Turkish Journal of Agriculture - Food Science and Technology, 6(3): 365-371, 2018

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X
www.agrifoodscience.com,
Turkish Science and Technology

Determination of *Listeria monocytogenes* and Serotypes in Modified Atmosphere Packed Ground and Cubed Beef Samples[#]

Adem Özkiraz, Ali Gücükoğlu[*]

Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Ondokuz Mayıs, 55200 Samsun, Turkey

A R T I C L E I N F O

[#]*This study was summarized from the master thesis.*

Research Articles

Received 08 December 2017
Accepted 02 February 2018

Keywords:
Listeria monocytogenes
mPCR
Serotype
Antibiotic resistance
MAP

[*]Corresponding Author:
E-mail: aligucuk@omu.edu.tr

A B S T R A C T

This study was conducted to determine the *Listeria monocytogenes*'s presence, serotypes and resistance against various antibiotics in modified atmosphere packaged (MAP) ground and cubed beef samples. Five of ground (5/50-10%) and 3 of cubed beef samples (3/50-6%) were identified as *L. monocytogenes* positive in MAP samples. Eleven *L. monocytogenes* isolates that obtained from samples being investigated in term of *hlyA* gene by PCR method have verified that this gene (100%). In serotyping results, 3 of 8 isolate that obtained from MAP ground beef samples were 1/2a, the other 3 isolate was 1/2b and the remaining 2 isolate was 4b. Also, 1 of 3 isolate that obtained from MAP cubed beef samples were 1/2b, the other one isolate was 1/2c and the last one was 4b. One isolate against (9%) ampicillin, 2 isolate against (18.2%) chloramphenicol, 3 isolate against (27.2%) erythromycin, 4 isolate against (36.3%) oxytetracycline and 4 isolate against (36.3%) penicillin G, 6 isolate against (54.5%) tetracycline and 3 isolate against (27.2%) vancomycin was resistant in 11 *L. monocytogenes* isolates that confirmed by PCR. The *L. monocytogenes* isolates were found to be resistant to one or more antibiotics in antibiotic-resistance test results. In conclusion, applying of national residue monitoring program by official authority for prevention of intensive antibiotic use in order to prevent the development of resistant strains to antibiotics has great importance.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 6(3): 365-371, 2018

Modifiye Atmosfer Paketli Sığır Kıyma ve Kuşbaşı Örneklerinde *Listeria monocytogenes* ve Serotiplerinin Belirlenmesi

M A K A L E B İ L G İ S İ

AraştırmaMakalesi

Geliş 08 Aralık 2017
Kabul 02 Şubat 2018

Anahtar Kelimeler:
Listeria monocytogenes
mPCR
Serotip
Antibiyotik direnç
MAP

[*]Sorumlu Yazar:
E-mail: aligucuk@omu.edu.tr

Ö Z E T

Bu çalışma modifiye atmosfer paketli sığır kıyma ve kuşbaşı örneklerinde *Listeria monocytogenes'in* varlığı, serotipleri ve çeşitli antibiyotiklere karşı dirençliliğinin belirlenmesi amacıyla yapılmıştır. 50 MAP sığır kıyma örneğinin 5'inin (%10), 50 MAP sığır kuşbaşı örneğinin ise 3'ünün (%6) *L. monocytogenes* pozitif olduğu belirlenmiştir. PCR yöntemi ile *hlyA* geni varlığı araştırılan MAP sığır kıyma ve kuşbaşı örneklerinden elde edilen 11 *L. monocytogenes* izolatının tamamının (%100) bu gene sahip olduğu belirlenmiştir. Yapılan serotiplendirme sonucunda MAP sığır kıyma örneklerinden elde edilen 8 izolatın 3'ünün *L. monocytogenes* 1/2a, 3'ünün 1/2b, 2'sinin ise 4b olduğu saptanmıştır. Ayrıca MAP sığır kuşbaşı örneklerinden elde edilen 3 izolattan ise 1'inin *L. monocytogenes* 1/2b, 1'inin 1/2c ve 1'inin de 4b olduğu tespit edilmiştir. PCR ile doğrulanan 11 *L. monocytogenes* izolatının, 1'inin (%9) ampisiline, 2'sinin (%18,2) kloromfenikole, 3'ünün (%27,2) eritromisine, 4'ünün (%36,3) oksitetrasikline, 4'ünün (%36,3) penisilin G'ye, 6'sının (%54,5) tetrasikline ve 3'ünün (%27,2) de vankomisine karşı dirençli olduğu belirlenmiştir. Elde edilen *L. monocytogenes* izolatlarının antibiyotik dirençlilik testi sonucunda bir veya birden fazla antibiyotiklere karşı dirençli olduğu saptanmıştır. Sonuç olarak antibiyotiklere dirençli suşların gelişmesinin önlenmesi amacıyla bilinçsiz antibiyotik kullanımı önlenmesine ilişkin resmi otorite tarafından ulusal kalıntı izleme programının etkin olarak yürütülmesi büyük önem taşımaktadır.

DOI: https://doi.org/10.24925/turjaf.v6i3.365-371.1749

Introduction

Listeria monocytogenes is one of the most frequently studied micro organisms since it is an intracellular pathogen that has recently been found to cause economic losses in areas where livestock are intense, exists in the flora of enterprises where animal foods such as meat and dairy products are produced, causing contamination of these products, causing contamination of these products, causing sensitivities in humans or in sensitive animals fed with contaminating feeds which cause very severe infections with sporadic or epidemic characteristics (Farber and Peterkin, 1991; Jemmi and Stephan 2006; Liu et al., 2006). It is known that the share of cattle in the main reservoir of the factor is large. It has been reported that in different countries, *L. monocytogenes* has been isolated in cattle's skins and carcasses in various countries and during the cutting and processing of animals, the agent in the skin of the cattle contaminates to carcass by various means such as slaughterhouse tools and slaughterhouse workers, thus joins food chain with contaminated meat and meat products and causes poisoning, serious illnesses and deaths in humans. However, it is emphasized that *L. monocytogenes* is detected more in processed meat products than carcass meat (Akkaya et al., 2008a; Akkaya et al., 2008b; Farber and Peterkin, 1991). The causative agent is invasive listeriosis (especially the pregnant uterus, central nervous system) and non-invasive (referred to as febrile listerial gastroenteritis) listerioses, which cause 20-30% mortality in pregnant women, newborns, elderly people and immunosuppressive drug users. Although the incidence of listeriolysis is lower than other food pathogens, it is reported that the mortality rate from listeriosity is around 30% (Liu et al., 2006). The virulence property of *L. monocytogenes* is dependent on 6 genes (*pRFA*, *plcA*, *hulya*, *mplA*, *actA* and *plCB*) and internalins. *L. monocytogenes* has 13 serotypes and serotypes of 1/2a, 1/2b and 4b are responsible for of 98% of listeriosis cases in humans (Holzapfel and Becker, 2007). *L. monocytogenes* can reproduce in aerobic, microaerophilic, anaerobic conditions. However, it has been reported that high levels of CO_2 use in modified-atmosphere packaging (MAP) and low-temperature storage of foods suppress the reproduction of *L. monocytogenes*, but not provide complete inhibition (Fernandez et al., 1997). Researchers have tended to determine the agent in meat procusts as the agent has shown itself in different years and in various countries as epidemics. But there is no study on the incidence and antibiotic resistance of *L. monocytogenes* in cattle meat minced and ground beef samples with modified atmospheric packaging (MAP) in our country.

In this study, the prevalence of *L. monocytogenes* in ground and cubed beef samples, known as one of the most important food pathogens in today's Modified Atmosphere Packaged (MAP) was investigated. IMS (Immunomagnetic Separation) based culture technique was used for isolation of the agent, Vitek 2 Compact (*BioMerieux*) automatic identification system was used for identification and PCR technique was used for confirmation and serotyping. Disc diffusion test was performed to determine the antibiotic resistance profiles of the isolates in the continuation of the study.

Material and Methods

In this study, a total of 100 MAP beef products (50 ground beef, 50 cubed meat), obtained from supermarkets in the Samsun-Turkey province between May-October 2013 were used. Collected samples weighed at least 500 grams and were brought to the laboratory under cold chain for analysis.

Isolation of the L. monocytogenes

For the isolation of *L. monocytogenes*, the IMS-based culture technique and Vitek 2 Compact (*BioMerieux*) automatic identification system (Pincus, 2010) proposed by ISO 11290-1 (The International Standards Organization) (Anon, 1995) and Dynal ® (Anon, 1996) was used. 25 g of samples, weighed under aseptic conditions, were diluted with 225 ml Half Fraser Broth (Oxoid, CM 895, SR 156, Hampshire, UK) and were homogenized at medium speed for 90 seconds in a stomacher (Interscience, Bagmixer 400). Then these samples were left for incubation at 30 °C for 24 hours.

Following the pre-enrichment process, the IMS technique was used in according to the manufacturering company's guide and 50 µl MOX (Modified Oxford Agar; Oxoid, CM856-SR 140) agar planting was made from the 100 µl Dynabeads-Listeria complex obtained from the result of the protocol and the plaques were left for incubation for 24-48 hours at 35°C. Following incubation, typical colonies that had grown in plaques with the following characteristics were selected: about 1-2 mm in diameter, middle part sunken with dark brown color having black halo as a surrounding, 5 of them were selected and transferred to TSA-YE (Tryptic Soy Agar-Yeast Extract, Fluka, 22091, Oxoid, LP0021) for biochemical testing and incubated for 24 hours at 30 °C. Those colonies that have grown in TSA-YE, confirmation was done by Vitek 2 Compact (*BioMerieux)* automatic identification system. For this purpose, the cultures produced in 24 hours in TBS-YE were taken into test tubes containing 3 ml sterile ester of 0.5% saline and the blurring was set to 0.5 McFarland (10^8 kob/ml) turbidity by McFarland densitometer device. Identification was completed in accordance with the software program in Vitek 2 Compact (*BioMerieux*) automatic identification system protocol.

Verification of L. monocytogenes by PCR and Serotyping

As the result of this analysis, isolates that contained *L. monocytogenes*, were verified by PCR. *hlyA* primers designed by Bohnert et al. (1992) were used (Table 1). For serotyping of *L. monocytogenes* isolates, primer squences of 1/2a (3a), 1/2b (3b), 1/2c (3c) and 4b (4d, 4e) serotypes that were designed by Doumith et al. (2004) were used (Table 1). To finalize the identification process, DNA extraction was performed by the boiling method. Accordingly, isolates had been incubated for 24 hours at 37°C in BHI broth (Oxoid, CM 0225) and then 1 ml of this was transferred to eppendorf tubes and centrifuged for 5 minutes at 10,000×g, later supernatant was attached with the addition of 500 µl PBS and were kept in a water bath at 95°C for 10 minutes. Ultimately, the supernatant was centrifuged again at 10.000 × g for 5 minutes and stored at -20°C until PCR process was performed as the template DNA.

Table 1 Primer sequence of *L. monocytogenes* and serotypes*

Target gene	Primer sequence (5'-3')	PPCR product (bp)	Serotype
hylA	F: GAATGTAAACTTCGGCGCAATCAG R: GCCGTCGATGATTTGAACTTCATC	388	All *L. monocytogenes*
Imo0737	F: AGGGCTTCAAGGACTTACCC R: ACGATTTCTGCTTGCCATTC	691	1/2a, 1/2c, 3a, 3c
Imo1118	F: AGGGGTCTTAAATCCTGGAA R: CGGCTTGTTCGGCATACTTA	906	1/2c, 3c
ORF2819	F: AGCAAAATGCCAAAACTCGT R: CATCACTAAAGCCTCCCATTG	471	1/2b, 3b, 4b, 4e, 4d
ORF2110	F: AGTGGACAATTGATTGGTGAA R: CATCCATCCCTTACTTTGGAC	597	4b, 4e, 4d

*Bohnert et al. (1992), Doumith et al. (2004).

Table 2 Prevalence of *Listeria spp*. and *L. monocytogenes* from MAP ground and cubed beef meat samples.

Samples	NS	Number of *Listeria spp.* positive samples (n-%)	Number of of *Listeria spp.* positive isolates (n)	Number of *L. monocytogenes* positive samples (n-%)	Number of *L. monocytogenes* isolates (n)
MAP ground meat	50	17- 34%	70	5- 10%	8
MAP cubed meat	50	15- 30%	42	3- 6%	3
Total	100	32- 32%	112	8- 8%	11

NS: Number of Samples (n)

Table 3 Serotypes of *L. monocytogenes* isolates.

Number of Samples	Number of *L. monocytogenes* positive isolates detected using IMS + culture and VITEK 2® technique	The number of *L. monocytogenes* positive isolates (hlyA gene region) determined using PCR technique	*L. monocytogenes* serotypes and numbers determined by PCR technique			
			1/2a (3a)	1/2b (3b)	1/2c (3c)	4 b (4d, 4e)
MAP ground meat (n:50)	8	8	3	3	-	2
MAP cubed meat (n:50)	3	3	-	1	1	1
Total (n:100)	11	11	3	4	1	3

PCR Amplification and Electrophoresis

PCR mixture for *hlyA* gene consisted of 1X PCR Buffer, 1.5 mM MgCl2, 0.1 mM dNTP, 0.5 U Taq-Polymerase (Sigma, D4545) and as 1 µM and 5 µl DNA from each primer in a total of 50 µl. The amplification of *hlyA* gene was determined in a Thermal Cycler (Bio-Rad MJ mini Gradient CA-USA) under the following conditions: intial denaturation at 94°C for 5 minutes and 35 cycles, denaturation at 94°C for 30 seconds, primer binding at 65°C for 45 seconds, primer extension at 72°C for 45 seconds and final extension at 72°C for 45 seconds.

For serotyping, PCR mixture, which contained 1× PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM dnTP, 0,5 U Taq-Polymerase at 50 µl total volume plus1 µM and 5 µl DNA from each primer. The amplification conditions of genes used in serotyping were cycled in a Thermal Cycler which was programmed as 5 minutes at 94°C, 30 seconds at 94°C, 45 seconds at 72°C, total 35 cycles and final extension for 5 minutes at 72°C. Program was revised and optimized in accordance with a different binding heat of primer sequences. Primer binding degrees were arranged as 55°C for *ORF2819*, 57°C for *ORF2110*, 57°C for *Imo0737* and 55°C for *Imo1118*. Amplification protocols was done step by step. Then, *ORF2819* and*ORF2110* primers, *Imo0737* and *Imo1118* primers were amplified at 56°C as multiplex. The isolated amplicons were subjected to 80-volt electric current in 2% agarose for electrophoresis.

Antibiotic Resistance Tests

Antibiotic resistance profiles of obtained isolates were determined by the disk diffusion method. For this purpose, fresh cultures produced on TSB-YE for 24 hours were set to 0.5 McFarland (10^8 kob/ml) turbidity with the help McFarland densitometer device and this suspension was inoculated to Mueller-Hinton Agar (Oxoid, CM337). The resulting zone diameters were compared to standards in CLSI (2012).

Results

Isolates identified as *L. monocytogenes* by IMS-based classical culture technique, were confirmed by PCR method using the *hlyA* gene as preference. According to the results obtained from the analysis (Table 2), all 11 (100%) *L. monocytogenes* isolates obtained from MAP ground beef and cubed beef samples of had this gene and were confirmed as *L. monocytogenes* (Figure 1).

Primer sequences of *Imo0737, Imo1118, ORF2819* and *ORF2110* developed by Doumith et al. (2004) were used in order to serotype *L. monocytogenes* isolates confirmed by PCR. The isolates were serotyped according to the presence of these genes (Table 3). The electrophoresis image obtained at the end of PCR process is shown in Figure 2.

Antibiotic resistance distribution of *L. monocytogenes* serotpypes; 3 of *L. monocytogenes* isolates obtained from

MAP packed ground beef samples were 4b serotypes and 3 them were found to be resistant to erythromycin (100%), 2 were found to be resistant to tetracycline (66.6%) and 2 were found to be resistant to vancomycin (66.6%). In adddition to this amongst 3 *L. monocytogenes* 1/2b isolates obtained from MAP packed ground beef samples, 1 was found to be resistant to ampicillin (33.3%), 2 were found to be resistant to chloramphenicol (66.6%), 3 were found to be resistant to oxytetracycline (100%), 1 was found to be resistant to penicillin G (33.3%), 2 were found to be resistant to tetracycline (66.6%) and 1 was found to be resistant to vancomycin (33.3%). Similarly, the resistance distribution for 2 *L. monocytogenes* 4b isolates obtained from MAP packed ground beef samples was detected to be as follows: 1 to oxytetracycline (50%), 1 to penicillin G (50%), 1 to tetracycline (50%). Nevertheless, amongst isolates obtained from MAP packed ground beef samples, 1 *L. monocytogenes* 1/2b isolate and 1 *L. monocytogenes* 1/2c isolate (100%) were found to be resistant to penicillin G whilst 1 *L. monocytogenes* 4b isolate (100%) was detected to be resistant to chloramphenicol. Antibiotic resistance distribution of all *L. monocytogenes* serotypes obtained from samples are shown in Table 4.

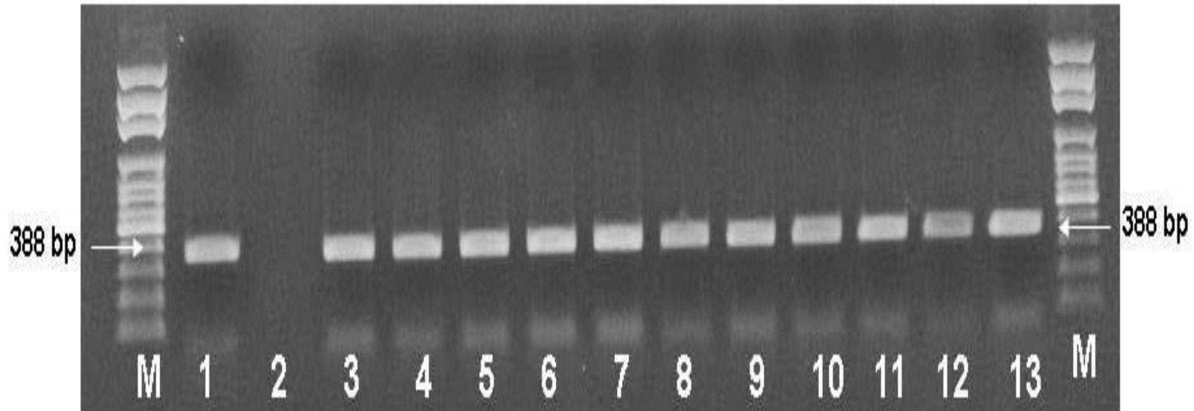

Figure 1 Electrophorese image of hylA gene from MAP ground and cubed beef meat samples isolates by PCR.
[M: 100 bp DNA marker, Lane 1: *L. monocytogenes* positive control (*L. monocytogenes* RSKK 471), Lane 2: Negative control (distileted water), Lanes 3-10: MAP ground beef meat samples isolates, Lanes 11-13: MAP cubed beef meat samples isolates.

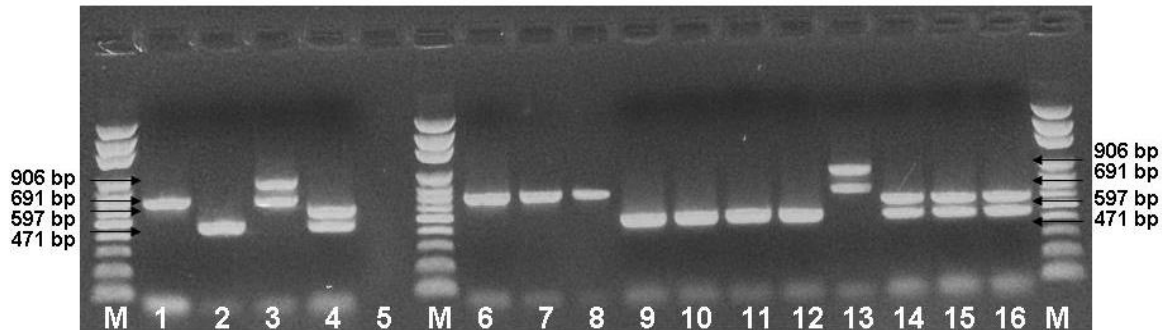

Figure 2 Electrophorese image of mPCR.
[M: 100 bp DNA marker, Lane 1: *L. monocytogenes* positive control serotype 1/2a, (*L. monocytogenes* RSKK 471), Lane 2: *L. monocytogenes* positive control serotype 1/2b, (*L. monocytogenes* RSKK 472), Lane 3: *L. monocytogenes* positive control serotype 1/2c, (*L. monocytogenes* ATCC 7644), Lane 4 : *L. monocytogenes* positive control serotype 4b, (*L. monocytogenes* RSKK 475), Lane 5: Negative control (distileted water), Lanes 6-8: Serotype ½ a isolates, Lanes 9-12: Serotype ½ b isolates, Lane 13: Serotype ½ c isolates, Lanes 14-16: Serotype 4b isolates]

Table 4 Antibiotic resistance profiles of *L. monocytogenes* serotypes.

AT	A	B	C	D	E	F	G	H	I	J	K
AMC	S	S	S	S	S	S	S	S	S	S	S
AMP	S	S	S	R	S	I	S	S	S	S	S
C	S	I	I	I	R	I	I	I	I	S	R
E	R	R	R	S	S	S	I	I	S	S	I
OT	S	S	S	R	R	R	S	R	S	S	S
PG	S	S	S	R	I	S	R	S	R	R	S
TE	I	R	R	I	R	R	R	I	I	S	R
VA	R	S	R	S	S	R	I	S	S	I	I

AT: Antibiotic Type, A: *L. monocytogenes* 1/2a (Isolate number: Cubed 52-1), B: *L. monocytogenes* 1/2a (Isolate number: Cubed 52-3), C: *L. monocytogenes* 1/2a (Isolate number: Cubed 52-5), D: *L. monocytogenes* 1/2b (Isolate number: Cubed 56-1), E: *L.monocytogenes* 1/2b (Isolate number: Cubed 74-1), F: *L. monocytogenes* 1/2b (Isolate number: Cubed 74-4), G: *L. monocytogenes* 4b (Isolate number: Cubed 33-4), H: *L. monocytogenes* 4b (Isolate number: Cubed 58-2), I: *L. monocytogenes* 1/2c (Isolate number: Ground 17-1), J: *L. monocytogenes* 1/2b (Isolate number: Ground 60-2), K: *L. monocytogenes* 4b (Isolate number: Ground 62-1), AMC: Amoxicillin and clavulonic acid (30 µg), AMP: ampicillin (10 µg), C: chloramphenicol (30 µg), E: erythromycin (15 µg), OT: oxytetracycline (30 µg), PG: penicillin G (10 µg), TE: tetracycline (30 µg), VA: vancomycin (30 µg). S: Sensitive, I: Intermediate, R: Resistance.

Discussion and Conclusion

It has been reported that slaughter animals may carry *L. monocytogenes* in their flora and the carcass may be contaminated with *L. monocytogenes* if hygienic conditions are not provided during the slaughter process (Farber and Peterkin, 1991). Various studies have conducted by different researchers on the prevalance of *L. monocytogenes* in meat and meat products. In his study, Ciftcioglu (1992) reported that amongst 100 ground meat samples, it was detected that 11% had *L. monocytogenes* and 34% had *Listeria* species, whilst amongst 100 spiced sausage meat samples, 2% had *L. monocytogenes* and 11% had other *Listeria* species. In the study of Niederhiauser et al. (1992) amongst 100 cooked and raw meat samples the existence of *L. monocytogenes* was detected in 14 samples. Pesavento et al. (2010) demonstrated in their study that, 21.4% of raw meat samples they used were contaminated with *Listeria* species and 23.6% of obtained isolates were *L. monocytogenes*. Samadour et al. (2006) reported that positive *L. monocytogenes* was detected in 18 samples (3.5%) amongst 1750 ground beef samples were taken from retail outlets, MCGowan et al. (1994) reported that *L. monocytogenes* was identified in 21 samples (65.6%) amongst 32 poultry products, in 9 samples (34.6%) amongst 26 samples taken from pieces of beef, in 8 samples (40%) amongst 20 samples taken from lamb meat, in 9 samples (28.1%) amongst 32 samples taken from pork meat and in 8 samples (34.7%) amongst 23 samples taken from sausage.

There are some notifications in literature about the presence of *L. monocytogenes* in modified atmosphere packaged other meat sources. Hart et al. (1991) reported that reproduction of *L. monocytogenes*in chicken breasts packaged with 100% CO_2 was inhibited. Pothuri et al. (1995) observed that crayfish tail fin meat samples that were packaged under modified atmospheric conditions (75%CO_2:10%O_2:15%N_2) compared to air or vacuum packaging had the lag phase lengthened for 8 days. Barakat and Harris (1999) stored cooked chicken meat, inoculed by *L. monocytogenes* (1.000 CFU/150-g) and packaged by 44%: 56% CO_2-N_2, at 3.5, 6.5 and 10°C for 5 weeks. By the end of storage, it is reported that *L. monocytogenes* reproduced in all test groups.

Closer values to findings of this research were reported by Barbuddhea et al. (2000) stating the contagion of *L. monocytogenes* with the ratio of 17.64% amongst 201 meat samples taken from sheep and goats in India. In 1988, food in 5779 retail outlets had been analysed and the highest ratio was found to be in fresh meat with the ratio of 7.5% of positive *L. monocytogenes* amongst 416 samples (Aznar and Alarcon, 2003). Similar values to findings of the study were reported amongst 1727 fresh beef meat samples collected from various places in USA in 39-month time with the ratio of 7.1% *L. monocytogenes* positive (Jay, 2000). Parallel to this, Luppi et al. (1998) found 9 (7.96%) *L. monocytogenes* amongst 113 meat samples in Italy in their study.

Samadpour et al. (2006) found out that 3.5% of packaged red meat samples sold in markets in USA were contaminated with *L. monocytogenes* as lower than the values of our study. Besides, Pesavento et al. (2010) reported that 21.4% of raw meat samples in Florence in Italy were contaminated with *Listeria* species and 23.6% of obtained isolates were *L. monocytogenes*.

In a study conducted in Switzerland, 43 (10.8%) samples amongst a total of 400 ground meat samples (211 beef and 189 pork) were found to be *L. monocytogenes* positive and it was reported that amongst isolated *L. monocytogenes* 9 were serotype 1/2a, 2 were 1/2b, 12 were 1/2c and 10 were 4b (Fantelli and Stephan, 2001). It is also stated that *L. monocytogenes* is commonly detected in raw pork (Norrung et al., 1999) and generally meat including pork has *L. monocytogenes* 1/2a, 1/2b and 1/2c as parallel to our findings (Hof and Rocourt, 1992; Thevenot et al., 2005).

L. monocytogenes is determined to have 13 serotypes according to somatic (O) and flagella (H) structures and together with this, it is reported that serotypes of *L. monocytogenes* 4b, 1/2a, 1/2b and 1/2c cause 98% of worldwide foodborne listeorisis cases (Seeliger and Höhne, 1979; Liu et al., 2006; Roberts et al., 2006). *Imo0737, Imo1118, ORF2819, ORF2110* and *prs* sequences developed by Doumith et al. (2004) were used for serotyping of *L. monocytogenes* isolates that we have obtained in our study and verified by PCR. As the result of analysis, it was detected that amongst 52 isolates examined, 38% were *L. monocytogenes* 4b, 38% were *L. monocytogenes* 1/2b, 12% were *L. monocytogenes* 1/2c and 12% were *L. monocytogenes* 1/2a. Serotypes of 1/2b, 1/2a and 4b were found to be dominant serotypes in our study.

In our country, Sireli and Erol (1999) have isolated 30% of *L. monocytogenes* by the method recommended by USDA/FSIS from frozen chicken carcasses sold in Ankara. As the result of their study, they reported that *L. monocytogenes* 1/2a was dominant with the ratio of 73% followed by 1/2b, 1/2c and 4b. In our study, unlike the researchers, serotype 4b was determined to be dominant with respect to 1/2a. Also, Ayaz and Erol (2011) reported as the result of serotyping the *L. monocytogenes* isolates they obtained from turkey meat that 4b (51.4%) was dominant serotype followed by 1/2a (27%) and 1/2b (21%).

The high prevalence ratios stated in the studies pictured above might be caused by weak sanitation and disinfection of both equipment and poor conditions of hygiene and/or owing to geographical reasons.

In this study, amongst 3 *L. monocytogenes* 4b isolates obtained from MAP packaged ground beef samples, 3 (100%) were found to be resistant to erythromycin, 2 (66.6%) to tetracycline and 2 (66.6%) to vancomycin. In addition to this, amongst 3 *L. monocytogenes* 1/2b isolates obtained from the MAP packaged ground beef samples, 1 (33.3%) were found resistant to ampicillin, 2 (66.6%) to chloromfenicole, 3 (100%) to oxytetracycline, 1 (33.3%) to penicillin G, 2 (66.6%) to tetracycline and 1 (33.3%) to vancomycin. Similarly, amongst 2 *L. monocytogenes* 4b isolates obtained from the MAP packaged ground beef samples, resistancies were as folloows; 1 (50%) to oxytetracycline, 1 (50%) to penicillin G and 1 (50%) to tetracycline. Amongst isolates obtained from the MAP packaged cubed beef samples, 1 isolate of *L. monocytogenes* 1/2b and 1 isolate of *L.*

monocytogenes 1/2c (100%) were found to be resistant to penicillin G, 1 (100%) isolate of *L. monocytogenes* was found to be resistant to 4b chloramphenicol whilst no resistance was observed against other antibiotics analyzed. Harakeh et al. (2009) reported that amongst *L. monocytogenes* isolates from dairy products in Lebanon, 93.33% were oxacillin and 90% were penicillin-resistant. Besides, Rahimi et al. (2010) reported that *L. monocytogenes* isolates isolated from milk and dairy products in Iran are resistant to various antibiotics such as nalidixic acid, ciprofloxacin, erythromycin, tetracycline, gentamycin, ampicillin, penicillin and chloramphenicol.

The result of the antibiotic sensitivity test showed that *L. monocytogenes* isolates obtained were found to be resistant to one or more antibiotics. Thus, it is of great importance to adhere to existing regulations in line with the EU directives on the use of antibiotics in animals and to ensure effective implementation of the national residue monitoring program. Following such regulations will prevent the development of antibiotic resistant strains, ensure food safety and protect public health in food production. Our findings suggest that future research be considered from an epidemiological perspective.

Acknowledgment

This work was supported by Ondokuz Mayıs University (Project No: PYO.VET.1904.12.002).

References

Akkaya L, Alisarlı M, Çetinkaya Z, Kara R, Telli R. 2008a. Occurrence of *Escherichia coli* O157:H7/O157, *Listeria monocytogenes* and *Salmonella* spp. in beef slaughterhouse environments, equipment and workers. Journal of Muscle Foods 19(3): 261-274.

Akkaya L, Alisarlı M, Cetinkaya Z, Telli R, Gök V. 2008b. The prevalence of *Escherichia coli* O157:H7/O157, *Listeria monocytogenes* and *Salmonella* spp. on bovine carcasses in Turkey, Journal of Muscle Foods 19(4): 420- 429.

Anonymous 1995. Microbiology of food and animal feeding stuffs Horizontal method for the detection and enumeration of L. monocytogenes Part1: Detection method. ISO11290-1.

Anonymous 1996. Cell separation and protein purification. Technical handbook. 2nd Ed. Dynal A.S., Norway Printed: 02 96,1996. http://tools.invitrogen.com/content/sfs/manuals/ 710%2006.Dynabeads%20anti%20 Listeria (rev004).pdf

Ayaz ND, Erol İ. 2011. Serotype distribution of Listeria monocytogenes isolated from turkey Meat by multiplex PCR in Turkey. Journal of Food Safety 31: 49-153.

Aznar R, Alarcon B. 2003. PCR detection of Listeria monocytogenes: a study of multiple Factors affecting sensitivity. Journal of Applied Microbiology 95: 958-966.

Barakat RK, Harris LJ. 1999. Growth of Listeria monocytogenes and Yersinia enterocolitica on cooked modified-atmosphere-packaged poultry in the presence and absence of a naturally occurring microbiota. Applied and Environmental Microbiology 65 (1): 342-345.

Barbuddhea SB, Malika SVS, Bhilegaonkar KN, Kumar P, Guptab LK. 2000. Isolation of *Listeria monocytogenes* and Anti-listeriolysin o detection in sheep and goats. Small Ruminant Research 38: 151-155.

Bohnert M, Dilasser F, Dalet C, Mengaud J, Cossart P. 1992. Use of specific oligonucleotides for direct enumeration of *Listeria monocytogenes* in food samples by colony hybridization and rapid detection by PCR. Research Microbiol 143: 271-280.

Ciftcioglu G.1992. İstanbul piyasasındaki kıyma, sucuk ve tavuk eti örneklerinde Listeria türlerinin mevcudiyetinin araştırılması. Istanbul Universitesi Saglık Bilmileri Enstitüsü. Doktora Tezi. Istanbul.

CLSI. 2012. Clinical and Laboratory Standards Institute. Methods for Antimicrobial Dilution and Disk Susceptibiltiy Testing of Infrequently Isolated or Fastidious Bacteria. Second edition. Approved Guideline M45-A2, Wayne, PA, USA.

Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P. 2004. Differentiation of the majör *Listeria monocytogenes* serovars by Multiplex PCR. Journal of Clinical Microbiology 42(8): 3819-3822.

Fantelli K, Stephan R. 2001. Prevalence and characteristics of shigatoxin-producing *Escherichia coli* and *Listeria monocytogenes* strains isolated from minced meat in Switzerland. International Journal of Food Microbiology 70: 63-69.

Farber JM, Peterkin PI. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiology Review 55: 476-511.

Fernandez PS, George SM, Sills CC, Peck MW. 1997. Predictive model of the effect of CO_2, pH, temperature and NaCl on the growth of Listeria monocytogenes. International Journal of Food Microbiology 37(1): 37-45.

Harakeh S, Saleh İ, Zouhairi O, Baydoun E, Barbour, Alwan N. 2009. Antimicrobia resistance of *Listeria monocytogenes* isolated from dairy based products. Science of the Total Environment 407: 4022-4027.

Hart CD, Mead GC, Norris AP. 1991. Effects of gaseous environment and temperature on the storage behaviour of *Listeria monocytogenes* on chicken breast meat. Journal of Applied Bacteriology 70: 40-46.

Hof H, Rocourt J. 1992. Is any strain isolated detected in food a health risk? International Journal of Food Microbiology 16: 683–692.

Holzapfel WH, Becker B. 2007. *Listeria monocytogenes*, an important concern for the meat industry, International Symposium on Meat safety: From Abattoir to Consumer, Valencia-Spain, 14–15 February 2007, Absracts of Invited Lectures. [http://www.meat-ims.org/symposium_proceeding.pdf].

Jay JM. 2000. Modern Food Microbiology. Sixth Edition, Maryland. pp: 485-510.

Jemmi T, Stephan R. 2006. *Listeria monocytogenes:* food-borne pathogen and hygiene indicator. Revue scientifique et technique-Office International des Epizooties 25(2): 571-580.

Liu D, Lawrence ML, Wiedmann M, Gorski L, Mandrell RE. 2006. *Listeria monocytogenes* subgroups IIIA, IIIB, and IIIC delineate genetically distinct populations with varied virulence potential. Journal of Clinical Microbiology 4229-4233.

Luppi A, Bucci G, Maini P, Rocourt J. 1998. Ecological survey of Listeria in the Ferrara area (northern Italy). Zentralbl Bakteriol Mikrobiol Hyg A 269: 266-275.

McGowan AP, Bowker K, McLauchlin J, Bennett PM, Reeves DS. 1994. The occurrence and seasonal changes in the isolation of *Listeria* spp. in shop bought food stuffs, human feces, sewage and soil from urban sources. International Journal of Food Microbiology 21: 325-334.

Niederhiauser C, Candrian U, Hofelein C, Jermini M, Buhler HP, Luthy J. 1992. Use of polymerase chain reaction for detection of *Listeria monocytogenes* in Food. Applied and Environmental Microbiology 58(5): 1564-1568.

Norrung B, Andersen JK, Schlundt J. 1999. Incidence and control of *Listeria monocytogenes* in foods in Denmark. International Journal of Food Microbiology 53: 195-203.

Pothuri P, Marshall DL, McMillin KW. 1995. Combined effects of packaging atmosphere and Lactic acid on growth and survival of *Listeria* monocytogenes in crayfish tail meat at 4°C. Journal of Food Protection 59: 253-256.

Pesavento G, Ducci B, Nieri D, Comodo N, Lo Nostro A. 2010. Prevelance and antibiotic susceptibilty of Listeria spp. isolated from raw meat and retaik foods. Food Control 21: 708-713.

Pincus DH. 2010. Microbial identification using the Biomerieux VITEK 2 system. Hazelwood, MO, USA www.pda.org/bookstore 2010.

Rahimi E, Ameri M, Momtaz H. 2010. Prevelance and antimicrobial resistance of Listeria Species isolated from milk and dairy products in Iran. Food Control 21: 1446-1452.

Roberts A, Nightingale K, Jeffers G, Fortes E, Kongo JM, Wiedmann, M. 2006. Genetic and phenotypic characterization of *L. monocytogenes* lineage III. Microbiology152: 685-693.

Samadpour M, Barbour MW, Nguyen T. 2006. Incidence of enterohemorrhagic *Escherichia coli, Escherichia coli O157, Salmonella and Listeria* monocytogenes in retail fresh ground beef, sprouts and mushrooms. Journal of Food Protection 69: 441-443.

Seeliger HPR, Höhne K. 1979. Serotyping of *Listeria monocytogenes* and related species. Methot Microbiology, 13: 31-49.

Sireli T, Erol İ. 1999. Hazır kıymalarda Listeria türlerinin araştırılması. Turkish Journal of Veterinary Animal Science, 23: 373-80.

Thevenot D, Delignette-Muller ML, Christieans S, Vernozy-Rozand C. 2005. Prevalence of *Listeria monocytogenes* in 13 dried sausage processing plants and their products. International Journal of Food Microbiology, 102: 85-94.