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Distribution of serotypes of *Listeria monocytogenes* in chicken meats in Turkey

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ABSTRACT. *Listeria monocytogenes* is one of the important causes of food-borne infections. This study was conducted to determine the presence of *L. monocytogenes* and its serotype distribution in a total of 400 packaged chicken meat products (drumstick, breast, wing, and whole chicken) from different national companies. *L. monocytogenes* contamination was detected in 26.5% (106 in 400) of all samples when the products considered, drumsticks, breasts, wings, and whole chickens showed 47%, 15%, 35, and 9% positivity respectively. Four important serotypes of *L. monocytogenes* in human listeriosis (1/2a, 1/2b, 1/2c and 4b) were identified, and serotype 1/2a (94.3%) was determined as predominant in packaged chicken meats. The present study revealed that *L. monocytogenes* 1/2a serotype is prevalent in chicken meats and this may cause public health problems in Turkey. Further studies in poultry meats should be conducted on a large scale such as regional or national big markets to determine the presence of the pathogen and its dominant serotypes.

Keywords: *Listeria monocytogenes*; food-borne pathogen; serotype 1/2a; mPCR, chicken meats.

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INTRODUCTION

As one of the important food-borne infectious agents, *Listeria monocytogenes* is still a serious concern for public health with its very high mortality rate (Farber and Peterkin, 1991; Oliveira et al. 2018). *L. monocytogenes* is a specific risk for the health of vulnerable people especially elderly people, immunocompromised individuals and pregnant women who show mortality rate up to 20% (CDC, 2016; Rothrock et al., 2019). *L. monocytogenes*, which is a facultative intracellular bacterium, is known to cause infections in both animals and humans. Food-borne infection controls are difficult because this agent is widely distributed in nature, can multiply in refrigerator environments, can tolerate a wide range of pH (4.3 to 9.6), and can multiply in high (10%) salt concentrations (Rocourt and Buchriser, 2007).

L. monocytogenes is a frequent contaminant of raw milk and an inhabitant of soil, water and other contaminated foods (Karthikeyan et al., 2015). It has been considered that nearly all cases of human listeriosis are foodborne and associated with consumption of contaminated dairy products, unwashed raw vegetables and under-cooked meat, seafood and poultry products (Dhama et al., 2015; Kurpas et al., 2018; Todd and Notermans, 2011). Recently, a noteworthy increase in chicken meat production is observed in Turkey like in other countries (TPMPBA, 2018). Consequently, the increase of infections and intoxications due to chicken meat may be due to improved rates of reporting and surveillance systems, or due to the increase in the population of elderly and immunocompromised people (EFSA, 2018). Birds may be an important vector and contributor to the contamination of the processing environment and transmission of *Listeria* to consumers via the food (Rothrock et al., 2017). According to the European Food Safety Authority (EFSA) report, 2.9% of all *L. monocytogenes* infections were caused by consumption of raw poultry products (EFSA, 2010).

The EFSA reported 2.480 confirmed invasive human cases of listeriosis in 2017. The EU notification rate was 0.48 cases per 100,000 population which was comparable with 2016. In the recent report published in 2018 by EFSA, *L. monocytogenes* was reported to be among the leading causes of food-borne infections with a mortality rate of 13.8% in 2017 (EFSA, 2018). Presence of *L. monocytogenes* in the food industry is a potential risk factor. Therefore, monitoring the microorganism through the food production and supply

chain is of primary importance.

Determining the serotype distributions of *L. monocytogenes* isolates is important for understanding food-borne infections, for withdrawing the risk imposing products and for differentiation of pathogenic and non-pathogenic strains (Liu, 2013). *L. monocytogenes* was divided into 13 different serotypes according to its somatic and flagellar antigens, which are known as 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7. Strains of 1/2a, 1/2b, 1/2c and 4b are known to be important for listeriosis in humans (Doumith et al., 2004; Jamali et al., 2013; Nho et al., 2015). The most common (91.8%) serotypes causing listeriosis in humans are 1/2a and 4b (EFSA, 2015). Serotype 1/2a is frequently detected in various animal-sourced foods including chicken meat and products (Guerra et al., 2001; Orsi et al., 2011; Praakle-Amin et al., 2006).

The aim of the present study was to determine the prevalence and serotype distribution of *L. monocytogenes* in packaged chicken meats in Turkey.

MATERIAL AND METHODS

Food samples

Samples belonging to the five biggest poultry production companies were selected for investigation. These companies (A, B, C, D, and E) have slaughtering capacity of average 10.000 broilers/h and are marketing their products in most parts of the country. A total of 400 retail chicken meat samples (80 samples per company) were bought from different markets from April 2015 to January 2016. Each set of 80 samples consisted of 20 drumsticks, 20 breasts, 20 wings, and 20 whole chickens.

Isolation and identification of *L. monocytogenes*

Each fresh sample was weighted in 25 g and the isolation of *L. monocytogenes* was conducted according to the classic culture method. This method includes two steps of pre-enrichment and selective enrichment in Half Fraser and Fraser Broth, respectively. After 225 ml of Half Fraser Broth (Merck 1.10398.0500) were added to samples (25 g), samples were homogenized in a stomacher (Interscience BagMixer 400 cc, France) for 2 to 3 min. The obtained homogenate was incubated for 24 h at 30°C for pre-enrichment. Following the pre-enrichment, 0.1 ml were taken from the culture and transferred into tubes containing 10 ml of Fraser Broth (Merck 1.10398.0500), then they were incubated for 24 h at 37°C. Inoculation into PALCAM Agar (Polymyxin Acriflavine Lithium Chloride Cefotaxime Aesculin

Mannitol Agar, Oxoid CM0877-SR0150E) was conducted and agar plates were left for incubation at 37°C for 48 h. At the end of the incubation period, 1-5 *Listeria* suspected colonies (black-centered, gray green in color with a black halo) in PALCAM Agar were selected and were transferred into TSA-YE (Tryptone Soy Agar with Yeast Extract, Oxoid CM0131-YE). TSA-YE grown colonies were transferred to TSB (Tryptone Soy Broth, Oxoid CM0129) containing 20% glycerin and were stored in -20°C for advanced analyses (Jeyaletchumi et al., 2010).

DNA extraction and PCR

Five hundred µl from cultures of each *L. monocytogenes* isolate in TSB were transferred to microcentrifuge tubes and tubes were centrifuged for 5 min at 10000 rpm (VMR International Galaxy 16DH). Supernatants were discarded and 300 µl sterile distilled water was added. Afterwards, 300 µl K-Buffer (20 mM Tris, 150 mM NaCl, 10 mM EDTA, 0.2% Sodium Dodecyl Sulphate (SDS)) and 5 µl Proteinase K (20 mg/ml) (Vivantis PC0712 (100 mg) were added to tubes. Tubes were incubated for 2 h at 56°C and were left in a water bath for 10 min at 95°C to inactivate proteinase K. Tubes were centrifuged for 10 min at 13000 rpm and the supernatants were discarded. A hundred µl sterile distilled water were added to remaining pellets and they were used as target DNAs in PCR.

Samples were analyzed first by using previously published primer sequences of *prs* and *prfA* genes, which are specific to *Listeria* spp. and *L. monocytogenes*, respectively. Afterwards, the serotypes of isolates were determined by multiplex PCR (mPCR) using specific primers *prs*, *lmo1118*, *lmo0737*, *orf2110* and *orf2819* (D'Agostino et al., 2004; Doumith et al., 2004).

For the detection of both *Listeria* spp. and *L. monocytogenes*, 50 µl PCR reaction mixtures were prepared. Each mixture was containing 5 µl 10X PCR Buffer (500 mM KCL, 100 mM Tris-HCl (pH 9.1) and 0.1% Triton X-100) (Vivantis, ViBufferA), 5 µl 25 mM MgCl₂ (Vivantis, 50 mM), 250 µM of each dNTP (Vivantis, NP2406, 100 mM), 20 pmol of each primer pair (Biomatik, Canada), 1.25 U Taq-polymerase enzyme (Vivantis PL1202, 500 U) and 5 µl (25 ng) of target DNA.

Serotyping of *L. monocytogenes* was conducted using the mPCR protocol of Doumith et al. (2004) in a thermal cycler device (Biorad-T100, Biorad, USA).

The PCR protocol was as follows: Initial denaturation at 94°C for 3 min, denaturation at 94°C for 40 sec, annealing at 53°C for 75 sec, extension at 72°C for 75 sec and a final extension at 72°C for 7 min. The protocol was conducted with 35 cycles. Reaction results were electrophoresed for 2 h in a 110 V in 1.5% agarose gel (Vivantis LE Grade Agarose Gel). Following the staining with ethidium bromide (10 mg/ml) (Merck 1.11608.0030), the gels were transilluminated (Vilber Lourmat Quantum ST4) to reveal DNA bands. *L. monocytogenes* ATCC 7644 (serotype 1/2c), RSKK 472 (serotype 1/2b), RSKK 471 (serotype 1/2a) and RSKK 475 (serotype 4b) were used as positive controls, while distilled water was used as negative control.

Statistical analysis

The statistical analyses were performed SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA). The chi-square test was used for analyzing the data obtained from the groups (products and companies) about the contamination with *L. monocytogenes*. The differences were considered significant at $p < 0.05$.

RESULTS

As shown in Table 1, 267 (66.75%) of the investigated chicken meat samples were detected by using classical culture method as suspect for *Listeria* spp. It is known that detection of non-pathogenic *Listeria* spp. can be challenging using classical culture methods due to the frequent presence of non-*Listeria* spp. background flora colonies that are β-glucosidase-positive (Angelidis et al., 2015). Hence, the PCR results for these isolates verified that a smaller fraction (62.75%) of the samples (251 in 400) were actually contaminated with *Listeria* spp.

Of the 251 suspect strains, 106 strains were determined in PCR as *L. monocytogenes*. The prevalence of *L. monocytogenes* in the investigated drumstick, breast, wing and whole chicken products were 47%, 15%, 35% and 9%, respectively (Table 1).

The highest isolation frequency for *L. monocytogenes* was noted in samples from company B (52.5%), whereas the lowest in company A (2.5%). Differences between company B and others (A, C, D, and E) were found as statistically significant ($p < 0.05$). The *L. monocytogenes* isolation frequency in samples from companies C, D, and E were 32.5%, 18.8% and 26.3%, respectively. Differences between these three companies were found to be non-significant ($p > 0.05$) (Table 2).

Table 1. Distributions of *Listeria* spp., *L. monocytogenes* and serotypes in chicken meats

Product	No. of samples	Culture results*	<i>Listeria</i> spp.	<i>L.monocytogenes</i>	<i>L. monocytogenes</i> serotype distribution (%)			
					1/2a	1/2b	1/2c	4b
Drumstick	100	%73 (73/100)	% 71 (71/100)	% 47 (47/100)	%93.6 (44/47)	%2.1 (1/47)	%2.1 (1/47)	%2.1 (1/47)
Breast	100	%67 (67/100)	% 66 (66/100)	% 15 (15/100)	%100 (15/15)	0	0	0
Wing	100	%82 (82/100)	% 80 (80/100)	% 35 (35/100)	%91.4 (32/35)	0	%5.7 (2/35)	%3.1 (1/35)
Whole Chicken	100	%45 (45/100)	% 34 (34/100)	% 9 (9/100)	%100 (9/9)	0	0	0
Total	400	%66.75 (267/400)	%62.75 (251/400)	%26.5 (106/400)	%94.3 (100/106)	%0.9 (1/106)	%2.8 (3/106)	%1.9 (2/106)

* Presumptive-positive results

Table 2. *L. monocytogenes* distribution according to company and product type

Product	Companies					Total
	A	B	C	D	E	
Drumstick	%0 ^d (0/20)	%100 ^{Aa} (20/20)	%70 ^{Ab} (14/20)	%20 ^d (4/20)	%45 ^{Ac} (9/20)	47/100
Breast	%5 (1/20)	%10 ^C (2/20)	%15 ^B (3/20)	%10 (2/20)	%35 ^A (7/20)	15/100
Wing	%5 ^b (1/20)	%85 ^{Ba} (17/20)	%35 ^{Bb} (7/20)	%30 ^b (6/20)	%20 ^{Ab} (4/20)	35/100
Whole chicken	%0 (0/20)	%15 ^C (3/20)	%10 ^B (2/20)	%15 (3/20)	%5 ^B (1/20)	9/100
Total	%2.5 2/80	%52.5 42/80	%32.5 26/80	%18.8 15/80	%26.3 21/80	%26.5 106/400

^{A-C} Differences between mean values of different letters are significant in the same column

^{a-d} Differences between mean values of different letters are significant in the same row

Four important serotypes of *L. monocytogenes* for human listeriosis (1/2a, 1/2b, 1/2c, and 4b) were investigated in *L. monocytogenes* isolates (n=106). Serotype 1/2a was determined as predominant with 94.3% in the isolates from chicken meats. Other determined serotypes in the isolates were 1/2c (n=3; 2.8%) and 1/2b (n=1; 0.9%). As for their distribution, *L. monocytogenes* serotypes 1/2a, 1/2b, 1/2c and 4b were determined in drumstick samples, and serotypes 1/2a, 1/2c and 4b were determined in wing samples (Table 1). All serotypes determined in breast and whole chicken samples were 1/2a. Serotype 1/2a, which was determined as predominant, was detected in 93.6% of drumsticks, 100% of breasts, 91.4% of wings and in 100% of whole chickens.

DISCUSSION

L. monocytogenes is an important pathogenic microorganism in terms of public health. Acquisition of

listeriosis is mainly due to consumption of contaminated (mostly ready-to-eat) food (Churchill et al., 2019; Lomonaco et al., 2015).

According to the other studies, the *L. monocytogenes* presence in different chicken meat products (drumstick, breast, and wings) in the world was reported to be between 7.14% in Malaysia and 71% in Spain (Goh et al., 2012; López et al., 2013). The prevalence of this pathogen in chicken meats was reported as varying between 8.4% and 38.4% in Turkey (Cetinkaya et al., 2015; Guven and Patir, 1998).

Chicken meat contamination with *L. monocytogenes* is known to be significantly increased during both slaughtering and processing of viscera and parts. Product comparisons in the study revealed that the *L. monocytogenes* isolation frequency in drumsticks was higher than other tested products. Drumsticks

are most preferred parts in our country and are used commonly in oven-baked meals. Companies lead products to more than one processing stage to make their products more appealing to the consumer and to improve long-term customer loyalty. These approaches may increase the level of cross contamination. The *L. monocytogenes* prevalence in drumsticks (47%) in this study was found higher than previously reported estimates of 11.27% (Goh et al., 2012) and 33.3% (Erol et al., 1999). This difference can be attributed to the company conditions, processing capacity, cross-contamination risks during the slaughtering and to the holding time of food before marketing (Goh et al., 2012).

In this study, the prevalence of *L. monocytogenes* in packaged chicken breast samples was 15% (Table 1). This was found to be similar to the previously reported estimates of 16.6% (Erol et al., 1999) and 18% (Soultos et al., 2003). Our estimate, however, was found lower than that reported (42.03%) in breast samples from wet markets by Goh et al. (2012). This difference is thought to be because of packaged and without skin breasts provided in markets.

The *L. monocytogenes* isolation frequency in wing samples was higher than those previously reported, i.e., 20% (Erol et al., 1999) and 18% (Soultos et al., 2003). Elmali et al., (2015) reported summer, autumn, winter, and spring *L. monocytogenes* prevalences in packaged wing samples as 60%, 73.3%, 33.3% and 13.3% respectively. In the study, *L. monocytogenes* in packaged wings was determined as 35%. Even though seasonal frequency was not investigated, most of the samples in the study were taken in the winter season. This is in agreement with the winter season findings of Elmali et al. (2015). In a study by Ayaz and Erol (2009) where *L. monocytogenes* presence was investigated in turkey meats, it was reported that seasonal effect was not present due to the psychrotrophic character of the bacteria.

In addition to reports from EFSA and CDC, various studies reported that most of the human listeriosis cases (more than 95%) are due to serotypes 1/2a, 1/2b, 1/2c, and 4b (CDC, 2014; Doumith et al. 2004; EFSA, 2015; Jamali et al. 2013; Nho et al. 2015). According to the report published by EFSA, the most commonly responsible serotype of human listeriosis cases is 1/2a (57.5%), which is followed by 4b, 1/2b, 1/2c, 3a and 3b (EFSA, 2015). In our study, the predominant serotype of *L. monocytogenes* in chicken meats (drumsticks, breasts, wings and whole chickens) was

1/2a (94.3%), which was followed by 1/2c, 4b and 1/2b (Table 1). Previous studies similarly reported that 1/2a is the most common serotype (Cetinkaya et al., 2014; Erol et al., 1999; Guerra et al., 2001; Praakle-Amin et al., 2006; Siriken et al., 2014).

Nevertheless, there are other studies with different findings. In a study conducted in the US, it was reported that 1/2b is the most common serotype; which is closely followed by serotype 4b (Zhang et al., 2007). In another study conducted in Iran, it was reported that serotype 4b is the most common, which is closely followed by serotype 1/2a (Fallah et al., 2012). In Turkey, infections by *L. monocytogenes* were reported as sporadic cases (Vardar et al., 2011); however, insufficient data is available about serotypes in human listeriosis cases.

CONCLUSIONS

In conclusion, the prevalence of *L. monocytogenes* in fresh chicken meats as a potential risk factor for humans was highlighted in this study. Since 26.5% of the investigated samples in the study were contaminated with *L. monocytogenes* and the most significant serotype in food-borne listeriosis cases, serotype 1/2a, was detected, it can be concluded that chicken meats may pose risk for public health. The present study provides an insight on the prevalence and serotype distribution of *L. monocytogenes* in chicken meats in Turkey. A baseline information is represented here, for further studies which may aim to improve microbiological safety procedures for raw foods including *L. monocytogenes*. To prevent listeriosis cases due to chicken meats, it is recommended to take preventive measures in general hygiene and disinfection applications through the production line and to take care in properly cooking such products.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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