



Listeria spp. in Raw Cow and Goat Meat in Port Harcourt, Nigeria

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Authors' contributions

Author SAO supervised the research; read and approved the manuscript.
Author OCE designed the study, wrote the draft, participated in the isolation, confirmation and handled the molecular aspect. Author JL handled the purchasing of samples, isolation and confirmation of isolates.

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ABSTRACT

Aims: To determine the occurrence of *Listeria monocytogenes* and other *Listeria* spp. in raw cow and goat meat.

Study Design: This work was based on a completely randomized design with two replication and the average values calculated for mean comparison.

Place and Duration of Study: Department of Microbiology, University of Port Harcourt, Nigeria and Nigeria Institute of Medical Research, Yaba, Lagos, Nigeria. Isolation of *Listeria* spp. was between March 2011 and February 2012.

Methodology: In this study, a total of 240 raw cow and goat meat samples were analyzed for the presence of *Listeria monocytogenes* and other *Listeria* species. The techniques recommended by the United State Department of Agriculture (USDA) revised and the Health Products and Food Methods of the Government of Canada were employed using Fraser broth and polymixin acriflavin lithium chloride ceftazidime aesculin mannitol (PALCAM) agar.

Results: The results of conventional and polymerase chain reaction (PCR) characterization of the isolates revealed that 81 samples (33.75%) were positive for *Listeria* spp. The highest prevalence of *Listeria* was found in cow flesh samples (19 of 36 samples; 52.78%) followed by cow intestine samples (8 of 20; 40%) and least was goat kidney samples (5 of 28; 17.86%). Out of the 310 characterized *Listeria* spp., *L.*

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monocytogenes were 4(1.29%). Other species isolated were *L. innocua* 20(6.45%), *L. ivanovii* 4(1.29%), *L. seeligeri* 72 (23.23%), *L. welshimeri* 139 (44.84%) and *L. grayi* 71(22.90%). No *L. monocytogenes* was isolated from cow samples. A higher incidence was noted during the raining season 216 (69.68%) than the dry season 94(30.32%).

Conclusion: This study demonstrated the occurrence and distribution of *Listeria* species in retail raw cow and goat meat in Port Harcourt, Nigeria.

Keywords: Cow meat; goat meat; listeria; listeriosis; PCR.

1. INTRODUCTION

Meat is the major source of protein and valuable qualities of vitamins for most people in many parts of the world, thus they are essential for the growth, repair and maintenance of body cells and necessary for our everyday activities [1]. Due to the chemical composition and biological characteristics, meats are highly perishable foods which provide excellent source for growth of many hazardous microorganisms that can cause infection in humans and spoilage of meat and economic loss [2]. Listeric infections, caused by microorganisms of the genus *Listeria*, occur worldwide and in a variety of animals including man [3]. Cases of listeriosis arise mainly from the ingestion of contaminated food and the disease is particularly common in ruminants fed on silage [3]. The genus *Listeria* comprises six species: *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. grayi*, *L. ivanovii* and *L. seeligeri* [4]. Two of these species, *L. monocytogenes* and *L. ivanovii*, are potentially pathogenic [5]. The other four *Listeria* species are essentially saprophytes that have adapted for survival in soil and decaying vegetation [4]. *Listeria monocytogenes*, a facultative intracellular pathogen, repeatedly found in meat and meat products, raw milk, soft cheese and pasteurized dairy products, vegetables, and fish and fish products [6] is responsible for severe foodborne infections in humans of all ages but especially pregnant women, infants less than four weeks old, the elderly and immunocompromised individuals and also cause invasive disease in many ruminants including, cattle, sheep, and goats species [7-9]. The bacterium possesses properties that favor it as a foodborne pathogen: at variance with most other pathogens it is relatively resistant to acid and high salt concentrations; it grows at low temperature, down to freezing point, which mean it may grow in refrigerated foods, measures commonly used to control the growth of pathogens in foods [10,11]. Before the development of *Listeria* selective media, this property was used for selective enrichment of the bacterium from complex matrices, with *Listeria* outnumbering the competing flora after incubation of the enrichment culture at refrigerator temperature for weeks or months [11].

Although the occurrence of *Listeria* in various foods has been investigated in several countries, there is limited information regarding its prevalence in raw meat and foods in Nigeria. The present study was conducted to determine the occurrence of *Listeria* species in raw cow and goat meat in Port Harcourt, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 240 raw cow and goat meat samples comprising 122 cow and 118 goat meat were randomly purchased from meat vendors in three strategic markets in Port Harcourt metropolis from March, 2011 to February, 2012. The samples were kept in ice box

containing ice packs and immediately transported to the Microbiology laboratory, University of Port Harcourt where they were kept in refrigerator until analyzed.

2.2 Isolation of *Listeria* sp

The techniques recommended by the United State Department of Agriculture (USDA) revised [12] and the Health Products and Food Methods of the Government of Canada [13] were employed using Fraser broth (Oxoid, England) and polymixin acriflavin lithium chloride ceftazidime aesculin mannitol (PALCAM) agar (Oxoid, England). Twenty-five grams of each meat samples was added to a stomacher bag containing 225ml of sterile half-Fraser broth and supplements. The mixture was homogenized using a stomacher (lab-blender, Seward medical, London) at high speed for 1-2min. The test portion was incubated at 30°C for 24h. From the pre-enrichment culture (half Fraser broth), 0.1ml was transferred into 10ml of full-strength Fraser broth with supplements added and was incubated at 35°C for 24-48h. From the culture obtained in Fraser broth showing evidence of darkening due to aesculin hydrolysis by *Listeria* sp, 0.1ml was transferred onto duplicate PALCAM plates. After spreading, plates were incubated at 37°C for 24- 48h. The plates were examined for the presence of characteristic colonies presumed to be *Listeria* sp- 2mm grey-green colonies with a black sunken centre and a black halo on a cherry-red background, following aesculin hydrolysis and mannitol fermentation. Five typical colonies were selected randomly from a pair of PALCAM plates for confirmation and subsequent identification.

2.3 Confirmation and Identification

Colonies suspected to be *Listeria* were transferred onto trypticase soy agar (Becton, Dickinson and company, France) with 0.6% yeast extract (Lab M, UK) and incubated at 37°C for 18 to 24h, before being subjected to the following standard biochemical tests: gram staining, catalase reaction, oxidase reaction, beta haemolysis on sheep blood agar and acid production from mannitol, rhamnose and xylose. Confirmed isolates on the basis of criteria suggested by Seeliger and Jones [14] were further identified using the polymerase chain reaction (PCR).

2.4 Extraction of *Listeria* DNA (Deoxyribonucleic acid)

DNA was extracted by the boiling method without triton x-100 [15]. Cells were harvested by centrifugation (Eppendorf centrifuge 5418, Germany) of overnight brain heart infusion broth culture of *Listeria* in 2ml eppendorf tube at 10,000rpm for 2min and the supernatants discarded. The pellets were re-suspended in 1ml sterile distilled water and centrifuged after vortexing (Vortexer 59a, Denville Scientific Inc, Taiwan) at 10,000rpm for 5min. The supernatants were again discarded and the pellets resuspended in 200µl sterile water and vortexed. The suspensions were heated for 10min in a boiling bath (100°C) (Grant GLS400, Grant Instrument, England). After cooling and vortexing, the mixtures were centrifuged at 10,000rpm for 5min. The supernatants were then transferred to a prelabelled 1.5ml eppendorf tube while the sediments were discarded. The DNA extracted was stored in deep freezer (-20°C) until further analysis.

2.5 Identification by PCR

Oligonucleotide primers described by Border et al. [16] for all *Listeria* species (U1[5'-CAGCMGCCGCGGTAATWC-3'] and LI1[5'-CTCCATAAAGGTGACCCT-3']) and for all serotypes of *L. monocytogenes* (LM1[5'-CTAAGACGCCAATCGAA-3'] and LM2 [5'-

AAGCGCTTGCAACTGCTC-3']) and Bubert et al. [17] for all *Listeria* species (primers MonoA [5'-TTATACGCGACCGAAGCCAAC-3'] and Lis1B [5'-TTATACGCGACCGAAGCCAAC-3']), all serotypes of *L. innocua* (Ino2 [5'-ACTAGCACTCCAGTTGTTAAAC-3'] and Lis1B), all serotypes of *L. grayi* (Mugra1 [5'-CCAGCAGTTTCTAAACCTGCT-3'] and Lis1B), all serotypes of *L. welshimeri* (Wel1 [5'-CCCTACTGCTCCAAAAGCAGCG -3'] and Lis1B), all serotypes of *L. ivanovii* (Iva1 [5'-CTACTCAAGCGCAAGCGGCAC -3'] and Lis1B), all serotypes of *L. seeligeri* (Sel1 [5'-TACACAAGCGGCTCCTGCTCAAC -3'] and Lis1B) while the grouped species, *L. seeligeri*, *L. ivanovii* and *L. welshimeri* were identified using Siwi2 (5'- TAACTGAGGTAGCGAGCGAA -3') and Lis1B, synthesized by Biomers.Net GMBH, Germany were employed.

The reactions involving U1, LI1, LM1 and LM2 were carried out in a final volume of 25µl, containing 2.5 µl 10×PCR buffer, 1.5 µl MgCl₂, 0.5µl dNTP (deoxynucleoside triphosphate), 0.25 µl each of appropriate primer, 0.1 µl AmphiTaq DNA polymerase(all products of Solis BioDyne, Estonia), 1.5µl of appropriate DNA preparation and 18.4µl sterile distilled water. amplification following an initial denaturation at 95°C for 3min was performed in 30 cycles, at 95°C for 30s, 50°C for 60s and 72°C for 60s in a thermo cycler (Mastercycler-Eppendorf, Vapo-product,Germany). A final extension was performed for 10min at 72°C. A 8µl aliquot of PCR product mixed with a loading dye (10mm, EDTA, 10% glycerol, 0.015% bromo phenol blue and 0.017% sodium dodecyl sulphate(SDS), made up to 100ml) were checked in an ethidium bromide stained 1.5% agarose(Fermentas, Life Science, Germany) and the gel read in a UV transilluminator (Genosens 1500, Clinx Science Instruments Co. Ltd, China). Reaction mixture with the DNA of *L. monocytogenes* PCM 2191 serovar 01/2 (Polish Collection of Microorganisms, Poland) template serve as positive control while a reaction mixture with no DNA template was incorporated as a negative control in each reaction.

The reactions involving MonoA, Iva1, Sel1, Wel1, Ino2, Mugra1, Siwi2, Lis1A and Lis1B were also carried out in a final volume of 25µl, containing 2.5 µl 10×pcr buffer, 1.5 µl MgCl₂, 0.5µl DNTP (deoxynucleoside triphosphate), 0.2 µl each of appropriate primer, 0.15 µl AmphiTaq dna polymerase (all products of Solis BioDyne, Estonia), 1.5µl of appropriate DNA preparation and 18.45µl sterile distilled water. amplification following an initial denaturation at 95°C for 3min was performed in 30 cycles, at 95°C for 30s, 58°C for 60s and 72°C for 60s in a thermo cycler (Mastercycler-Eppendorf, Vapo-product,Germany). A final extension was performed for 10min at 72°C. a 8µl aliquot of pcr product mixed with a loading dye (10mm, EDTA, 10% glycerol, 0.015% bromo phenol blue and 0.017% sodium dodecyl sulphate (SDS), made up to 100ml) were checked in an ethidium bromide stained 1.5% agarose (Fermentas, Life Science, Germany) and the gel read in a UV transilluminator (Genosens 1500, Clinx Science Instruments Co. Ltd, China). Reaction mixture with the DNA of *L. monocytogenes* PCM 2191 serovar 01/2 (Polish Collection of Microorganisms, Poland) template serve as positive control while a reaction mixture with no DNA template was incorporated as a negative control in each reaction.

2.6 Statistical Analysis

The distribution of the *Listeria* species in the various meat types and parts were subjected to analysis of variance (ANOVA) and Duncan [18] to determine means that differed.

3. RESULTS AND DISCUSSION

Out of a total of 240 meat samples analyzed, 81(33.75%) were positive for *Listeria*. *Listeria* species were isolated from all the beef and goat meat parts examined (Table 1). The level of

contamination of meat samples by *Listeria monocytogenes* and other *Listeria* species varied and was highest in cow flesh (19 of 36 samples, 52.78%), followed by cow intestine (8 of 20 samples, 40%) and least was goat kidney (5 of 28 samples, 17.86%).

Table 1. Detection of *Listeria* species in raw cow and goat meat

Meat type	Number of samples		%
	Examined	Positive	
Cow flesh	36	19	52.78
Cow intestine	20	8	40.00
Cow kidney	32	9	28.13
Cow liver	34	12	35.29
Goat flesh	36	11	30.56
Goat intestine	18	5	27.78
Goat kidney	28	5	17.86
Goat liver	36	12	33.33
Total	240	81	33.75

out of the 310 characterized *Listeria*, 74(23.87%) were from cow flesh, 29(9.35%) from cow intestine, 41(13.23%) from cow kidney, 46(14.84%) from cow liver, 33(10.65%) from goat flesh, 19(6.13%) from goat intestine, 17(5.48%) from goat kidney and 51(16.45%) from goat liver (Table 2).

Table 2. Distribution of *Listeria* species isolated from various meat types/parts.

<i>Listeria</i> sp.	Number of <i>Listeria</i> species isolated from each meat type/parts								Total
	CF	CI	CK	CL	GF	GI	GK	GL	
<i>L. monocytogenes</i>	0	0	0	0	3	1	0	0	4
<i>L. innocua</i>	5	3	1	3	1	0	1	6	20
<i>L. seeligeri</i>	18	1	13	8	9	4	7	12	72
<i>L. ivanovii</i>	2	0	1	1	0	0	0	0	4
<i>L. grayi</i>	14	8	10	11	9	6	1	12	71
<i>L. welshimeri</i>	35	17	16	23	11	8	8	21	139
Total	74	29	41	46	33	19	17	51	310

CF= cow flesh; CI= cow intestine; CK= cow kidney; CL= cow liver; GF= goat flesh; GI= goat intestine; GK= goat kidney; GL= goat liver.

All 310 *Listeria* isolates identified as *L. monocytogenes* 4(1.29%), *L. innocua* 20(6.45%), *L. seeligeri* 72(23.23%), *L. ivanovii* 4 (1.29%), *L. welshimeri* (139%) and *L. grayi* 71(22.90%) by conventional method were also positive using the PCR assay (Fig. 1). There were no significant difference ($p=0.05$) in the occurrence of *Listeria* among the different meat parts examined.

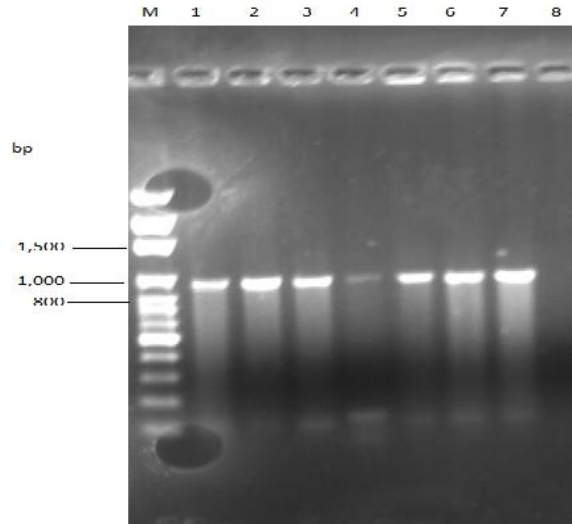


Fig. 1. Products obtained when total genomic DNA from the reference *L. monocytogenes* PCM 2191serovar 01/2 strain and representative of each isolated *Listeria* species were subjected to PCR using LI1 and U1 primer combination for all *Listeria* species. A PCR product of 938bp was observed. Lanes: m, molecular weight standard; 1, *L. innocua*; 2, *L. welshimeri*; 3 *L. seeligeri*; 4, *L. grayi*; 5, *L. ivanovii*; 6, *L. monocytogenes*; 7, *L. monocytogenes* PCM 2191serovar 01/2 (positive control); 8, control reaction (all reagent ingredients except chromosomal DNA). PCR products were separated in a 1.5% agarose gel and stained with ethidium bromide.

The highest occurrence of *Listeria* was in July (15.81%); it was also high in August (12.58%), both being the peak of the raining season in Port Harcourt (Fig. 2). Overall, a higher incidence was noted during the raining season 216 (69.68%) than the dry season 94(30.32%).

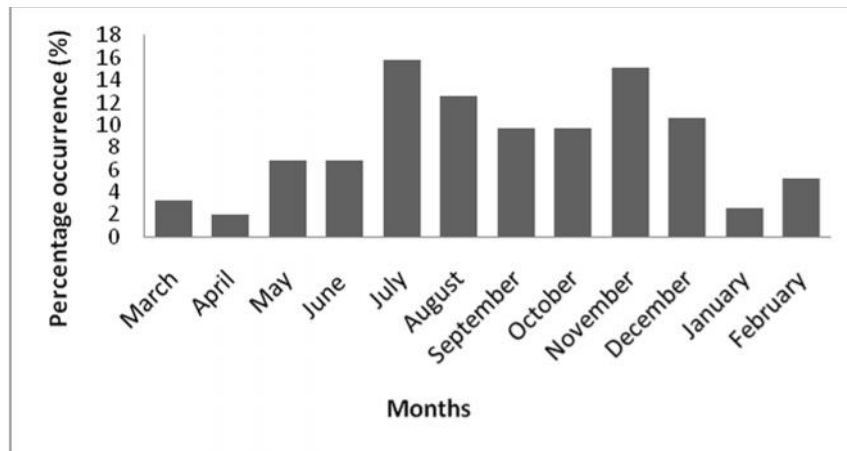


Fig. 2. Monthly Distribution of Isolated *Listeria* Species

The importance of raw meat and meat products as a vehicle for the transmission of various diseases, especially in countries where hygienic standards are not strictly enforced has been well documented [19]

It is not surprising that *L. monocytogenes* and other *Listeria* spp. were found in meat considering its widespread occurrence in nature, especially in plants, and their associations in nature with other bacteria that are well established in meats make it reasonable to find it in meats [20].

Overall, 33.75% (81 of 240) of all meat samples in this study were contaminated with *Listeria* species. This incidence of *Listeria* in the meat examined coincides with the report of a 0 to 68% prevalence of *Listeria* in fresh meat [21]. This can be attributed to the consumption of contaminated silage or other feeds, fecal contamination during evisceration and from handlers and slaughterhouses [3,22-24].

In this study, *L. welshimeri* was the predominant *Listeria* species isolated from the beef and goat meat samples; other *Listeria* species were less common (Table 2). Our result differed from those already reported [20,25,26] that stated that *L. monocytogenes* and *L. innocua* are most often reported with most investigators reporting one or the other to be most predominant and *L. welshimeri* coming third or next after *L. innocua*, followed by *L. seeligeri* and lastly *L. ivanovii* in meat among this five related species. *L. monocytogenes* accounted for 4(1.29%) of the 310 *Listeria* isolated. None was from the beef samples. Previous studies in Nigeria, India, Serbia and Bangkok, reported the inability to isolate *L. monocytogenes* in raw-goat, -beef and – meat [22,27-29]. However, a number of authors reported a 4.65% and 6.4% (Bulgaria), 5.1% (Ethiopia), 6.66% (India), 17.7% (Portugal), 20% (Greek), 31% (Denmark) and 35% (Spain) prevalence of *L. monocytogenes* from raw- beef, -goat and– meat [10,30-35], making obvious that the occurrence of *L. monocytogenes* varies from one place and one author to the other.

The distribution of the *Listeria* species (Fig. 2) shows that the highest occurrence was in July (15.81%). The occurrence was also high for August (12.58%), both being the peak of the raining season in Port Harcourt, Nigeria. This is comparable with a high incidence of *Listeria* in beef and goat meat during fall in Iran [36].

The inability of the Lis1A and Lis1B and MonoA and Lis1B primer combinations to produce the expected PCR product for all *Listeria* species and *L. monocytogenes* respectively, occasioned the use of primer combination; LI1 and U1 (Fig. 1) and LM1 and LM2 targeting the more conserve genomes of all *Listeria* and *L. monocytogenes* respectively. However, the others produced the expected PCR product, amongst them Siwi2 and Lis1B (Fig. 3) for *L. seeligeri*, *L. ivanovii* and *L. welshimeri*, the most predominant of the *Listeria* species isolated.

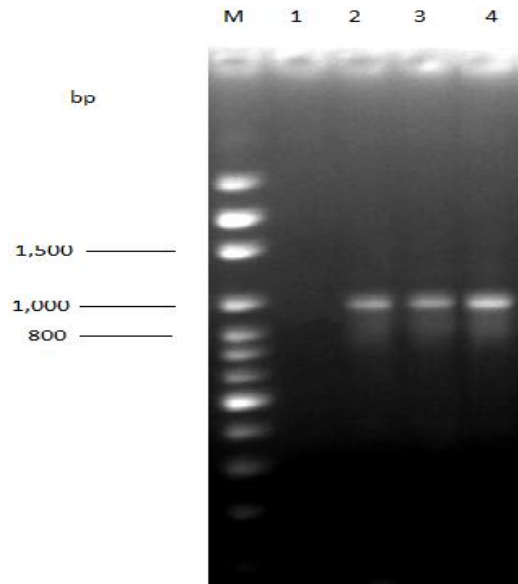


Fig. 3. The PCR products obtained when total genomic DNA from representative of each isolated *Listeria* species were subjected to PCR using siwi2 and Lis1B primer combination for *Listeria seeligeri*, *L. ivanovii* and *L. welshimeri*. A PCR product of 1,200bp was observed. Lanes: m, molecular weight standard; 1, control reaction (all reagent ingredients except chromosomal DNA); 2, *L. ivanovii*; 3, *L. welshimeri*; 4, *L. seeligeri* . PCR products were separated in a 1.5% agarose gel and stained with ethidium bromide.

4. CONCLUSION

This study has demonstrated the presence and distribution of *L. monocytogenes* and other *Listeria* species in raw meat examined in Port Harcourt. The high percentage of the isolation of *Listeria* suggests the need for improved food safety through proper hygienic measures during processing of food and meat products.

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COMPETING INTERESTS

Authors have declared that no competing interests exists

REFERENCES

1. Adzitey F, Huda N. *Listeria monocytogenes* in foods: incidence and possible control measures. Afr. J. Microbiol. Res. 2010;4(25):2848-2855.
2. Barbuddhe SB, Malik SVS, Bhilegaonkar KN, Kumar P, Gupta LK. Isolation of *Listeria monocytogenes* and anti-listeriolysin o detection in sheep and goats. Small Rum. Res. 2000;38(2):151-155.
3. Border PM, Howard JJ, Plastow GS, Siggins KW. Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. Lett. Appl. Microbiol. 1990;11(3):158-162.
4. Bubert A, Hein I, Raugh M, Lehner A, Yoon B, Goebel W, Wagner M. Detection and differentiation of *Listeria* spp. by a single reaction based on multiplex PCR. Appl. Environ. Microbiol. 1999;65(10):4688-4692.
5. Filiouis G, Johansson A, Frey J, Perreten V. Prevalence, genetic diversity and antimicrobial susceptibility of *Listeria monocytogenes* isolated from open-air food markets in Greece. Food Control. 2009;20(3):314-317.
6. Hassan EL-S, Ferage M, Nahla T. Lactic acid and ph as indication for bacterial spoilage of meat and some meat products. J Appl. Sci. Res. 2006;2(8):522-528.
7. Hitchins AD, Jinneman KC, Yoshitomi KJ, Blackstone GMK, Thammasouk K, Johnson JM, et al. Multiplex real-time PCR to simultaneously detect *Listeria* spp. and *L. monocytogenes* from a variety of food enrichments, Abstr. 49. XV International Symposium on problems of listeriosis (ISOPOL), Uppsala; 2004.
8. Ikeh MAC, Obi SKC, Ezeasor DN, Ezeonu IM, Moneke AN. Incidence and pathogenicity profile of *Listeria* sp. isolated from food and environmental samples in Nsukka, Nigeria. Afr. J. Biotech. 2010;9(30):4776-4782.
9. Indrawattana N, Nibaddhasobon T, Sookrung N, Chongsa-Nguan M, Tungtrongchitr A, Makino S, et al. Prevalence of *Listeria monocytogenes* in raw meats marketed in Bangkok and characterization of the isolates by phenotypic and molecular methods. J. Health Popul. Nutr. 2011;29(1):26-38.
10. Ivanović S, Radanović O, Pavlović I, Žutić J. Goats –source of *Listeria monocytogenes*. Proceedings, IV International Symposium of livestock production, Struga, Makedonija; 2009.
11. Jay JM. Prevalence of *Listeria* spp. in meat and poultry products. Food Control. 1996;7(4/5):209-214.
12. Johnson JL, Doyle MP, Cassens RG. *Listeria monocytogenes* and other *Listeria* spp. in meat and meat products. J. Food Protect. 1990;53(1):81-91.
13. Kamat AS, Nair PM. Incidence of *Listeria* species in Indian seafoods and meat. J. Food Safety. 1993;14(2):117-130.
14. Karakolev R. Incidence of *Listeria monocytogenes* in beef, pork, raw-dried and raw-smoked sausages in BULGARIA. Food Control. 2009;20(10):953-955.
15. Kim JW, Rajagopal SN. Antimicrobial activities of *Lactobacillus crispatus* ATCC 33820 and *Lactobacillus gasseri* ATCC 33323. The J. Microbiol. 2001;39(2):146-148.
16. Kinnean PR, GRAY CD. SPSS for windows made simple. psychology press, Hove, UE; 1999.
17. Kosek- Paszkowska K, Bania J, Bystron J, Molenda J, Czerw M. Occurrence of *Listeria* sp. in raw poultry meat and poultry meat products. Bull. Vet. Inst. Pulawy. 2005;49:219-222.

18. Laboratory Quality Assurance Division (LQAD). Isolation and identification of *Listeria monocytogenes* from red meat, poultry, egg, and environmental samples. MLG 8.06; 2008.
19. Liu D, Lawrence LM, Austin FW, Ainsworth AJ. A multiplex pcr for species- and virulence-specific determination of *Listeria monocytogenes*. J. Microbiol. Methods. 2007;71(2):133-140.
20. Low JC, Donachie W. A review of *Listeria monocytogenes* and listeriosis. The Vet J. 1997;153(1):9-29.
21. Mena C, Almeida G, Carneiro L, Teixeira P, Hogg T, Gibbs PA. Incidence of *Listeria monocytogenes* in different food products commercialized in Portugal. Food Microbiol. 2004;21(2):213-216.
22. Molla B, Yilma R, Alemayehu D. *Listeria monocytogenes* and other *Listeria* species in retail meat and milk products in Addis Ababa, Ethiopia. Ethiop. J. Health Dev. 2004;18(3):208-212.
23. Nightingale KK, Schukken YH, Nightingale CR, Fortes ED, Ho AJ, Her Z, et al. Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. Appl. Environ. Microbiol. 2004;70(8):4458-4467.
24. Nørrung B, Andersen JK, Schlundt J. Incidence and control of *Listeria monocytogenes* in foods in Denmark. Int. J. Food Microbiol. 1999;53(2-3):195-203.
25. Okutani A, Okada Y, Yamamoto S, Igimi S. Overview of *Listeria monocytogenes* contamination in Japan. Int. J. Food Microbiol. 2004;93(2):131-140.
26. Pagotto F, Daley E, Farber J, Warburton D. Isolation of *Listeria monocytogenes* from all food and environmental samples. Government of Canada. MFHPB-30; 2001.
27. Pesavento G, Ducci B, Nieri D, Comodo N, Lo Nostro A. Prevalence and antibiotic susceptibility of *Listeria* spp. isolated from raw meat and retail foods. Food Control. 2010;21(5):708-713.
28. Rahimi E, Yazdi F, Farzinezhadizadeh H. Prevalence and antimicrobial resistance of *Listeria* species isolated from different types of raw meat in Iran. J. Food Protect. 2012;75(12):2223-2227.
29. Ramaswamy V, Cresence VM, Rejitha JS, Lekshmi MU, Dharsana KS, Prasad SP, et al. *Listeria*-review of epidemiology and pathogenesis. J. Microbiol. Immunol. Infect. 2007;40(1):4-13.
30. Seeliger HPR, Jones D. *Listeria*. In Bergey's Manual of Systematic Bacteriology, Edited by P. H. A. Sneath, N. S. Nair, N. E. Sharpe & J. G. Holt. Baltimore: Williams and Wilkins. 1986;2:1235-1245.
31. Swaminathan B, Gerner-Smidt P. The epidemiology of human listeriosis. Microbes Infect. 2007;9(10):1236-1243.
32. Uyttendaele M, De Troy P, Debevere J. Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. Int. J. Food Microbiol. 1999;53(1):75-80.
33. Vazquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Dominguez-Bernal G, Goebel W, et al. *Listeria* pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. 2001;14(3):584-640.
34. Vitas AI, Aguado V, Garcia-Jalon I. Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). Int. J. Food Microbiol. 2004;90(3):349-356.

35. Wiedmann M, Mobini S, Cole JR, Watson CK, Jeffers G, Boor KJ. Molecular investigation of a listeriosis outbreak in goats caused by an unusual strain of *Listeria monocytogenes*. J. Am. Vet. Med. Assoc. 1999;215(3):369–371.
36. Yuçel N, Citak S, Onder M. Prevalence and antibiotic resistance of *Listeria* species in meat products in Ankara, Turkey. Food Microbiol. 2005;22(2-3):241–245.

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