (1972): A numerical study on the relationships of *Listeria* and *Erysipelothrix*. *J Gen Microbiol*., 73: 551-565.
120. Stuart, S.E. and Welshimer, H.J., (1974): Taxonomic re-examination of Listeria Pirie and transfer of *Listeria grayi* and *Listeria murrayi* to a new genus Murrayi. INt. *J. System. Bact.*, 23: 30-66.
121. Swaminathan, B. and Gerner-Smidt, P. (2007): The epidemiology of human *listeriosis. J. Microbes Infect.*, 9: 1236-1243.
122. Tasci, F., Turutoglu, H. and Ogutcu, H. (2010): Investigation of *Listeria* species in milk and silage produced in Burdur province. *J. Kafkas Univ Vet Fak Derg*., 16:93 – 97 Tefera, H. (2014): Prevalence and Antibiotic Susceptibility of *Listeria* Species in Raw Milk and Dairy Products from North Shewa Zone, Oromia Regional State, Ethiopia. Master of Science in Microbiology School of Graduate Studies Haramaya University.
123. Teuber, M., (1999): Spread of antibiotic resistance with food borne pathogens. *J. Cell Mol Life Sci*., 56: 755–63.
124. Thrusfeld, M. (2007): Veterinary Epidemiology, 3rd ed .Singapore, Black well Science, 233.
125. Todar's online textbook of Bacteriology (2003): Listeria monocytogenes and Listeriosis. Kenneth Todar University of Wisconsin-Madison Department of Bacteriology USFDA/CFSAN, (2003): Detection and Enumeration of *L. monocytogenes* in foods, Bacteriological Analytical Manual (BAM); January, http://www.cfsan.fda.gov [Chapter 10].
126. Uyttendael, M., Neyts, K., Lips, R. and Debevre, J., (1997): Incidence of *Listeria monocytogenes* in poultry and poultry products obtained from Belgian French abattoir. *Int. J Food Microbiol*., 14: 339-343.
127. Uyttendaele, M., Troy, P.D. and Debevere, J. (1999): Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. *Int J Food Microbiol*., 53: 75-80.
128. Valk, H., de, Jacquet, C., Goulet, V., Vaillant, V., Perra, A., Simon, F., Desenclos, J.C. and Martin, P., (2005): Surveillance of Listeria infections in Europe. *J. Euro Surveill*., 10: 251-5.
129. Vázquez-Boland, J.A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., González-Zorn, B., Jürgen, Wehland, J. and Kreft, J. (2001): *Listeria* Pathogenesis and Molecular Virulence Determinants. *J. Clin .Microbiol.*, 14: 584-640.
130. Vitas, A.I., Aguadoe, V. and Garcia-Jalon, I. (2004): Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). *Int J Food Microbiol.*, 90: 349-356.
131. Vogel, B.F, Jorgensen, L.V., Ojeniyi, B., Huss, H.H. and Gram, L. (2001): Diversity of *Listeria monocytogenes* isolates from cold-smoked salmon produced in different smokehouses as assessed by random amplified polymorphic DNA analyses. *Int. J. of Food Microbiol*., 65: 283–292.
132. Volokhov, D., Rasooly, A., Chumakov, K. and Chizhikov, V. (2002): Identification of *Listeria species* by microarray-based assay. *J Clin Microbiol*, 40: 4720–4728.
133. Wang, R. F., Cao, WWW ang, H. and Johnsonm, M. G. (1993): A 16S rRNA-based DNA probe and PCR method specific for Listeria ivanovii. *J.FEMS Microbiol*., 106: 85–92.
134. Wang, R.F., Cao, W. W. and Johnson, M. G. (1992): 16S rRNA-based probes and polymerase chain reaction method to detect *Listeria monocytogenes* cells added to foods. *Appl Environ Microbiol.*, 58: 2827–2831.
135. White, S. L. (2010): Virulence Attenuation and Relative Fitness of *Listeria monocytogenes* from the Processing Plant Environment. A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, North Carolina, 3-4p.
136. WHO (1988): Food borne listeriosis: Report of a WHO informal working group, World Health Organization. Geneva. Switzerland Pp. 2-18.
137. WHO (2009): Codex Alimentarius, Food hygiene Basic texts, Fourth edition. Rome.
138. WHO/FAO (2004): Risk assessment of *Listeria monocytogene*s in ready-to-eat foods. Technical Report. Series; No. 5 Pp. 1-265.
139. Wing, E.J. and Gregory, S.H. (2000): An updated model of cell mediated immunity – listeriosis: clinical and research aspects. *J. Allergy Asthma Proc*, 21: 209–214.
140. Yadav, M.M., Roy, A., Bhanderi, B. and Joshi, C. (2010): Pheno–genotypic characterization of *Listeria monocytogenes* from bovine clinical mastitis, Buff. Bull, 29: 29 – 38.

7. APPENDICE

Appendix 1: Listeria Enrichment Broth (LEB).

Peptone mixture	20 gm
Yeast extracts	6.0 gm
Sodium chloride	5.0 gm
Potassium dihydrogen phosphate	2.5 gm
Glucose	2.5 gm
Esculin	1g
Distilled water	1000 mL
Cyclohexamide	0.05gm
Nalidixic Acid 2% solution in 0.1M NaOH)	0.04 gm
Acriflavin HCL	0.015gm

Preparation of culture media used for isolation and identification of *L. monocytogenes.* Sterilize at 121oC for 15 minutes. Do not overheat; cool at once after removal from the sterilizer.

Appendix 2: Oxford Agar (OXA).

Columbia blood agar base	39.0 g
Esculin	1.0 g
Ferric ammonium citrate	0.5 g
Lithium chloride	15.0 g
Cycloheximide	0.4 g
Colistin	0.02 g
Acriflavin	0.005 g
Distilled or deionised water	1000 Ml

Suspend the ingredients in the water. Bring to a boil to dissolve completely. Sterilize by autoclaving at 121oC for 15 min. Cool to 50oC.

Appendix 3: Trypticase Soy Agar with 0.6% Yeast Extract (TSA-YE).

Trypticase soy agar	40.0 g
Yeast extracts	6.0 g

Appendix 4: Blood Agar Base.

Heart muscle infusion from (solids)	2.0
Pancreatic digest of casein	13.0
Yeast extract	5.0
Sodium chloride	5.0
Agar	15.0

Suspend 40g of powder in to one litter of purified water and mix thoroughly. And heat with frequent a agitation and boil for one minute to completely dissolves the powder and autoclave at 121oC for 15minutes(N.B do not over heat).Then Cool the base to 45^{o}C -50^{o}C and add 5% sterile, defibrinated sheep blood and finally dispense in to sterile Petri dishes.

Appendix 5: Carbohydrate utilization broths (Purple broth base).

Peptone from casein	5.0g
Peptone from meat	5.0g
Sodium chloride	5.0g
Phenol red	0.018g

Dissolve 15g of powder in to one litre of purified water and autoclave at 121^{o}C for 15minutes and cool to 60^{o} C.

Appendix 6: Carbohydrate solutions.

Rhamnose	5g
Xylose	5g
Mannitol	5g

Dissolve 5g of each carbohydrate in 100ml of water separately and sterilize by filtration

For each carbohydrate, add aseptically 0.5ml of filter sterilized carbohydrate solutions to 4,5ml of phenol red solutions prepared.

Appendix 7: Procedure Isolation and identification procedure of Listeria species

Each 25 ml or gm of samples to add 225 ml LEB broth. Incubate at 30oC for 48 h. Streak positive LEB onto selective agar plates (Oxford & PALCAM). Re-incubate negative LEB for an additional 24 h. Incubate plates for 24-48h.

Streak 5 isolates for each positive sample onto TSA. Incubate at 350 C for 24h.

Appendix 8: Values of antibiotic susceptibility test of l. monocytogenes were performed according to Clinical and Laboratory Standards Institute standard reference procedure.

Antibiotics	**Disc code**	**Disc potency**	**Inhibition zone diameter to nearest mm**		
			Resistance	**Intermediate**	**Susceptible**
Ampicilline	AM		≤13	14-16	≥17
Sulfamethoxazole	SXT		≤10	11-15	≥16
Amoxicillin	AML		<13	14-17	>18
Streptomycin	S		<14	15-20	>21
Penicillin	P		≤14	15	>16
Tetracycline	TE		≤14	15-18	≥19