

Isolation, Identification and Antimicrobial Susceptibility of *Listeria* Species from Raw Bovine Milk in Debre-Birhan Town, Ethiopia

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Abstract

Listeriosis is a disease of humans and animals, in which it is one of the important emerging bacterial zoonotic diseases worldwide. Among the different species of *Listeria*, *Listeria monocytogenes* is the most common causes of Listeriosis in humans and animals, as a result of food and environmental contamination with low incidence but high case fatality rate. The present study was undertaken to isolate *Listeria* species in raw bovine milk. A cross-sectional study was conducted from January to September 2015 to determine the presence of *Listeria monocytogenes* and other *Listeria* species from raw milk samples originated from dairy producers and vendors and determining antimicrobial resistance profile of *Listeria monocytogene*. A total of 407 raw milk samples of which, 384 from dairy producers were collected by using simple random sampling technique and 23 from vendors were also collected. *Listeria* species isolations were performed according to the standard bacteriological techniques by using *Listeria* enrichment broth, Modified Fraser broth and Oxford Agar medium as well as confirmatory tests: carbohydrate utilization (rhamnose, xylose, mannitol); blood agar (hemolysis) and Christie Atkins Munch Peterson (CAMP) test. The antimicrobial resistance profile of *Listeria monocytogenes* was also assessed by using the standard disk diffusion method (Kirby Bauer techniques) and it was tested against 9 antimicrobial drugs (Cephalothin 30 µg, Chloramphenicol 30 µg, Kanamycin 30 µg, Nalidixic acid 30 µg, Streptomycin 10 µg, Tetracycline 30 µg, Vancomycin 30 µg, Gentamicin 10 µg and Ampicillin 10 µg). Overall isolated *Listeria* species were *Listeria monocytogenes* 36 (8.84%), *Listeria innocua* 28 (6.88%), *Listeria seeligeri* 14 (3.4%), *Listeria grayi* 3 (0.74%), *Listeria welshimeri* 2 (0.49%) and *Listeria murrayi* 2 (0.49%). Antimicrobial susceptibility test was conducted on 36 isolated *Listeria monocytogenes*. *Listeria monocytogenes* were found to be resistant to two or more antimicrobial. The presence of *Listeria monocytogenes* in raw milk and drug resistant isolates of the bacteria is an indication of public health hazards to the consumers, particularly to the high risk groups. Therefore awareness creation on milk safety and implementations of regulations about the use of antimicrobials in humans and animals should be strongly practiced.

Keywords: Antimicrobials; Debre-Birhan; Detection; Isolation; *Listeria monocytogene*; Raw milk; Susceptibility

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Introductions

The genus *Listeria* is a group of closely related Gram-positive, facultative anaerobic, non-spore forming, rod shaped, and motile bacteria. Genus *Listeria* comprises ten species, i.e.,

L. monocytogenes, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. grayi*, *L. murrayi*, *L. marthii*, *L. fleischmannii*, and *L. weihenstephanensis*, [1]. *Listeria monocytogenes* is the principal pathogen in humans and animals. *Listeria ivanovii* is a pathogen of animals but is occasionally implicated in human disease. The

other *Listeria* species are generally considered as non-virulent [2].

Listeria monocytogenes, the causative agent of Listeriosis is ubiquitous in the environment and has been recognized as animal pathogen since the 1920s but in the past two decades, it has been implicated in several outbreaks of food-borne illness in humans [3,4]. Contamination of silage leads to infection of farm animals resulting in possible human infection by way of the food chain [5,6]. Food-borne transmission constitutes the main acquisition route of Listeriosis [7].

In humans, food-borne *L. monocytogenes* causes large outbreaks of Listeriosis, with a mortality rate of 9% to 44% [8]. Certain sections of the population are predisposed to the development of Listeriosis due to presence of existing chronic illness, suppression of the immune system, pregnancy, or extreme youth or age (under 1 year or over 60 years) [5]. This presents a significant public health problem because in such population, Listeriosis is fatal in up to 30% of cases [9] which may increase up to 75% in high risk groups such as pregnant women, neonates and immune-compromised persons [10].

L. monocytogenes the leading cause of death among bacterial pathogens acquired primarily through the consumption of contaminated foods [11]. Transmission is generally through eating contaminated food, particularly dairy products made from unpasteurized milk and ready-to-eat meat and fish products [12]. The incidence of *L. monocytogenes* in soft and semi-soft cheese varied from 0.50% to 46.00% [3,13,14].

Raw milk can be contaminated from the environment or by direct excretion into the milk; therefore, consumption of raw milk is associated with increased risk factors. *L. monocytogenes* has been largely studied in the past decades because of its importance as a food-borne human pathogen [15,16]. Ongoing efforts are needed to further reduce the incidence of listeriosis, due to the manifestation of its high mortality rate [17].

Listeria species detection and identification in foods traditionally involve culture methods based on selective pre-enrichment, enrichment and plating. This is followed by the characterization of *Listeria* species using colony morphology, sugar fermentation and haemolytic properties [18,19]. In the last years there has been a notable development of new culture media for the improved detection of *L. monocytogenes* in foods, and efficient methods based on antibodies or molecular techniques have also been developed [18,19].

In developing countries most of the time, there have been few or no reports on *L. monocytogenes*. This is true because no one has given it attentions or awareness on the occurrence of *L. monocytogenes* in food [20]. In Ethiopia, there is inadequate published information regarding to the prevalence and antimicrobial susceptibility patterns of *Listeria* species in both Veterinary and Public health sectors [21]. Therefore, the present study was undertaken to isolate *Listeria* species from raw milk and to determine the antimicrobial resistance profile.

Materials and Methods

Study area

The study was conducted in Debre-birhan town, North Shoa, Ethiopia. The Town is located 130 km away from the capital city (Addis Ababa) at 9°41' N latitude and 39°32' E longitude and the elevation is ranged from 2700 to 2800 meter above sea level. The area receives annual rain fall ranges from 814 to 1080 mm with about 70% rain falling between June and September. The annual average temperature of the town ranges between 4°C in the coldest month (August) to 26°C in the hottest month (April) which is categorized under “dega” or tropical climate weather condition [22].

Study design

A cross-sectional study was conducted at Debre-Birhan town, to determine the prevalence of *L. monocytogenes* and other *Listeria* species, and also the antimicrobial susceptibility profile of *L. monocytogenes*.

Sample size determination

Since there was no previous study in the area on raw milks from dairy producers, sample size was estimated by taking 50% expected prevalence with 95% confidence interval and 5% desired absolute precision [23]. Accordingly a total of 384 dairy producers were included in this study. In addition, 23 venders were also sampled for the detections of *L. monocytogenes*.

Sampling method and collection

Study samples were selected using simple random sampling technique and 384 dairy producers that deliver their milk to 13 milk collection centers were sampled. All dairy producers encountered in this study were smallholder dairy farms having one to five, and some are more than five lactating dairy cows. Each raw milk samples were collected in sterile snap-cap milk (sterile plastic containers). 30 ml of raw milk were collected aseptically in sterile plastic containers according to Jorgensen [24], from milk containers of the dairy producers at the sites of milk collection centers. 23 milk samples from bulk milk container of vendor's were also taken. The collected samples were identified by date of collection, code of the sample and their source. All milk samples were transported immediately by using icebox to Debre-brihan agricultural research center microbiology laboratory and stored at 4°C until analysis.

Isolation and identifications of *Listeria* species

Bacteriological examination was done to isolated *L. monocytogenes* and other species of *Listeria* from raw milk samples. For the isolations and identifications of *Listeria* species in food samples, the techniques recommended by the International Standards Organization (ISO 11290-1, 1996) [25] and the French Association for Standardization [26], were employed. Oxford agar was used for selective plating and identification of *Listeria* species.

Primary and secondary selective enrichment

Twenty five milliliters of each sample from the composite mixture was measured and transferred into 225 ml of previously prepared half Fraser broth followed by mixing the suspended sample for two minutes using a laboratory blender at 2400 rev/min. Finally the samples were incubated at 30°C for 24 hrs. After 24 hrs of incubation, the inoculated half Fraser suspension was mixed by swirling the Erlenmeyer flask and 0.1 ml was drawn and inoculated into 10 ml of Fraser broth and then incubated for 48 hrs at 35°C. Growth in the Frazer broth was very apparent, with colour change from the initial golden yellow to dark green or black; unlike in the half Fraser which has no showed colour change.

Isolation and identification

As a continuation of the procedure from the selective enrichment broth, a positive Fraser broth media with black /dark brown or dark green color was inoculated by taking a loop-full of the suspension into selective media Oxford agar (OXA) and the plate were incubated at 37°C for 48 hrs. Growth of 1-2 mm diameter black or black green colony with a black halo and black sunken center was taken as positive for *Listeria* species. The colonies of *Listeria* were identified as per Falana [27], on Oxford agar colonies appeared brown black or greenish black with a depressed center and a surrounding black halo. When the colonies were grown and well isolated on the selective media, about 3-5 colonies from each medium was picked and inoculated into pre dried plates of Tryptone Soya Yeast Extract Agar and incubated for 18 to 24 hrs at 37°C to obtain pure culture. Any grey-whitish colony was suspected to be positive for *Listeria*. Typical colonies (1 mm to 2 mm in diameter, convex, colorless and opaque), were used for further biochemical tests. Gram staining, Catalase test, Motility test, and CAMP tests were also carried out. For the carbohydrate utilization test, the isolated colonies from TSYEA were transferred into test tubes that contain Xylose, Rhamnose and Mannitol and incubated at 37°C for up to 5 day. A positive reaction (acid formation) was indicated by yellow color production and occurred mostly within 24 h to 48 h (Table 1) [28].

Antimicrobial susceptibility test

Antimicrobial susceptibility test were performed for *L. monocytogenes* isolates by using Muller Hinton Agar. The common conventional antimicrobial drugs (cephalotin, Nalidixic acid, chloramphenicol, kanamycin, steptomycin, tetracycline,

vancomycin, gentamicin and Ampicillin) were tested. The method applied for antimicrobial testing was agar plate antibiotic disk diffusion method by adjusting to 0.5 McFarland turbidity standards [29,30]. About 2-3 pure colonies of the isolates were taken from the Tryptone Soya Yeast Extract Agar and suspended in Muller Hinton broth and then, incubated at 37°C for 1-2 hrs. The suspension was checked for the development of slight turbidity. It was inoculated to Mueller-Hinton agar plate by using a sterile cotton swab. Plates were seeded uniformly by rubbing the swab against the entire agar surface. After the inoculums were dried, antibiotics impregnated disc were firmly placed on the surface of the inoculated plates. The plates were incubating at 37°C for 24 hrs. After incubation the zones of inhibition around each disc were measured and the results were interpretate as sensitive, intermediate and resistant using a standard zone interpretative chart [31].

Quality control

The correct performance of all stages of the analysis, including enrichment, screening tests, plating and all confirmatory tests were verified through the use of *L. monocytogenes* (ATCC 11911) reference strains. *Staphylococcus aureus* (*S. aureus*) (American type culture collection (ATCC) 25923), *L. monocytogenes* (ATCC 11911) kindly obtained from the Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia.

Data management and analysis

Microsoft Excel was utilized for data entry and storage and analysis were done by using statistical package for social science (SPSS 2007, version 16). Descriptive statistics were used to describe and process the data. Comparisons between detection rates of groups were analysed by using Chi square (χ^2). For all tests, p-value less than 0.05 were considered to be significant.

Result

A total of 407 samples of raw bovine milk, out of which 384 from Dairy producers and 23 from Vendors were sampled for isolation and identifications of *Listeria* species by bacteriological examination, 85 (20.88%) proved positive for *Listeria*. The identification results showed that raw bulk milk from vendors was highly contaminated with *Listeria* species with the detection rates of 30.4% and raw milk from dairy producers were 20.3%. Statistically there was no significant difference ($P>0.05$) with *Listeria* isolation among dairy producers and vendor at the study area (Table 2).

Table 1: Differentiations of main *Listeria* species [28].

Species	Production of acid from			Hemolysis	CAMP test <i>S. aureus</i>
	Xylose	Rhamnose	Mannitol		
<i>L. monocytogenes</i>	-	+	-	+	+
<i>L. innocua</i>	-	+/-	-	-	-
<i>L. ivanovii</i>	+	-	-	+	-
<i>L. seeligeri</i>	+	-	-	(+)	(+)
<i>L. welshimeri</i>	+	+/-	-	-	-
<i>L. Grayi</i>	-	-	+	-	-
<i>L. Murrayi</i>	-	+	+	-	-

+/-: Variable; +: Weak reaction; >: positive reaction; -: no reaction

In this study, both pathogenic and nonpathogenic *Listeria* species were isolated from raw milk samples of dairy producers and vendors in the study area. The detections of *L. monocytogenes* in both dairy producers and vendors were 8.6% and 13% respectively (Table 3).

As depicted in the above Table 3, the level of identification of *L. monocytogenes* in vendor was 1.6 times (OR=1.6, CI=0.45-5.65) higher compared to milk samples originated from dairy producers. But it was not statistically significant ($p=0.75$).

The bacteriological analysis indicated that *L. monocytogenes* and *L. innocua* were found with higher prevalence followed by *L. seeligeri*. The frequency of isolation of *Listeria* species from raw milks of the dairy producers and vendor are summarized as depicted below in (Table 4).

Antimicrobial susceptibility test

Of the total 36 *L. monocytogenes* species subjected for antimicrobial susceptibility test, 11 (30.5%) exhibited resistance for Nalidixic acid, 9 (25%), 8 (22.2%) and 4 (11.1%) of *L. monocytogenes* were resistance to Tetracycline, Chloramphenicol and Streptomycin's respectively. All of *L. monocytogenes* isolated in this study were sensitive to Cephalotin, Kanamycin, Vancomycin and Ampicillin (Table 5).

Of these *L. monocytogenes* exhibited resistance for one or more antimicrobials, one (2.7%) *L. monocytogenes* were resistance to four antimicrobials (Streptomycin, Chloramphenicol, Tetracycline

and Nalidixic acid), Nine (25%) *L. monocytogenes* were resistance to two antimicrobials and Ten (27.8%) of *L. monocytogenes* were resistance to one antimicrobial.

Discussion

In this finding raw milk samples were analyzed for the presence of *L. monocytogenes* and other *Listeria* species. The overall isolations of *Listeria* species were 20.88% from 407 raw milk samples examined. The present finding was comparable with other reports in Ethiopia; Addis Ababa 22 % and Gondar 25% from raw milk samples [21,32], respectively. In others countries like Uganda, 60% of bulk raw milk were positive for *Listeria* species [33], which is with higher prevalence compared to the present studies (20.88%). Such differences are might be due to poor hygiene and sanitation activities in the milk production, processing and supplying chains, since the origins of contaminations of milk with *Listeria* are mainly faeces [34]. In Ethiopia relatively low prevalence's of *Listeria* were reported in Jimma town 14 % and in Addis Ababa 8.3% from raw milk samples [35,36], respectively. These different isolations might be due to either by the difference of samples sizes or hygienic practicing and /or sample collecting origin. The isolated species of *Listeria* in the present study was *L. monocytogenes* 36 (8.8%), *L. innocua* 28 (6.88%), *L. seeligeri* 14 (3.4%), *L. grayi* (0.74%), *L. welshimeri* (0.49%) and *L. murrayi* (0.49%) indicated as diminishing orders.

In this study almost from both (dairy producers and vendors) sampling origin the presence of *L. monocytogenes* in raw milk,

Table 2: Isolation and detections of *Listeria* species in raw milk from Dairy producers and Vendors.

Sources of raw milk sample	No. of sample examined	No. of Samples positive for <i>L. mono.</i> (%)	No. of Samples positive for other <i>Listeria</i> sps (%)	Total positive (%)	χ^2	P-value
Dairy p.	384	33(8.59)	45(11.7)	78(20.3)	0.55	0.75
Vendors	23	3(13.04)	4(17.39)	7(30.4)	-	-
Total	407	36 (8.8)	49(12)	85(20.88)	0.55	0.75

Dairy p: Dairy producer; *L. mono.*: *L. Monocytogenes*; Sps: Species

Table 3: The prevalence of *L. monocytogenes* from different sources of samples.

Source of sample	Total examined	Prevalence (%)	OR	95% CI of OR		P-value
				Lower	Upper	
Dairy producer	384	33(8.6)	REF* 1.6	0.4503 - 5.6523		0.75
Vendor	23	3(13)				
Total	407	36(8.8)				

OR: Odds Ratio; CI: 95% Confidence interval, REF*: Reference

Table 4: Numbers of *Listeria* species isolated from raw milks of dairy producers and Vendors.

<i>Listeria</i> species Isolated	Dairy producers	Vendor	Total isolated spp.	Percentage (%)
<i>L. monocytogenes</i>	33	3	36	8.8
<i>L. ivanovii</i>	0	0	0	0
<i>L. innocua</i>	25	3	28	6.88
<i>L. seeligeri</i>	13	1	14	3.4
<i>L. welshimeri</i>	2	0	2	0.49
<i>L. grayi</i>	3	0	3	0.74
<i>L. murrayi</i>	2	0	2	0.49
Total <i>Listeria</i> isolates	78	7	85	20.88

Table 5: Antimicrobial resistance profiles of *Listeria monocytogenes* from milk samples.

Antimicrobial agent	Symbol	Disc Content	Numbers of isolates (%)		
			R	I	S
Cephalothin	KF	30 µg	-	-	36(100%)
Chloramphenicol	C	30 µg	8(22.2%)	10(27.7%)	18(50%)
Kanamycin	K	30 µg	-	-	36(100%)
Nalidixic acid	NA	30 µg	11(30.5 %)	9(25%)	16(44.4%)
Streptomycin	S	10 µg	4(11.1%)	5(13.8 %)	27(75%)
Tetracycline	T	30 µg	9(25%)	5(13.8%)	22(61.1%)
Vancomycin	VA	30 µg	-	-	36(100%)
Gentamicin	G	10 µg	-	7(19.4%)	29(80.5%)
Ampicillin	AMP	10 µg	-	-	36(100%)

R: Resistance; I: Intermediate; S: sensitive

higher bacterium isolation were observed in vendors. This might be due to supply chain contamination from dairy producer to the retailer transportations or might be the retailers' handling exposed to contaminations. Because of *L. monocytogenes* are found everywhere; in water, soil, vegetables and in healthy persons without showing symptom [2]. One of the main reasons for lower detection of *Listeria* in the dairy producers might be due to early supplying of the milk to collection centers where there are few chances of contamination from handlers and the environment [36].

Money researchers have been reported the occurrence of *Listeria* species in raw milks, according to Yakubu [37], report, out of 192 raw milk samples 76 (39.58%) were positive for *Listeria* species. Out of which 39 (20.3%) were *L. innocua*, 14 (7.3%) *L. ivanovii*, 17(8.9%) *L. monocytogenes*, 4(2.1%) *L. welshimeri* and 2(1%) *L. seeligeri*. Another studies conducted in Algeria by Hamdi [38], from raw milk samples, the isolations of *L. monocytogenes* were 7.7%. The detections of *L. monocytogenes* from raw milk was 6.5% in Ireland and 5.5% in Finland [39,40], respectively. A total of 240 milk samples from farm bulk milk tanks collected in Tehran province, Iran, 54 (22.5%) were positive for *Listeria* species out of which *L. monocytogenes* 13(5.4%), *L. welshimeri* 6(2.5%) *L. innocua* 33 (13.8%) and *L. seeligeri* 2 (0.8%) [41]. From these reports of *Listeria* species isolation, *L. monocytogene* were comparable with the present findings. Whereas other more recent reports in the isolations of *Listeria* species from unpasteurized raw milk were 13.46% and *L. monocytogenes* 4.81% [42], relatively lower detection compared to the present studies. Similarly D'costa [43], study showed that the isolations of *L. monocytogenes* was 4.82% in raw milk samples from different markets of Mumbai city, this study result was lower compared to the present findings.

Antimicrobial resistance profiles of *L. monocytogenes* were exhibited in this study. Of these 58.3% of isolated *L. monocytogenes* were resistances at least for one or more antimicrobials tested. One (2.7%), Nine (25%) and Ten (27%) of *L. monocytogenes* were resistance to four antimicrobial (Streptomycin, Chloramphenicol, tetracycline and Nalidixic acid), two antimicrobial and one antimicrobials respectively. Further analysis of antimicrobial susceptibility tests result showed that 25% of the isolated *L. monocytogenes* were resistance to tetracycline which might

be ascribed to high level utilization of this antibiotic due to its relatively cheaper price and easily accessible. Tetracycline is one of the most frequently prescribed drugs in the current study area for most of infectious diseases in veterinary medicine that could be mentioned as one of the reasons for the development of such higher resistance profile. Recent study conducted by saha [44] *L. monocytogenes* were 100% sensitivity to penicillin, tetracycline and gentamicin. In our finding, majority of *L. monocytogenes* isolated were susceptible to Cephalotin and Vancomycin which was in line with the recent study in Gondar, Ethiopia [21].

Resistance of *L. monocytogenes* to nalidixic acid, tetracycline and chloramphenicol were observed in the present study which is similar with studies conducted by [3,43,44]. The presence of antimicrobial resistant *L. monocytogenes* in raw food products has an important public health implication especially in developing countries where there is a wide spread and uncontrolled use of antimicrobials [45]. The problem can be higher in Ethiopia since consumption of raw meat, raw milk and raw milk products are very common and large number of high-risk population are found in the country. The increased antimicrobial administration to animals can cause the development of antimicrobial resistance *L. monocytogenes* [46]. Multidrug resistant *L. monocytogene* isolated from foods and human Listeriosis has been previously reported by Safdar and Marian [47-49]. The overall frequencies and patterns of resistance can vary remarkably from one country to another and also one reports to other even within a country.

Conclusion and Recommendations

In this study the results of bacteriological assessment showed that milk from dairy producers and vendors are positive for *Listeria*. In this finding both pathogenic *L. monocytogenes* and nonpathogenic *Listeria* species were isolated. The presence of *L. monocytogenes* in food can poses a threat to human life and can cause outbreaks of morbidity and mortality. Isolations of *L. monocytogenes* in raw milk and its antimicrobial resistant is an indication of public health hazards to the consumer, particularly to the high risk group such as young, elder, pregnant women and immune-compromised individuals. Raw milk intended for human consumption must be subjected for pasteurization or heat treatment at equivalent to pasteurization temperature. Further studies on the risk factors, molecular characterization

and serotyping of the bacteria should be carried out. Awareness creation on food safety and implementation of regulations about the use of antimicrobials in humans and animals should be strongly practiced.

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References

- Meloni D (2014) Focusing on the main morphological and physiological characteristics of the food-borne pathogen *Listeria monocytogenes*. *J Veterinary Sci Res* 1: 1-5.
- Liu D (2006) Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important food borne pathogen. *J Medical Microbio* 55: 645-659.
- Pintado C, Oliveira A, Pampulha M, Ferreira M (2005) Prevalence and characterization of *L. monocytogenes* isolated from soft cheese. *London J Food Microbio* 22: 79-85.
- Yucel N, Citak S, Onder M (2005) Prevalence and antibiotic resistance of *Listeria* species in meat products in Ankara, Turkey. *Food Microbio* 22: 241-245.
- Ramaswamy V, Cresence M, Rejitha J, Lekshmi M, Dharsana K, et al. (2007) *Listeria*-review of epidemiology and pathogenesis. *J Microbio Infection* 40: 4-13.
- Sleator R, Watson D, Hill C, Gahan C (2009) The interaction between *Listeria monocytogenes* and the host gastrointestinal tract. *J Microbiology* 155: 2463-2475.
- Churchill R, Lee H, Hall J (2006) Detection of *Listeria monocytogenes* and the toxin listeriolysin O in food. *Journal of Microbiology* 64: 41-70.
- Clark C, Farber J, Pagotto F, Ciampa N, Dore K, et al. (2010) Surveillance for *Listeria monocytogenes* and listeriosis, 1995-2004. *Epidemiology of Infection* 138: 559-72.
- Aurora R, Parakash A, Prakash S, Rawool D, Arbuddhe S (2008) Comparism of PIPLC based assay and PCR along with in vivo pathogenicity tests for rapid detection of pathogenic *Listeria monocytogenes*. *Food Control* 19: 641-647.
- Jalali M, Abedi D (2008) Prevalence of *Listeria* species in food products in Isfahan, Iran. *Int J Food Microbiology* 122: 336-340.
- Jemmi T, Stephan R (2006) *Listeria monocytogenes*: Food-borne pathogen and hygiene indicator, revue scientifique technique (International Office of Epizootics). *J Food-borne Pathogen* 25: 571-580.
- European Food Safety Authority (EFSA) (2009) The community summary report on trends and sources of zoonoses and zoonotic agents in the European Union. *EFSA Journal* 223: p320.
- Manfreda G, Cesarede A, Stella S, Cozzi M, Cantoni C (2005) Occurrence and ribotypes of *Listeria monocytogenes* in Gorgonzola cheeses, Amsterdam. *Int J Food Microbiology* 102: 287-293.
- Colak H, Hampikyan H, Bingol E, Ulusoy B (2010) Prevalence of *L. monocytogenes* and *Salmonella* spp. in Tulum cheese, London. *Food Control* 18: 576-579.
- Ryser E, Marth E (2004) *Listeria, listeriosis and food safety*. Third edition, Marcel Dekker Inc., New York, USA.
- Saran T (2013) Listeriosis and *Listeria monocytogenes* in India. *Wudpecker Journal of Food Technology* 1: 98-103.
- ILSI Research Foundation-Risk Science Institute (2005) Achieving continuous improvement in reductions in foodborne listeriosis. A risk-based approach. *Journal of Food Protection* 68: 1992-1994.
- Gasanov U, Hughes D, Hansbro M (2005) Methods for the isolation and identification of *Listeria* spp. and *L. monocytogenes*. A review. *Federation of Eur Microbiol Societies* 29: 851-875.
- Paoli G, Bhunia A, Bayles D (2005) *Listeria monocytogenes*. In: *Foodborne pathogens, microbiology and molecular biology*. Caister Academic Press, Wymondham, Norfolk, UK. PP: 295-325.
- Molla B, Yilma R, Alemayehu D (2004) *Listeria monocytogenes* and other *Listeria* species in retail meat and milk products in Addis Ababa, Ethiopia. *J Health Development* 18: 131-212.
- Garedew L, Taddese A, Biru T, Nigatu S, Kebede E, et al. (2015) Prevalence and antimicrobial susceptibility profile of *Listeria* species from ready-to-eat foods of animal origin in Gondar Town, Ethiopia. *J Bio-Medical Central Microbio* 15: 1-6.
- North Shoa zone Agricultural office (2015) Annual report.
- Thrusfield M (2007) *Veterinary Epidemiology*. Wiley-Blackwell publisher, Hoboken, New Jersey, USA; PP: 151-208.
- Jorgensen H, Mørk T, Høgasen H, Rørvik L (2005) Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. *Journal of Applied Microbiology*. 99: 158-166.
- International organization for standardization (ISO 11290-1) (1996) Microbiology of Food and feed stuffs-Horizontal method for the detection and enumeration of *Listeria monocytogenes*. ISO Geneva PP: 1-16.
- French Association for Standardization (AFNOR) (1993) Food microbiology detection of *Listeria monocytogenes* routine method. AFNOR PP: 8-55.
- Falana A, Denloye S, Mainasara O, Ubiaru M, Udegunaam C, et al. (2003) Determinations of profiles of human bacteria pathogens in Nigerian fish and seafood for export. *Food Africa Internet Forum*.
- World Organization for Animal Health (OIE) (2014) *Listeria monocytogenes*. OIE Terrestrial Manual 2014 PP: 1-18.
- Antunes P, Reu C, Jousa J, Pestana N, Peixe L (2002) Incidence and susceptibility to antimicrobial agents of *Listeria* species and *Listeria monocytogenes* isolated from Poultry Carcasses in Porto, Portugal. *Journal of food protection* 65: 1883-1893.
- Hansen M, Gerner-Smidt P, Bruun B (2005) Antibiotic susceptibility of *Listeria* moonocytogenes in Denmark 1958- 2001. *APMIS* 113: 31-36.

- 31 National Committee for Clinical Laboratory Standards (NCCLS) (2004) Interpretative Chart of Zone Inhibition Sizes (Kirby-Bauer disc diffusion method).
- 32 Gebretsadik S, Kassa T, Alemayehu H, Huruy K, 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 Public health* 81: 1-8.
- 33 Mugampoza D, Muyanja C, Ogwok K, Serunjogi M, Nasinyama G (2011) Occurrence of *Listeria monocytogenes* in bulked raw milk and traditionally fermented dairy products in Uganda. *African Journal of Food, Agriculture, Nutrition and Development* 11: 4610-4622.
- 34 Uhitil S, Jaksic S, Petrak T, Medic H, Gumhalter KL (2004) Prevalence of *Listeria monocytogenes* and other *Listeria* species in cakes in Croatia. *Food contrl* 5: 213-216.
- 35 Muhammed W, Muleta D, Deneke Y, Gashaw A, 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 Research* 32: 35-39.
- 36 Abera F (2007) Prevalence and antimicrobial profile of *Listeria monocytogenes* in retail meat and dairy products in Addis Ababa and its surrounding towns, Ethiopia. P: 81.
- 37 Yakubu Y, Salihu M, Faleke O, Abubakar M, Junaidu A, et al. (2012) Prevalence and antibiotic susceptibility of *Listeria monocytogenes* in raw milk from cattle herds within Sokoto Metropolis, Nigeria. *Sokoto J Veterinary Sci* 10: 13-17.
- 38 Hamdi T, Naïm M, Martin P, Jacquet C (2006) Identification and molecular characterization of *Listeria monocytogenes* isolated in raw milk in the region of Algiers (Algeria). *International J Food Microbio* 116: 190-193.
- 39 Fox E, Hunt K, O'Brien M, Jordan K (2011) *Listeria monocytogenes* in Irish Farm house cheese processing environments. *Inter J Food Microbio* 145: 39-45.
- 40 Ruusunen M, Salonen M, Pulkkinen H, Huuskonen M, Hellstrom S, et al. (2013) Pathogenic bacteria in Finnish bulk tank milk. *Food borne Pathogens and Disease* 10: 99-106.
- 41 Hossein J, Behrad R, Kwai-lin T (2013) Prevalence, characterization, and antimicrobial resistance of *Listeria* species and *Listeria monocytogenes* isolates from raw milk in farm bulk Tanks. *J Food Control* 34: 121-125.
- 42 Saha M, Debnath C, Pramanik A, Murmu D, Kumar R, et al. (2015) Studies on the Prevalence of *Listeria Monocytogenes* in Unpasteurized Raw Milk Intended for Human Consumption in and Around Kolkata, India. *Inter J Current Microbio Applied Sci* 4: 288-298.
- 43 D'Costa D, Bhosle S, Dhuri R, Kalekar S, Rodrigues J, et al. (2012) Occurrence and characterization of *Listeria* species isolated from the milk production chain. *Milchwissenschaft* 67: 43-46.
- 44 Navratilova P, Schlegelova J, Sustackova A, Napravnikova E, Lukasova J, et al. (2004) Prevalence of *Listeria monocytogenes* in milk, meat and food stuff of animal origin and the phenotype of antibiotic resistance of isolated strains. *Veterinary Medicine Czech* 49: 243-252.
- 45 Sharma D, Sharma P, Saharan B, Malik A (2012) Isolation, identification and Antibiotic susceptibility profiling of antimicrobial resistant *Listeria monocytogenes* from dairy milk. *International J Microbial Res Technology* 1: 1-4.
- 46 Maha A, AL-Ashmawy M, Mayada M, Khaled H (2014) Prevalence, Detection Methods and Antimicrobial Susceptibility of *Listeria monocytogenes* Isolated from Milk and Soft Cheeses and its Zoonotic Importance. *World applied Sci J* 29: 869-878.
- 47 Safdar A, Armstrong D (2003) Antimicrobial activities against 84 *Listeria monocytogenes* isolates from patients with systemic listeriosis at a comprehensive cancer (1955-1997). *J Clinical Microbio* 41: 483-485.
- 48 Marian M, Sharifah A, Zuraini M, Son R, Maimunah M, Lee H (2012) MPN-PCR detection and antimicrobial resistance of *L. monocytogenes* isolated from raw and ready to eat food in Malaysia. *Food Control* 28: 309-314.
- 49 Mengesha D (2005) Study on *Listeria monocytogenes* and other *Listeria* species in meat and milk products in retail markets of Addis Ababa, Ethiopia. Msc Thesis. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit Pp: 88.