

**Original Article****Prevalence and Antibiotic Susceptibility of *Listeria Monocytogenes* Isolated from Retail Ready-to-Eat Meat Products in Gorgan, Iran**Vaez Nemati^{*1}, Morteza Khomeiri¹, Alireza Sadeghi Mahoonak¹, Ali Moayedi¹

1- Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Received: August 2019

Accepted: October 2019

ABSTRACT

Background and Objectives: *Listeria monocytogenes* are known as an emerged foodborne pathogen and considered as a severe health risk. In the present study, prevalence and antibiotic resistance of *L. monocytogenes* isolated from ready-to-eat meat products in Iran were assessed.

Materials and Methods: A total of 200 ready-to-eat meat products, including chicken meat (wing, breast, and leg), lamb and fish, were collected, and *L. monocytogenes* was isolated according to ISO 11290–1. All of the isolates were verified using polymerase chain reaction and serotyping methods. Antibiotics susceptibility of *L. monocytogenes* isolates was assessed using the broth microdilution method.

Results: The presence of *L. monocytogenes* was verified in 13% of the samples. The presence of *L. monocytogenes* was reported in 26% of roast chicken meat, 5% of roast fish and 8.33% of cooked beef. Serology showed that Serotype 1/2a (48.13%) was the dominant serotype, followed by 4b (38.4%), 1/2c (6.99%) and 1/2b (6.48%). The result showed that 37 out of 100 *L. monocytogenes* isolates were resistant to all tested antibiotics. Furthermore, eight isolates were intermediately multi-resistant to the antibiotics. The rate of antibiotic resistance in *L. monocytogenes* was 52% in Serotype 1/2a, 39% in Serotype 4b, and 35% in Serotype 1/2c. Isolates of *L. monocytogenes* were mostly resistant to penicillin, ampicillin and erythromycin, but highly susceptible to tetracycline and gentamycin.

Conclusions: The high level of *L. monocytogenes* prevalence and its resistance to antibacterial agents in meat products may result in severe human listeriosis. Therefore, it is necessary to use efficient monitoring protocols for antibiotic administration and further safety management systems in food production units.

Keywords: *Listeria monocytogenes*, Lamb, Chicken, Antibiotic, Fish

Introduction

Listeria monocytogenes, the agent of listeriosis, is a food-borne pathogen that can cause fatal health problems, especially in immunocompromised patients. The *L. monocytogenes* infections are associated with mortality rates of nearly 12%, which is the highest rate within foodborne pathogens (1). This pathogen is found in soil and water as well as infected animals. In general, *L. monocytogenes* is transmitted to humans through contaminated foods (2). The bacterial strains differ in their epidemic potentials and serotypes as Serotypes 4b, 1/2a and 1/2b are responsible for nearly 90% of the human listeriosis (3). Other abilities of *L. monocytogenes* are linked to the formation of biofilms, which can grow at

refrigerator temperatures and high concentration tolerances to NaCl (4). Several studies reported *L. monocytogenes* from meat products (5,6). The prevalence of *L. monocytogenes* in ready-to-eat (RTE) meat products mostly ranged from 2.60 to 28% in other studies (7–10). Moreover, the contamination rate is different, affected by the food type and geographical area. Naturally, *L. monocytogenes* is sensitive to a wide range of antibiotics (2). For several years, the resistance rate of *L. monocytogenes* to antibiotics was constant (2). Nevertheless, new reports suggest increases in antibiotic resistance of the bacteria isolated from foods such as dairy products and meats (11,12). In the present study, the

*Address for correspondence: Vaez Nemati, Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. E-mail address: vaez.nematy@gmail.com

prevalence of *L. monocytogenes* isolates from RTE meats such as chicken meat (wing, breast and leg), lamb and smoked fish was assessed to report their genetic affiliation and antibiotic susceptibility using serological typing methods.

Materials and Methods

Collection of the *L. monocytogenes* isolates

A total of 200 RTE meat product samples were collected from 40 restaurants and retail supermarkets in Gorgan, Iran, from September to November 2018. These samples were transported to the Microbiology Laboratory at Gorgan University of Agricultural Science and Natural Resources under appropriate conditions. Samples were analyzed for *L. monocytogenes*, according to ISO 11290-1 (13). Briefly, 25 g of each sample was homogenized with 225 ml of *Listeria* enrichment broth and incubated at 37 °C for 24 h. Then 0.1 mL of pre-enrichment media was added into the CHROM agar *Listeria* and incubated for 24 h at 37 °C. Four suspicious *Listeria* colonies were chosen from every selected agar, and then secondary enrichments were streaked into a new CHROM agar and then incubated for 24 h at 37°C. Confirmation tests were performed using pure culture obtained from BHI (Merck, Germany) agar. Several biochemical tests were used for confirmation, including Gram staining, blood agar test, qualifying the consumption of rhamnose and xylose and mannitol.

Extraction of DNA from the *L. monocytogenes* isolates

In the following, the genomic DNAs were separated from 1 mL of the tested bacterial culture mediums, using a “Bacterial Genomic DNA Purification Kit” (DENA Zist Asia, Iran). DNA samples were re-suspended in 50 mL ddH₂O and then were stored at -20 C.

Polymerase chain reaction (PCR) assay

The PCR reactions were proceeded under optimized conditions using *L. monocytogenes* PTCC 1298 as control. The PCR was carried out in a 25- μ l volume of the mixture containing ten mM of tris-HCl, 50 mM of KCl, 1.5 mM of MgCl₂, 0.21 M of each primer, 0.2 mM of each dNTP, 1 μ l of DNA template and 0.5 unit of Taq polymerase (Takara, Japan). The forward primer included 5'-

TTGCGCAACAACTGAAGC-3' and the reverse primer included 5'-GCTTTTACGAGAGCACCTGG-3'. The PCR condition respectively included 35 cycles of denaturation, annealing and extension at 95 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min. The final extension was carried out at 72 °C for 7 min. The PCR products were electrophoresed to verify expected bands of 346 bp (5).

Serotyping of the *L. monocytogenes* isolates

All of the isolates were serotyped based on a protocol by Seeliger and Hohne (14). The isolates were analyzed using *Listeria* SEIKEN Antisera Kit (set code: 294616) for O and H antigens according to the kit manufacturer's instruction (Denka-Seiken, Japan).

Antibiotic resistance of the *L. monocytogenes* isolates

Antibiotics susceptibility of the *L. monocytogenes* isolates was assessed using the broth microdilution method (15). The antibiotics included enrofloxacin (5 μ g), ciprofloxacin (5 μ g), ampicillin (10 μ g), erythromycin (15 μ g), penicillin (5 μ g), clindamycin (2 μ g), tetracycline (30 μ g), gentamycin (10 μ g) and vancomycin (30 μ g). The minimum inhibitory concentration (MIC) was calculated using broth microdilution susceptibility and serial microdilution assays using Sensititre Vizion System (Trek, UK). Antibiotic susceptibility of the isolates was assessed according to instructions by the Clinical and Laboratory Standards Institute (CLSI) (16).

Statistical analysis

Rates of *L. monocytogenes* contamination in various RTE meat products were compared with each other using the chi-square test of the SPSS Software v.9.0 (SPSS Inc., Chicago, IL, USA). Furthermore, distributions of various serotypes of *L. monocytogenes* in each season were compared with each other using the same test. Differences were considered significant at $P < 0.05$.

Results

Prevalence and serotype distribution of the *L. monocytogenes* isolates in RTE meat products

From a total of 200 RTE meat product samples, 29 samples (14.5%) were contaminated with *L. monocytogenes* (Table 1). Roasted chicken meats included the highest *L. monocytogenes* contamination

rate with 13 (26%) out of 50 positive samples. Moreover, 5% of the roasted fish and 8.33% of the cooked beef were contaminated. Results of the serology showed that Serotype 1/2a was the most common serotype (45.4%) in the present study, majorly similar to other studies (17–19), followed by Serotypes 4b (38.4%), 1/2c (6.99%) and 1/2b (6.58%).

Table 1. Prevalence of *Listeria monocytogenes* in RTE meat products collected from retail markets in Iran

Sample	Food item	<i>L. monocytogenes</i> (%)
1	Cooked beef	8.33 (5/60)
2	Roast chicken	26.25 (21/80)
3	Roast fish	5 (3/60)

Antibiotic susceptibility

A total of 100 *L. monocytogenes* isolated from RTE meat products were tested for resistance against nine antibiotics (Table 2). Results showed that 37 isolates (37%) were resistant to all antibiotics. Moreover, all isolates were resistant to at least one (40%), two (18.3%), three (12.4%), four (4.20%) or five (3.25%) antibiotics. The bacterial resistance to ampicillin (64.71%) was the most common finding. Resistances to erythromycin, vancomycin, enrofloxacin, and ciprofloxacin were detected in 52.94, 35.29, 29.41 and 16.64% of the *L. monocytogenes* isolate, respectively (Table 2). The *L. monocytogenes* Serotypes 1/2a was the most common serotype in the present study, mostly resistant to ampicillin, penicillin and tetracycline. Serotype 4b was mostly resistant to vancomycin and penicillin, Serotype 1/2b to erythromycin and penicillin and Serotype 1/2c to ampicillin (Table 2).

Table 2. Number and proportion of the *Listeria monocytogenes* isolates resistant to nine antimicrobial agents

Antimicrobial agent	<i>L. monocytogenes</i> (n = 100)				Total no. (%)
	1/2a no. (%)	1/2b no. (%)	1/2c no. (%)	4b no. (%)	
Vancomycin	26 (27.4)	0 (0.0)	8 (16.66)	1 (18.8)	35 (35)
Ampicillin	43 (39)	12 (9.09)	7 (18.18)	2 (18.18)	64 (64)
Erythromycin	41 (37.8)	8 (8)	1 (11.11)	2 (22.22)	52 (52.94)
Enrofloxacin	13 (8.2)	0 (0.0)	2 (20)	3 (20)	18 (29.41)
Ciprofloxacin	2 (1.91)	0 (0.0)	0 (0.0)	1 (33.33)	3 (16.64)
Tetracycline	1 (0.96)	0 (0.0)	0 (0.0)	3 (54.3)	4 (5.78)
Penicillin	5 (4.83)	6 (20)	0 (0.0)	3 (30)	14 (58.82)
Clindamycin	2 (50)	0 (0.0)	1 (25)	1 (25)	4 (24.52)
Gentamycin	6 (50)	0 (0.0)	0 (0.0)	3 (26.32)	9 (11.76)

Discussion

Results of prevalence and serotype distribution of *L. monocytogenes* in the current study are mostly similar to those in other studies. Manios et al. (2015) isolated *L. monocytogenes* from 28% of RTE meat samples. The contamination level of *L. monocytogenes* in roasted chicken could be linked to fecal contamination during slaughtering or contact with hands (22). The high rate of *L. monocytogenes* contamination in roasted chicken were demonstrated in previous studies. For example, *L. monocytogenes* was isolated in 6.9% of the samples (9/131) in China (23) and 24% of the samples in Turkey (5). Although other studies reported a lower prevalence of *L. monocytogenes*. For example, bacteria were detected in 5.3% of meat products in China (24), 4% in Egypt (25) and 0.7% in Estonia (26). In the current study, the prevalence of *L. monocytogenes* in roasted fish, roasted chicken meat and cooked beef were similar to that in Shi et al. study (23); in which, *L. monocytogenes* was recovered from 6.9% of Chinese roast chicken meats and 6.5% of Chinese cooked beef. Results were also similar to results by Fallah et al. (7), where *L. monocytogenes* was recovered from 4% of Iranian RTE fish products.

In this study, resistance to penicillin was the most common resistance in *L. monocytogenes* isolates. Issa et al. (2011) showed high levels of resistance to penicillin and ampicillin in *L. monocytogenes*. Other researches (10,28,29) reported susceptibility of *L. monocytogenes* to ampicillin and penicillin. Ampicillin and penicillin are the most common antibiotics in listeriosis treatment (30,31). The current report on *L. monocytogenes* sensitivity to tetracycline and gentamycin is in agreement with the report of Gomez et al. for isolates from meats and animal-derived foods (10,32). Reports are available on relationships between serotype distribution and antibiotic susceptibility of the *L. monocytogenes* isolates. Lemes-Marques et al. (2007) report that the human *L. monocytogenes* serotypes (1/2a, 1/2b and 4b) were resistant to ampicillin, vancomycin, gentamycin and trimethoprim (33). In Turkey, isolates of *L. monocytogenes* Serotype 1/2c were highly resistant to antibiotics, more than isolates of Serotypes 1/2a, 1/2b and 4b (34). The results of this study showed differences in antibiotic susceptibility of *L. monocytogenes* isolated from Iran. Furthermore,

the results of the current serology study were similar to those of other serology studies (17–19).

Conclusion

The high rate of *L. monocytogenes* contamination in RTE meat products can be linked to the fact that these meats were not cooked thoroughly, which may cause severe health risks to consumers. Furthermore, great resistance of the *L. monocytogenes* isolates to antibiotics can lead to serious health problems and needs public awareness of meat producers and consumers.

Financial disclosure

The authors declare no financial interest.

Funding/Support

This study did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sector.

References

1. European Food Safety Authority. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA Journal*. 2017;15(12).
2. Schlech WF, Acheson D. Foodborne listeriosis. *Clin Infection Diseases*. 2000;31(3):770–5.
3. Kathariou S. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *Journal Food Protection*. 2002;65(11):1811–29.
4. Gandhi M, Chikindas ML. *Listeria*: A foodborne pathogen that knows how to survive. *International Journal Food Microbiology*. 2007;113(1):1–15.
5. Abay S, Irkin R, Aydin F, Mustak HK, Diker KS. The prevalence of major foodborne pathogens in ready-to-eat chicken meat samples sold in retail markets in Turkey and the molecular characterization of the recovered isolates. *LWT - Food Science and Technology*. 2017;81:202–9.
6. Yu T, Jiang X. Prevalence and characterization of *Listeria monocytogenes* isolated from retail food in Henan, China. *Food Control*. 2014 ;37:228–31.
7. Fallah AA, Saei-Dehkordi SS, Mahzounieh M. Occurrence and antibiotic resistance profiles of *Listeria monocytogenes* isolated from seafood products and market and processing environments in Iran. *Food Control*. 2013;34(2):630–6.
8. Ayaz ND, Ayaz Y, Kaplan YZ, Dogru AK, Aksoy

- MH. Rapid detection of *Listeria monocytogenes* in chicken carcasses by IMS-PCR. *Annual Microbiology*. 2009;59(4):741–4.
9. Abay S, Irkin R, Aydin F, Mustak HK, Diker KS. The prevalence of major foodborne pathogens in ready-to-eat chicken meat samples sold in retail markets in Turkey and the molecular characterization of the recovered isolates. *LWT - Food Science and Technology*. 2017;81:202–9.
 10. Gomez D, Azon E, Marco N, Carraminana JJ, Rota C, Arino A, et al. Antimicrobial resistance of *Listeria monocytogenes* and *Listeria innocua* from meat products and meat-processing environment. *Food Microbiology*. 2014;42:61–5.
 11. Srinivasan V, Nam HM, Nguyen LT, Tamilselvam B, Murinda SE, Oliver SP. Prevalence of Antimicrobial Resistance Genes in *Listeria monocytogenes* Isolated from Dairy Farms. *Foodborne Pathogen Disease*. 2005;2(3):201–11.
 12. Conter M, Paludi D, Zanardi E, Ghidini S, Vergara A, Ianieri A. Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. *Int J Food Microbiolgy*. 2009;128(3):497–500.
 13. ISO-11290-1: Microbiology of food and animal feeding stuffs—horizontal method for the detection and enumeration of *Listeria monocytogenes*—Part 1. 2017.
 14. Seeliger HPR, Höhne K. Chapter II Serotyping of *Listeria monocytogenes* and Related Species. *Methods Microbiolgy*. 1979;13:31–49.
 15. Wiczorek K, Dmowska K, Osek J. Characterization and Antimicrobial Resistance of *Listeria monocytogenes* Isolated from Retail Beef Meat in Poland . *Foodborne Pathogen Diseases*. 2012
 16. Clinical and Laboratory Standards Institute (CLSI). Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline. *Clin Lab Stand Inst*. 2006;(26).
 17. Vasilev V, Japheth R, Breuer R, Andorn N, Ben Abraham R, Yoni Y, et al. A survey of *Listeria monocytogenes* strains, isolated from ready-to-eat foods in Israel over a period of 10 years, 1998–2007. *Food Control*. 2010;21(8):1179–81.
 18. Wang X-M, Lu X-F, Yin L, Liu H-F, Zhang W-J, Si W, et al. Occurrence and antimicrobial susceptibility of *Listeria monocytogenes* isolates from retail raw foods. *Food Control*. 2013;32(1):153–8.
 19. Kramarenko T, Roasto M, Meremäe K, Kuningas M, Põltsama P, Elias T. *Listeria monocytogenes* prevalence and serotype diversity in various foods. *Food Control*. 2013;30(1):24–9.
 20. Stonsaovapak, S., &Boonyaratanakornkit M. Prevalence and antimicrobial resistance of *Listeria* species in food products in Bangkok, Thailand. *Journal of Food Safety*. 2010;30(1):154–61.
 21. Manios SG, Grivokostopoulos NC, Bikouli VC, Doultzos DA, Zilelidou EA, Gialitaki MA, et al. A 3-year hygiene and safety monitoring of a meat processing plant which uses raw materials of global origin. *International Journal of Food Microbiology*. 2015;209:60–9.
 22. Fenlon DR, Wilson J, Donachie W. The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. *Journa of Applied Bacteriology*. 1996;81(6):641–50.
 23. Shi W, Qingping W, Jumei Z, Moutong C, Zéan Y. Prevalence, antibiotic resistance and genetic diversity of *Listeria monocytogenes* isolated from retail ready-to-eat foods in China. *Food Control*. 2015;47:340–7.
 24. Wang G, Qian W, Zhang X, Wang H, Ye K, Bai Y, et al. Prevalence, genetic diversity and antimicrobial resistance of *Listeria monocytogenes* isolated from ready-to-eat meat products in Nanjing, China. *Food Control*. 2015;50:202–8.
 25. Mohamed Y, Reda WW, Abdel-Moein K, Abd El-Razik KA, Barakat AMA, El Fadaly HA, et al. Prevalence and phylogenetic characterization of *Listeria monocytogenes* isolated from processed meat marketed in Egypt. *Journal of Genetic Engireeng Biotechnolgy*. 2016;14(1):119–23.
 26. Kramarenko T, Roasto M, Keto-Timonen R, Maesaar M, Meremae K, Kuningas M, et al. *Listeria monocytogenes* in ready-to-eat vacuum and modified atmosphere packaged meat and fish products of Estonian origin at retail level. *Food Control*. 2016;67:48–52.
 27. Issa ZM, Mohamed SAS, Muda NM, Mustakim M, Yen LH, Radu S. Antibigram Profiles of *Listeria monocytogenes* isolated from foods. Vol. 7, 2nd International Conference on Biotechnology and Food Science. 2011.
 28. Abdollahzadeh E, Ojagh SM, Hosseini H, Ghaemi EA, Irajian G, Naghizadeh Heidarlo M.

- Antimicrobial resistance of *Listeria monocytogenes* isolated from seafood and humans in Iran. *Microbial Pathogens*. 2016;100:70–4.
29. Chao G, Zhou X, Jiao X, Qian X, Xu L. Prevalence and Antimicrobial Resistance of Foodborne Pathogens Isolated from Food Products in China. *Foodborne Pathogen Diseases*. 2007;4(3):277–84.
 30. Sakaridis I, Soultos N, Iossifidou E, Papa A, Ambrosiadis I, Koidis P. Prevalence and Antimicrobial Resistance of *Listeria monocytogenes* Isolated in Chicken Slaughterhouses in Northern Greece. *Journal of Food Protection*. 2011;74(6):1017–21.
 31. Allen KJ, Walecka-Zacharska E, Chen JC, Katarzyna K-P, Devlieghere F, Van Meervenne E, et al. *Listeria monocytogenes* – An examination of food chain factors potentially contributing to antimicrobial resistance. *Food Microbiol.* 2016;54(54):178–89.
 32. Altuntas EG, Juneja VK, Ayhan K, Materon L, Cosansu S, Kocan D. Antibiotic and Bacteriocin Sensitivity of *Listeria monocytogenes* Strains Isolated from Different Foods. *Food Nutrition Science*. 2012;03(03):363–8.
 33. Lemes-Marques EG, Cruz CD, Destro MT. Pheno- and genotypic characterization of *Listeria monocytogenes* clinical isolates from the southwestern region of the State of São Paulo, Brazil. *Brazilian Journal of Microbiol.* 2007;38(2):287–92.
 34. Ayaz Nd, Erol I. Relation between Serotype Distribution and Antibiotic Resistance Profiles of *Listeria monocytogenes* Isolated from Ground Turkey. Vol. 73, *Journal of Food Protection*.