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RESEARCH ARTICLE

Determining Existance and Antibiotic Susceptibility Status of *Listeria monocytogenes* Isolated from Dairy Products, Serological and Moleculer Typing of the Isolates [1]

Emek DÜMEN * ✎ Ghassan ISSA * Serkan İKİZ ** Funda BAĞCIGİL **
Yakut ÖZGÜR ** Tolga KAHRAMAN * Sevgi ERGİN *** Osman YEŞİL *

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* İstanbul University, School of Veterinary Medicine, Department of Food Hygiene and Technology, TR-34320 Avcılar, İstanbul - TURKEY
** İstanbul University, School of Veterinary Medicine, Department of Microbiology, TR-34320 Avcılar, İstanbul - TURKEY
*** İstanbul University, Cerrahpaşa School of Medicine, Department of Clinical Microbiology, TR-34098 Cerrahpaşa, İstanbul - TURKEY

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Summary

In this study, 700 samples of dairy and its products were collected from İstanbul and Trakya region and the samples were analyzed for *L. monocytogenes* by using the reference methods. 20 samples of 700 were *L. monocytogenes* positive. The positive samples were confirmed by using PCR. Antibiotic susceptibility tests were applied to the positive samples by using 16 different kinds of antibiotic to expose the sensitivity profiles of the agents. Serotyping procedures were applied to the positive samples and all the agents were determined as *L. monocytogenes* serovar 4d. PCR confirmed samples were sequenced and specific DNA sequences of the agents were determined. Afterwards, Lineage groups of the positive samples were investigated and all the positive samples were exposed to be in Lineage I. The last step of our study was to compare our samples with the reference samples that are isolated from different origins which were belonged to same and different Lineages and to discuss all procedures that were applied in the study.

Keywords: *Listeria monocytogenes, Milk, Dairy products, Serotyping, Lineage*

Süt ve Süt Ürünlerinde *Listeria monocytogenes* Varlığının Belirlenmesi, Antibiyotik Duyarlılık Profillerinin Saptanması, Izolatların Serolojik ve Moleküler Yöntemlerle Tiplendirilmesi

Özet

Bu çalışmada İstanbul ve Trakya bölgelerinden toplanan 700 adet süt ve süt ürünleri örnekleri *L. monocytogenes* yönünden referans yöntemler ile analiz edilmiştir. Analiz edilen toplam 700 adet örnekten 20 adedi *L. monocytogenes* yönünden pozitif olarak saptanmıştır. Saptanan pozitif örneklere PCR analizi yapılmış ve örneklerin tamamı *L. monocytogenes* pozitif olarak doğrulanmştır. Pozitif örneklere antibiyotik duyarlılık testleri yapılarak etkenlerin toplam 16 adet antibiyotiğe karşı duyarlılık profilleri ortaya konmuştur. Daha sonra etkenlere serotiplendirme prosedürü uygulanmış ve etkenlerin 4d serotipine dahil oldukları belirlenmiştir. PCR prosedüründe doğrulanan örneklere DNA dizileme işlemi uygulanmış ve etkenlerin spesifik bölge DNA dizileri tespit edilmiştir. Sonraki aşamada ise etkenlerin Lineage (soy) grupları belirlenmiştir. Pozitif olarak tespit edilen etkenlerin tamamının Lineage I'e dahil olduğu saptanmıştır.

Anahtar sözcükler: *Listeria monocytogenes, Süt, Süt ürünleri, Serotiplendirme, Lineage (Soy)*

✎ **İletişim (Correspondence)**
☏ +90 212 4737070/17154
✉ emekdumen@yahoo.com

INTRODUCTION

Listeria monocytogenes, is an important food borne pathogen that cause gastroenteritis, septicemia, central nervous system infections, materno - fetal infections and abortions in humans. *L. monocytogenes* is a Gram positive facultative anaerobic microorganism and can be isolated from almost all foods. The agent is identified as the main factor of epidemic and sporadic listeriosis since 1980s by the medical literatures. However much frequencies of listeriosis outbreaks are relatively less than the other food-borne pathogens based outbreaks, because of high rate of mortality (up to 40%) *L. monocytogenes* is one of the most dangerous pathogens that threat public health [1,2].

Fourteen different serovars of the agent were determined. *L. monocytogenes* serovar 1/2a, serovar 1/2b and serovar 4b are generally identified as the main responsible serovars from human and animal listeriosis. Human isolates are generally included into 4b serovar while food isolates of the agent are included into 1/2 serogroups. Nevertheless the studies performed in the last years indicate that the isolation rate of 1/2 serogroups increases from humans, too [3].

Serotyping of *L. monocytogenes* with conventional methods are important but indicated to be insufficient for epidemic aspect. Analysis methods such as ripotyping, comparative virulence characterization and virulence gene allelity, seperate *L. monocytogenes* serogroups into 3 lineages according to their genetic structure. Serogroups included into Lineage I (flagellar antigen type b and d) are isolated more frequent from humans than Lineage II (antigen type a or c) and lineage III (serovars 4a and 4c, occasionally isolated). Determining lineages of the isolates is very important for both establishing the origins of the agents and exposing the potential virulence of the agents [4-6].

L. monocytogenes can easily cross contaminate from animal to animal and from animal to human by using feco -oral pathway because of its survival potential in soil, vegetables, milk and dairy products, drinkable and waste water [5-8]. Milk and dairy products, cheeses, meat and its products and vegetables are indicated to be the main food sources of human listeriosis [2]. *L. monocytogenes* outbreaks are reported in almost all countries of the world, but especially in The United States [1]. *L. monocytogenes* is the primer species which is responsible from meningitis. Besides, rarely *L. ivanovii* can cause meningitis, too [9]. Pregnant women, pediatric and geriatric cases, the cases whose immune system are depressed as the people infected with HIV or hepatitisis and cancer patients are the primer risk groups for *L. monocytogenes*. Agent can cause invasive or non - invasive forms of listeriosis in both animals and humans. Most evident symptom of non - invasive listeriosis is gastroenteritis combined with high fever. Invasive listeriosis can lead infected cases to death with symptoms as meningitis septicemia, endocarditis, non -meningial central nervous system infections, conjunctivitis, and influenza like symptoms [10]. According to a study performed in Australia in the year 2004, listeriosis incidence was 0.24% for every 100.000 persons, and the mortality rate or *L. monocytogenes* infected case was 21% [11,12].

L. monocytogenes is frequently isolated from unpasteuraized milk and dairy products. Also it is indicated that the agent can cross contaminate to humans, especially to the staff of dairy plants, from equipments and asymptomatical porters [13]. Because the agent is highly zoonotic and can cause abortus, septicemia and encephalitis in cattle too, it is very important to provide sufficient hygienic conditions in stables and the plants that produce milk and/or dairy products [14]. Fernandez - Garayzabal et al.[2] and Ryser et al.[15], point out that *L. monocytogenes* can even resist to UHT (ultra high temperature) process by infiltrating of the agent into the somatic cells and/or leukocytes of milk. According to a study performed in the year 1987, it was determined that the *L. monocytogenes* infected raw milk rate samples were as 0.32% in Germany, 4.2% in France, 4.4% in Netherlands, 7% in Massachusett - United States [16].

Conventional microbiological analysis methods are still accepted as referance methods [17-19]. In addition to the conventional microbiological methods, molecular - genetic analysis methods are becoming very important for verification, further scientific studies and fast isolation procedures of the pathogens. According to European Union (EU) norms, development of molecular - genetic methods are encouraged by support of the government in almost all fields of the science, but especially in medicine [20,21]. Partial sequencing of *act*A gene is accepted as a very economic and effective method for classification of the lineages of *L. monocytogenes* [22,23]. Aim of this study was to expose incidence differences of *L. monocytogenes* originated from milk and different dairy products. Besides, we aimed to determine antibiotic susceptibility profiles of the agent. Also it was aimed subtyping the serovars of *L. monocytogenes* we isolated with molecular subtyping methods. As the final stage of the study we aimed to determine Lineage groups of the isolated serovars by DNA sequencing and form a comparative dendogram shows genetic similarities of the isolates we got from our study and different origin based isolates verified by the reference laboratories of the world.

MATERIAL and METHODS

Sampling

Trakya is the main milk producer region and İstanbul is the most crowded and biggest city of Turkey. In this aspect, 700 milk and dairy product samples were collected from 3 different cities of Trakya (Tekirdağ, Kırklareli, Edirne) besides İstanbul. Properties, origins and kinds of the samples are shown in *Table 1*.

Table 1. *Details of the samples collected according to their numbers due to their origins and cities*

Tablo 1. *Toplanan örneklerin illere ve il bazındaki sayılarına göre detay veri dağılımı*

Number of Samples Collected	Origin	City
60	Milk (Raw)	Tekirdağ
30	White Pickled Cheese (Open)	Tekirdağ
30	Kashar Cheese (Open)	Tekirdağ
20	Cream (Open)	Tekirdağ
60	Milk (Raw)	Kırklareli
30	White Pickled Cheese (Open)	Kırklareli
30	Kashar Cheese (Open)	Kırklareli
20	Cream (Open)	Kırklareli
60	Milk (Raw)	Edirne
30	White Pickled Cheese (Open)	Edirne
30	Kashar Cheese (Open)	Edirne
20	Cream (Open)	Edirne
60	Milk (Raw)	İstanbul (European Side)
30	White Pickled Cheese	İstanbul (European Side)
30	Kashar Cheese	İstanbul (European Side)
20	Cream	İstanbul (European Side)
60	Milk	İstanbul (Asian Side)
30	White Pickled Cheese	İstanbul (Asian Side)
30	Kashar Cheese	İstanbul (Asian Side)
20	Cream	İstanbul (Asian Side)
TOTAL 700		

Microbiological Analysis

Microbiological analysis of the collected samples were performed according to International Standardization Organization (ISO) procedures [17]. 25 gr/ml sample was inoculated into 225 ml Buffered Listeria Enrichment Broth Base (BLEB) and the samples were incubated at 30°C for 4 h. At the end of the 4th h 25 mg/L natamycine was added to each sample and the incubation period was continued up to 48 h at 30°C. At the 24th h of the incubation samples were inoculated onto Oxford Agar and Palcam Agar Plates and the plates and were again inoculated for 48 h at 35°C. After 48 hours, all the samples (both from Oxford and Palcam Agars and from BLEB) were inoculated onto Chromogenic Listeria Agar. Then, suspected colonies were passaged onto Triptic Soy Agar with Yeast Extract (TSA) for purification. Suspected isolates which matched to all identification parameters according to reference method (Gram staining, catalase activity, motility test, fermentation of maltose, rhamnose, mannitol, and xylose, hydrolization of esculin, reduction of nitrate) were evaluated as positive. CAMP test with *Staphylococcus aureus* and Henry illumination tests were also applied to all suspected samples.

Antibiotic Susceptibility Tests

Antibiotic susceptibility tests of the suspected isolates were determined according to the standards of Clinical and Laboratory Standards Institute (CLSI) with disc diffusion method [24]. Amicazine (30 µg), amoxicillin (15 µg), sefazoline (30 µg), chloramphenicole (30 µg), eritromycine (15 µg), flumequine (30 µg), gentamycine (10 µg), canamycine (30 µg), lincomycine (10 µg), oxitetracycline (30 µg), rifampycine (5 µg), spiramycine (100 µg), streptomycine (10 µg), tetracycline (30 µg), tobramycine (30 µg), and vancomycine (30 µg), discs were used.

Serotyping

Denka Seiken (Ref 294616) ready to use antiserums were used according to serotyping procedure of the manufacturer [24].

I - Antigen - O Determining with Slide Agglutination: Suspensions were prepared in sodium chloride (0.2% w/v) with a density of 10 mg/ml from the isolates grown in BHI (Brain Heart Infusion Agar). Suspensions were heated 121°C 30 min and they were centrifuged at 3.000 rpm during 20 min. Supernatant of the suspensions was removed and precipitate was again suspended in a very little amount of sodium chloride (0.2%w/v) again.

II - Slide Agglutionation Test with Polyvalant Type O Antiserum: Bacterial suspensions placed on the slide after dropping OI/II, OV/VI antiserums with physiological saline water (one drop for each). Antiserum and bacterial suspension were mixed on the slide and agglutination position was observed. Existance of agglutination in 1 min was evaluated as positive

I - a Slide Agglutination Test with Monovalant O - antiserum: Positive evaluated isolates with antiserum OI/II were tested with OI and OIV. Isolates agglutinated with OV/VI antiserums were tested with OVI, OVII and IX antiserums.

I - b H Antigen Determining with Tube Agglutination Method: It is adviced to be passaged the isolates to a half solid agar for increasing capability of *L. monocytogenes* (because *L. monocytogenes* serovars has 1-4 flagella). For this purpose, cultures were placed into Craigie's tubes included half solid BHI. After 3 times passaging, the liquid inoculated again, but into petri dishes with BHI. Bacterial suspension was prepared adding 1v/v physiological saline water with formaline with the culture grown after overnight incubation of BHI included Petri dishes at 30°C. For each sterile tube, 2 drops of H antiserum was put and 0.5 ml bacterial suspension was added. The tubes that did not include antiserum were used as control. Each tube was mixed for homogenization and they were incubated water bath 1 h at 52°C. At the end of the incubation period, agglutinated tubes were evaluated as positive.

PCR

DNA of all isolates were extracted according to the protocol of the manufacturer (Macherey-Nagel, Nucleospin®

Tisue). All the extracts were stored at -20ºC until they are used as target DNA for the PCR procedure.

L. monocytogenes specific *act*A gene was reproduced by using specific designed primers (01: 5'- GCTGATTTAAGA GATAGAGGAACA-3' and 02: 5'-TTTATGTGGTTATTTGCTGTC -3') (GenBank accession no. NC-003210). Each PCR mixture was 100 µl and consisted of 4 µl of each primers (forward & reverse), 10 µl 10X buffer (Kapabiosystems, 1.5 mM Mg for the final concentration), 8 µl 25 mM dNTP mixture (TaKaRA), 0.5 µl Taq polymerase (5 u/ul - Kapa), 69.5 µl distilled water and 4 µl target DNA and the mixtures were put to the thermal cycler. After 1st denaturation at 94ºC 2 min, total of 35 cycle (1 min at 94ºC, 1 min at 50ºC, 1 min at 72ºC) and final synthesis step (10 min at 72ºC was applied). PCR products were stored at +4°C up to electrophoretic seperation.

Electrophoresis procedure was applied to 10 ml. of PCR products with 2 µl 6X loading buffer at 1.5% agarose with ethidium bromide and existance of specific bands were explored via U.V. Observed bands at 827 bp were evaluated as positive.

DNA Sequencing, Linagesand Dendograms

Lineages of all *L. monocytogenes* isolates were determined by sequencing *act*A gene according to the methods of Zhou et al.[23]. Sequencing procedure was applied to the 3' - terminal region of DNA zone of approximately 800 bp interval of *act*A gene.

RESULTS

In the study total of 700 milk and dairy product samples were investigated (300 raw milk, 150 white pickled cheese, 150 kashar cheese and 100 cream samples). Twenty samples were evaluated as *L. monocytogenes* positive (12 samples of raw milk, 3 samples of white pickled cheese, 4 samples of kashar cheese and 1 sample of cream). *Table 2* shows the city origins of positive samples according to their sources.

Table 3 shows antibiotic resistance of *L. monocytogenes* isolates

As a result of serotyping procedures, all the positive samples were included to serotype 4d. Conventional microbiological methods were totally parallel with the PCR results. PCR results of the analysed samples is shown in *Fig. 1*.

Positive samples were included to Lineage I. Results, then compared with different isolates belonged to Lineage II and III confirmed from different origins by the international medical literatures according to genetic similarities and dendogram table was prepared *(Table 4)*.

Table 2. *Subunit details of positive isolates according to the cities which they were collected and their origins*
Tablo 2. *Pozitif izolatların toplandıkları şehirlere ve elde edildikleri kaynaklara göre detay altkırılımları*

Row Number of Positive Isolates	Origin	City	Number of Positive Samples Isolated
1	Milk	Tekirdağ	3
2	Milk	Kırklareli	2
3	Milk	Edirne	2
4	Milk	İstanbul (European Side)	3
5	Milk	İstanbul (Asian Side)	2
6	White Pickled Cheese	Tekirdağ	1
7	White Pickled Cheese	Kırklareli	None
8	White Pickled Cheese	Edirne	1
9	White Pickled Cheese	İstanbul (European Side)	1
10	White Pickled Cheese	İstanbul (Asian Side)	None
11	Kashar Cheese	Tekirdağ	1
12	Kashar Cheese	Kırklareli	None
13	Kashar Cheese	Edirne	None
14	Kashar Cheese	İstanbul (European Side)	2
15	Kashar Cheese	İstanbul (Asian Side)	1
16	Cream	Tekirdağ	None
17	Cream	Kırklareli	None
18	Cream	Edirne	1
19	Cream	İstanbul (European Side)	None
20	Cream	İstanbul (Asian Side)	None

Table 3. *Antibiotic susceptibility test results of Listeria monocytogenes positive samples*
Tablo 3. *L. monocytogenes pozitif örneklerin antibiyotic duyarlılık testi sonuçları*

Antibiotic/Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Tetracycline	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	İ	R
Streptomycine	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Chloramphenicol	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Tobramycine	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Vancomycine	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Spiramycine	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Amikacin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Flumequine	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R
Oxytetracycline	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S
Gentamycine	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S
Sefazoline	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Amocycyline	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Rifampicine	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S
Eritromycine	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Linkomycine	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Canamycine	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

S: *Sensitive* ***R:*** *Resistant* ***I:*** *Intermediate Sensitive*

Table 4. *Comparison of different genetically confirmed L. monocytogenes isolates obtained from different origins with the present study-findings*
Tablo 4. *Çalışmanın sonuçları ile dünyanın değişik bölgelerinden farklı kaynaklardan köken almış ve referans laboratuvarlarca doğrulanmış L. monocytogenes izolatlarının karşılaştırılması*

Isolate Number	Source	Origin	Serotype	*hly* type	*act A* Type	*EcoRI* Ribotype	Lineage
IUSVM 91038	IUSVM	Kashar Cheese	4d	1	4	DUP 1042	I
IUSVM 91039	IUSVM	Milk	4d	1	3	DUP 1042	I
IUSVM 91040	IUSVM	Kashar Cheese	4d	1	4	DUP 1042	I
IUSVM 91041	IUSVM	Milk	4d	1	4	DUP 1052	I
IUSVM 91042	IUSVM	Cream	4d	1	3	DUP 1052	I
IUSVM 91043	IUSVM	White Pickled Cheese	4d	1	4	DUP 1042	I
J 2003	CUVDL	Cattle	1/2a	2	3	DUP 1030	II
J 2017	CUVDL	Cattle	1/2a	2	4	DUP 1030	II
J 1022	CDC	Human	1/2c	2	4	DUP 1030	II
J 1047	CDC	Human	1/2c	2	4	DUP 1039	II
C 1117	CTDOH	Human	1/2a	2	4	DUP 1039	II
J 2068	CUVDL	Horse	4c	1b	4	DUP 1059	III
X 1002	CDC	Food	4a	4a	3	DUP 1059	III
W 1110	CDC	Non specific	4c	4a	3	DUP 1055	III
W 1111	CDC	Non specific	4c	4b	3	DUP 1032	III
J 1158	CUFSL	Goat	4b	4b	3	DUP 1042	III

** All data of the isolates compared with our findings in Table 2 were taken from international reference sources [25,26].*

CDC: Centers For Disease Control and Prevention - USA
CUVDL: Cornell University Veterinary Diagnostic Laboratory/Ithaca - USA
CTDOH: Connecticut Department of Health/Connecticut - USA
CUFSL: Cornell University Food Safety Laboratory/Ithaca - USA
IUSVM: *Istanbul University, School of Veterinary Medicine, Dept. of Food Hygiene & Tech. İstanbul - TURKEY*

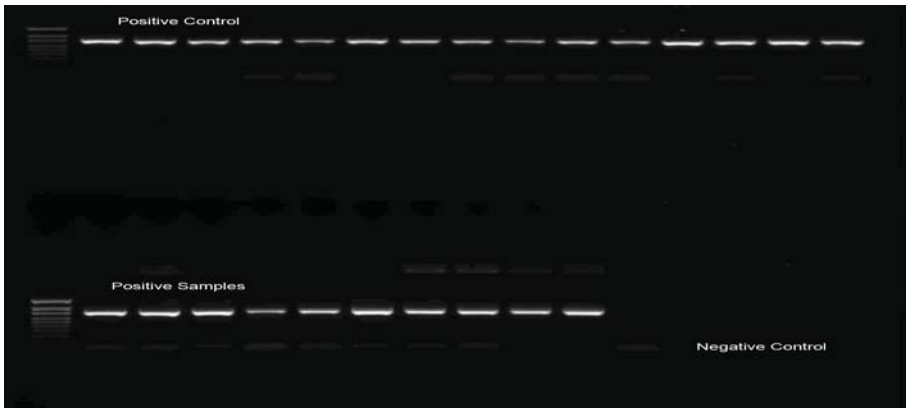

Fig 1. PCR results of *L. monocytogenes* positive samples

Şekil 1. *L. monocytogenes* pozitif örneklerin PCR sonuçları

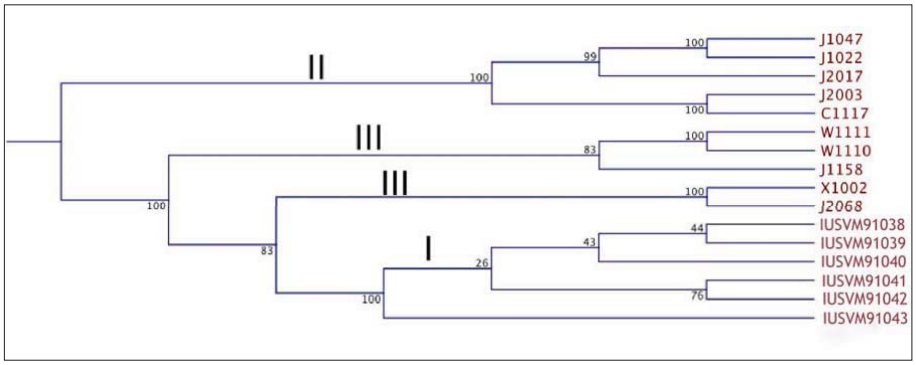

Fig 2. Comparision of *L. monocytogenes* isolates verified by the reference laboratories from different origins in the world included to Lineage II and III with the present study findings according to genetic similiarities by dendogram

Şekil 2. Farklı kaynaklardan ve dünyanın farklı coğrafyalarından orijin almış ve referans laboratuvarlarca genetik olarak doğrulanmış Lineage II ve Lineage III'e dahil *L. monocyotogenes* izolatları ile araştırma bulgularının genetik yakınlık açısından karşılaştırılmasının dendogram ile gösterilmesi

DISCUSSION

L. monocytogenes is a Gram positive and facultatively anaerobic food borne pathogen that cause gastroenteritis, septicemia, central nervous system infections, materno-fetal infections and aborts in humans. The agent can be isolated from almost all foods and can cause sporadic and epidemic outbreaks. The agent is identified as the main factor of epidemic and sporadic listeriosis since 1980's by the medical literatures. However much frequencies of listeriosis outbreaks are relatively less than the other food borne pathogens based outbreaks, because of high rate of mortality (up to 40%) *L. monocytogenes* is one of the most dangerous pathogens that threat public health [1,2,27].The agent threats public health by its easy growing characteristic in all foods but especially in milk and dairy products, meat and its products, vegetables and seafoods because of its high survival capacity in natural environment such as soil, water and silage [28].

Gungor et al.[29] indicated that they diagnosed a case as *L. monocytogenes* infected who had symptoms of nausea, vomiting, headache, dizziness and high fever and had a story of fresh cheese consuming habit. This situation states the importance of application of decontaminating processes in foods especially in dairy products. However, *L. monocytogenes* can also survive in salt treated dairy products such as white pickled cheese besides short aged and fresh cheese. Akkaya and Alisarli [30] indicated that they explored 100 white pickled cheese samples obtained from open bazaars of Afyon and 6 samples were positive In this study, *L. monocytogenes* was from 3 white pickled cheese.

In The United States and Canada, 6 big listeriosis outbreaks were reported between the years 1979 - 1999. According to the litarature, hospitalized cases were contaminated from different foods such as carrot, potato, pasteurized and raw milk, pork, hotdog, chocolate and various kinds of cheese. Infected cases had symptoms as meningitis, encephalitis and/or septicemia and mortality rate of the infected cases was reported as 20% [31].

L. monocytogenes generally follow animal feed to animals /foods to humans contamination pathways and the major serovars of the agent that cause human listeriosis can be listed as 1/2b, 1/2 and 4b. Serovar 4b is responsible from human outbreaks and sporadic cases in general. In spite of using food - human contamination pathway, it is still unclear that why serovars 1/2a and 1/2b are isolated from foods while serovar 4b is isolated from humans generally [32].

However, presence of differantial proteins said to be relate/interact with other proteins of 4b isolates are being explored by means of developing comperative sub-proteomic analysis technics. Before development of sub-proteomic technics, it has been considered that asymtomatic 4b serovars originated from clinical and environmental sources, however now it is being evaluated that 4b serovars are probably integrated from different serovars by gaining various differential proteins [32].

Serovar 4b is generally included to Lineage I and lineage III, but specific antigen surfaces related to changing numbers (generally increasing) and rows of differential proteins could diverge with an unclear mechanism and this situtation can reveal misleading results for phenotypically serotyping of *L. monocytogenes* [33]. For the reasons mentioned above, it is being thought that serovars 4d and 4e are derivated from serovar 4b with a complex conjugated mechanism and "serovar 4b complex" concept is being used for serovar 4d and 4e [34].

According to the results, it has been considered that our serovars (4d) also could include some differential proteins and/or have capability of corelation with differential proteins with other serovars of *L. monocytogenes*. Studies indicate that serovar 4d could be isolated from various foods such as water and seafood, meat and its products, milk and dairy products, vegetables and infected human beings [35]. The authors think that isolated serovars could seriously threat stable hygiene (especially infected milk samples), staff health of the dairy plants (especially infected cheese and cream samples) and puclic health (all infected samples). It is considered that high infection, survival and cross contamination capacity of the agent may increase the risk factors for public health.

Antibiotic susceptibility results are parallel to results of other studies [36-38]. In spite of sensitiveness of *L. monocytogenes* to many antibiotics, blood - placenta and blood - brain barriers can decrease the efficiency of the applied antibiotics at meningitis, encephalitis, amnionitis and fetus infections cases and this situation can increase the mortality rates of the infected cases. Besides, *L. monocytogenes* infections can lead with septicemiae at the cases mentioned above, so prognosis can be effected more negatively and application of the antibiotics can be useless [39]. Zhang et al.[39], indicate that human listeriosis originated from food become more resistant to antibiotics regularly with an increasing rate since 1988.

Food based microorganisms resistant to antibiotics are generally said to be saphropyhtes. However, resistence genes of different saphrophytes can be transferred to *L. monocytogenes* and many pathogens with mobile genetic structures as plasmids and transposons in the gastrointestinal tract [41]. This situtation can generate a strong possibility of formation of common genes of *L. monocytogenes* with many other pathogens but especially with *Streptococcus* spp. and *Enterococcus* spp. Besides, resistance genes can be transferred to *L. monocytogenes* and the other foodborne pathogens by changeable conjugative mobilization property from different habitat originated gram positive and gram negative microorganisms [39].

Gene transfer among different microorganisms can also lead the transfer of the virulence genes that have high level polymorphic protein to *L. monocytogenes* serovars. This situation cause to diverge the genetic structure of *L. monocytogenes* serovars. Due to these genetic diverging, antibiotic resistance properties to of *L. monocytogenes* serovars from different, even same Lineages from different ecologies and/or different geographic regions can be highly variable. Aureli et al.[41], indicated that *L. monocytogenes* they isolated were resistant to lincomycine, but according to our results, our serovars were highly sensitive to the same antibiotic. Besides, same scientists found out that their serovars showed the most sensitivity to streptomycine. However our all serovars were resistant to streptomycine.

According to these different results of various literatures, it is thought that a plenty of variables (environment, geographic regions, even the rate and number of saphrophytes grown in the infected food) affect genetic variations and resistance to antibiotics of *L. monocytogenes* serovars. In spite of our serovars were sensitive to many antibiotics according to the results we obtained from the antibiotic susceptibility tests, it is hard to estimate the resistance circumstances of *L. monocytogenes* serovars in our country in future. Because of these reasons trace of presence of *L. monocytogenes* must be taken into consideration, especially in foods very carefully and regularly.

L. monocytogenes is pathogen microorganism gathered under 3 lineages (Lineage I, II and III) according to ribotyping procedures due to *hly* and *act*A allelic genes which are reported to be responsible from contamination and spreading from cell to cell [24]. Lineage I is identified as main responsible lineage from symptomatic and/or asymptomatic human listeriosis outbreaks while Lineage II is generally isolated from various foods and Lineage III is originated from animals and humans with clinical symptoms [43,44]. In this study, obtained serovars can seriously risk the public health in the studied region because of explained properties. Missing routine medical controls for asymptomatic cases and delay time of applications to the hospitals and/or mistakes about diagnosis procedures can seriously increase the risk factors for public.

Researchers evaluate the serovars included to Lineage I as highly virulent serovars relatively to serovars belonged to Lineage II and III [44]. They estimate this hyper virulent situation is based from an unclear contamination mechanism especially during the period of reproduction [42,44]. Another possible reason of this hyper virulent situation, could be genetic relation of especially 4d and 4e serovars with 4b serovar by interaction of differential proteins. "4b complex" concept and including 4d serovars to Lineage I [32], could cause the formation of much more resistant and pathogen new generation serovars because of high interaction of polymorphic proteins among different serovars even different microorganism species. Another reason that support this idea is preventing the clonal Lineages of the gene flow among the serovars belonged to different lineages [43].

*Act*A gene is identified as a gene which carry high polymorphic virulent genes. This study results expose that

the serovars belonged to Lineage I and II had a high variation defense/survival capacity against hard physical conditions by the help of changable aminoacid sequence structures. In United States, human and food isolates are generally belonged to Lineage I while the same origin isolated serovars belonged to Lineage II in China [41,42]. Molecular subtyping methods and virulence analysis provide hopeful developments for us to understand ecologies and chracteristics of foodborne pathogens like all microorganisms. High variation ability according to environmental conditions by help high polymorphic *Act*A gene of isolates identified in this study put forward the importance of comprehensive epidemiological researches and exploring the contamination pathways of new clonal groups in both our country and the world.

As a conclusion it is important to expose Lineage groups of *L. monocytogenes* serovars obtained from different origins for our country. Studies about determining phylogenetic status of *Act*A gene would be very important for exposing epidemiological profile of *L. monocytogenes,* controlling and prevention procedures, finding out the contamination pathways of the agent so for the food security chain and of course for public health both for the world and our country.

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