



Research Article

Prevalence and Antibiotic Resistance of *Salmonella* sp., *E. coli* O157, and *L. monocytogenes* in Meat and Dairy Products

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ABSTRACT

Bacteria-related food poisoning is the most prevalent cause of food borne diseases, reaching up to 33 percent of the incidents. The study aimed to provide data for the risk assessment processes focusing on poisoning due to the consumption of foods of animal origin. Bovine meat, chicken meat, white cheese, and kosher samples were collected from five different stores in Tekirdağ, Turkey, monthly for twelve consecutive months, from January 1 to December 31, 2019. Ten samples of bovine meat, ten samples of chicken meat, ten samples of white cheese, ten samples of kosher cheese, weighing 300 grams each, were collected at each monthly visit. The total number of samples collected for the study was 480. Results showed that 4,16% of the samples (n=12) were contaminated with *Salmonella* sp. (n=12), *Escherichia coli* O157 (n=4), and *Listeria monocytogenes* (n=6). Antibiotic sensitivity studies revealed that the microorganisms were resistant to 8 different antibiotics. Despite the low number of pathogens isolated, their presences with the high antibiotic resistance rates pose a significant threat to public health.

Keywords: Antibiotic resistance, *E. coli* O157, *L. monocytogenes*, *Salmonella* sp.

Et ve Süt Ürünlerinde *Salmonella* sp., *E. coli* O157 ve *L. monocytogenes*'in Prevalansı ve Antibiyotik Direnci

ÖZET

Dünya çapında önemli sorunlardan biri gıda zehirlenmeleridir. Çalışmalar gıda zehirlenmelerinin yaklaşık %33'ünün bakterilerden kaynaklandığını göstermiştir. AB Fasil 12 kapsamındaki risk değerlendirme konusunda kaynaklık edebilecek veri hazırlamak amacıyla Tekirdağ ilindeki hayvansal ürünlerden *Salmonella* sp., *E. coli* O157, *L. monocytogenes* izolasyonu ve suşların antibiyotik duyarlılıklarının tespiti yapılmıştır. Bu amaçla materyal olarak 2019 yılı içerisinde her ay 10'ar adet ve her biri 300'er gram sığır eti, tavuk eti, beyaz peynir ve kaşar peyniri örnekleri Tekirdağ ilindeki marketlerden alınmıştır. Çalışmada toplam 480 numune üzerinde çalışılmıştır.Yapılan analizler neticesinde 12 adet *Salmonella* sp., 4 adet *E. coli* O157, 6 adet *L. monocytogenes* izole edilmiştir. Çalışmanın ikinci aşamasında izole edilen bakterilerin 8 farklı antibiyotiğe direnç gösterdiği tespit edilmiştir.Çalışma sonucunda Tekirdağ ili için izole edilen bakterilerin sayısal olarak düşük olması sevindirici olarak değerlendirilse de, yüksek antibiyotik dirençliliği halk sağlığını tehdit etmesi bakımından önemli bir sonuç olarak karşımıza çıkmaktadır.

Anahtar Kelimeler: Antibiyotik dirençliliği, *E. coli* O157, *L. monocytogenes*, *Salmonella* sp.

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Introduction

Food poisoning is a significant problem in all countries. The general definition is the ill condition developed by consuming food or water contaminated with pathogenic microorganisms. The reports show that bacteria-related food poisoning is the most prevalent cause of foodborne illnesses, reaching up to 33 percent of the incidents (Çakıcı et al., 2015).

The increase in the food variety and distribution accelerates food poisoning incidence. The *Salmonella* sp., *Escherichia coli* O157, and *Listeria monocytogenes* are the leading causes of food poisoning (Dorman et al., 2010).

Salmonella is a genus of rod-shaped Gram-negative bacteria belonging to the family *Enterobacteriaceae*. *Salmonella* species are intracellular pathogens and can invade different cell types, including epithelial cells, M cells, macrophages, and dendritic cells (Jantsch et al., 2011). The microorganism has an optimum growth temperature of 37 °C with a vast range between 7 and 48 °C and resistance to destruction by freezing (Sorrells et al., 1970). The optimum pH for the growth of *Salmonella* is between 6.5 and 7.5, ranging between 4.5 and 9.0 (Keerthirathne et al., 2016). The species might cause severe infections with severities varying from mild to fatal. Food consumption contaminated with *Salmonella* sp. develops different poisoning conditions, including a mild course or a severe form (Yildirim et al., 2016).

Salmonella species can be found in the digestive tracts of animals. Food or water, previously contacted with the faeces of infected animals, becomes a significant source for transmission of the microorganism if consumed by humans (Goldrick, 2003).

Dairy products and inadequately heat-treated derivatives are the risk-bearing food products for *Salmonella* poisoning (Akkaya & Alişarlı, 2006). The reports indicate that *Salmonella* causes an economic burden of 3.4 billion USD and a billion USD for the USA and Canada, respectively (Yildirim et al., 2016).

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobic, nonspore forming, rod-shaped bacteria belonging to the *Enterobacteriaceae* family. The *E. coli* responsible for gastrointestinal diseases are called DEC, meaning diarrheagenic. Intestinal pathotypes are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), Vero-toxin or Shiga-toxin producing *E. coli* (VTEC-STEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffuse adherent *E. coli* (DAEC) (Omerović et al., 2017).

Among over a hundred serotypes, *E. coli* O157 of the EHEC group is the most prevalent serotype causing food poisoning. Bovine and sheep are the main reservoirs. *E. coli* O157 causes hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TP) in humans. Besides, due to the resemblance to the toxin of *Shigella dysenteriae* type I, the verotoxin produced by the microorganism is called Shiga like toxin (SLT) increases its significance (Temelli, 2002).

The primary source of *E. coli* O157 is the gastrointestinal tract of the bovine and sheep. The organism's isolation rate in the faeces of the bovine and sheep might rise to 10%. *E. coli* O157 infections are caused by consuming water or food contaminated with faeces (Akkaya et al., 2007).

Listeria monocytogenes (*L. monocytogenes*) is a pathogenic, Gram-positive, rod-shaped, motile between 18-26 °C, nonspore forming, non-capsulated, intracellular bacteria (Berktas et al., 2006). There are 13 serotypes of *L. monocytogenes* able to develop the disease. However, more than 90% of

human isolates belong to serotypes 1/2a, 1/2b, and 4b. The serotype 4b strains are responsible for 33 to 35% of sporadic human cases (Ward et al., 2004). The microorganism can be isolated from humans, domestic animals, raw agricultural and fishery products (Lakicevic et al., 2015). The organism can be found in the animal's flesh, blood, and milk, regardless of the listeriosis symptoms. The infection might occur with raw and undercooked food, including cross-contamination following cooking procedures. Vegetables contaminated with sewage or soil fertilized with animal manure are other transmission sources with *L. monocytogenes* (Ekici et al., 2018).

The resistance to antibiotics has become a global public health concern. Besides the acceleration in the resistance to commonly used broad-spectrum antibiotics, the reports show that new drugs have been quickly followed by the emergence of resistant strains (Hoge et al., 1998). In addition to developing resistant pathogenic bacteria, the administration of an antibiotic alters the gut microbiota and creates antibiotic-resistant intestinal bacteria that enable genetic transfer to the pathogenic bacteria and facilitate multi-drug resistance. Seemingly paradoxically, the broad and intensive use of antibiotics creates a bigger drug-resistant world in which the antimicrobials are less useful (Atabey, 2011).

Aiming to provide data for the risk assessment processes on poisoning due to the consumption of foods of animal origin, the study analyzed *Salmonella* sp., *E. coli* O157, and *L. monocytogenes* by isolation of the organisms from the meat and dairy products sold in Tekirdağ, Turkey and the analysis for antibiotic resistance.

Material and Methods

The study included samples collected from five different stores in Tekirdağ, Turkey, monthly for twelve consecutive months, from January 1 to December 31, 2019.

Ten samples of bovine meat, ten samples of chicken meat, ten samples of white cheese, ten samples of kosher cheese, weighing 300 grams each, were collected at each monthly visit. The total number of samples collected for the study was 480.

Bovine, chicken, white cheese and kosher cheese samples were taken in original packages and carried to the laboratory in cold-chain (4 °C) containers.

Salmonella sp. isolation and identification were performed according to the ISO 6579-1 standard. *E. coli* O157 isolation and identification was performed according to the ISO 16654:2001 standards. *L. monocytogenes* isolation and identification was performed according to the ISO 11290-1 standard.

The isolated pathogens were cross-checked, and verification was confirmed by the VIDAS method. VIDAS verification was performed according to the ISO 16140-1:2016 standards. An automated, multi-parametric immunoassay system which uses ELFA (Enzyme-Linked Fluorescent Assay) Phage and Immuno concentration technology, VIDAS® *Salmonella* (SLM) Immunoassay Method with Rappaport-Vassiliadis (RV) Medium, VIDAS *Listeria monocytogenes* II (LMO2), and VIDAS® *E. coli* O157 (ECO) and O157:H7 Plate Method were used for qualitative analysis.

Antibiotic sensitivity test

The antibiotic resistance of the pathogens obtained within the scope of the EUCAST 2018 standard was examined using the disk diffusion technique. Samples yielding positive results were incubated in Mueller-Hinton agar at 37 °C for 24 hours. The

Table 1. Samples with positive results by months

	<i>Salmonella</i> sp.				<i>E. coli</i> O157				<i>L. monocytogenes</i>			
	Bovine	Chicken	White cheese	Kosher cheese	Bovine	Chicken	White cheese	Kosher cheese	Bovine	Chicken	White cheese	Kosher cheese
January		1										
February											1	
March	1											
April	1				1							
May		2							1		1	
June	1				1							
July		1										
August		1			1							
September		2			1						1	1
October												
November		1							1			
December		1										

bacterial inoculum was adjusted with SSF to the Mac Farland N° 0,5 turbidity standard. Every inoculum was spread over plates containing Mueller-Hinton agar and a paper filter disc (6 mm). The inhibitory zone diameter of the discs were measured in mm scale. The diagnostic discs used in antibiogram tests were Ampicillin (AMP 25 µg), Ciprofloxacin (CIP 10 µg), Nalidixic acid (NA 30 µg), Streptomycin (S 25 µg), Gentamicin (CN 30µg), Sulphafurazole (SF 300 µg), Trimethoprim (W 5µg), Chloramphenicol (C 30µg), Tetracycline (TE 30µg), Colistin (CT 25µg), Cefotaxime (CTX 30µg), Ceftazidime (CAZ 30µg), Cefoxitin (FOX 30µg), Cefepime (FEP 30µg), Meropenem (MEM 10µg).

Results

Among the 480 samples, 22 (4.6%) was contaminated with the study microorganisms. *Salmonella* sp. was found in 12 (2.5%), *E. coli* O 157 was found in 4 (0.83%), and *L. monocytogenes* was found in 6 (1.25%) of the samples. In Table 1, the samples with positive results by months were presented.

Salmonella sp. was isolated from 3 (2.5%) bovine samples and 9 (7.5%) chicken samples. There were no white cheese and kosher cheese samples with positive results for *Salmonella* sp. The identification of *Salmonella* species isolated from the bovine samples showed that two samples included *S. Typhimurium*, and one specimen had *S. Bongori*. In chicken samples, *S. Enteritidis* and *S. Typhimurium* were identified in four and three samples, respectively. *S. Infantis* and *S. Dublin* were identified in one specimen for each.

E. coli O 157 was found in four (3.33%) of the bovine specimen and was not isolated from the chicken meat, white and kosher cheese samples.

L. monocytogenes was isolated from two (1.66%) bovine, three (2.5%) white cheese and one (0.83%) kosher cheese samples. There were no chicken samples positive for *L. monocytogenes*.

The isolated pathogens were cross-checked, and 100% verification was confirmed by the VIDAS method.

The antibiotic resistance study showed that the *S. Enteritidis* and *L. monocytogenes* strains had the maximum variety of antibiotic resistance with 11 different antimicrobials. *S. Typhimurium* was found resistant to 8 different antibiotics. *S. Infantis* and *S. Dublin* strains showed resistance to 5 different antibiotics each. *E. coli* O157 strains showed resistance to 10 and *L. monocytogenes* to 11 different antimicrobials. From

the antibiotic resistance point of view, Streptomycin was the only antimicrobial to which all strains were found resistant. *S. Enteritidis* was the only microorganism that presented antibiotic resistance to gentamycin, ceftazidime and cefepime. Presenting the least number of antimicrobial resistance, *S. Dublin* was the only microorganism resistant to cefotaxime. *E. coli* O157 strains presented resistance to colistin, and *L. monocytogenes* was the only isolate detected to be resistant to chloramphenicol. In Table 2, the samples with strains showing antibiotic resistance were presented.

Discussion

In our study, *Salmonella* sp. were isolated from 3 (2.5%) bovine samples. The reports conducted in Turkey show that the *Salmonella* sp. isolation rate found in the study were similar to the rates in others ranging between 2.5 and 6%. Büyükcinal et al., (2015), Kahraman T & Aydın, (2009), and Yildirim et al., (2016) included an approximately similar number of samples and isolated *Salmonella* spp. from the bovine samples with rates of 1%, 2.5% and 6%. The studies from other countries presented similar rates with bovine specimen numbers ranging from 200 to 72292 samples, between 1.5% to 5% (Madden et al., 2001),(Zarei et al., 2013). It was worth noting that the isolation rates inclined as the sample size increased in the researches.

Salmonella sp. were isolated from 9 (7.5%) chicken samples. Studies conducted in Turkey with approximate sample sizes indicate comparable results. Tanoğlu & Gümüşsoy, (2008), Kahraman & Aydın, (2009) and Acaröz et al., (2018) reported similar isolation with 9.7%, %3, and %2.9, respectively. The researches from other countries with similar sample sizes present parallel to our results. One high rate was reported from South Africa, indicating that 19% of the samples were contaminated with *Salmonella* (van Nierop et al., 2005).

In white and kosher cheese samples, *Salmonella* sp. was not detected. There were studies conducted in Turkey reporting the same results Turantaş et al., (1989), Gülmez & Güven, (2001). However, Kahraman et al., (2010) and Akkaya & Alişarlı, (2006), have presented similar rates of *Salmonella* presence in cheese samples with 1.9 and 2%.

In our study, *E. coli* O157 was detected only in 4 (3.33%) bovine samples. The researches of Bingöl et al., (2013), Fantelli, (2001) and Büyükcinal et al., (2015) reported that no *E. coli* O157 was found in bovine samples. However, Ahmed & Shimamoto,

Table 2. The number and percentages of samples with strains showing antibiotic resistance

	<i>S. Enteritidis</i>		<i>S. Typhimurium</i>		<i>S. Bongori</i>		<i>S. Infantis</i>		<i>S. Dublin</i>		<i>Total Salmonella sp.</i>		<i>E. coli O157</i>		<i>L. monocytogenes</i>	
	<i>n</i>	4	<i>n</i>	5	<i>n</i>	1	<i>n</i>	1	<i>n</i>	1	<i>n</i>	12	<i>n</i>	4	<i>n</i>	6
		%		%		%		%		%		%		%		%
Ampicillin	4	100,00%	3	60,00%	1	100,00%	0	0,00%	1	100,00%	9	75,00%	4	100,00%	6	100,00%
Ciprofloxacin	1	25,00%	1	20,00%	0	0,00%	1	100,00%	1	100,00%	3	25,00%	3	75,00%	6	100,00%
Nalidixic Acid	2	50,00%	1	20,00%	0	0,00%	0	0,00%	1	100,00%	4	33,33%	2	50,00%	6	100,00%
Streptomycin	3	75,00%	2	40,00%	1	100,00%	1	100,00%	1	100,00%	7	58,33%	3	75,00%	6	100,00%
Gentamycin	2	50,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	2	16,67%	0	0,00%	0	0,00%
Sulphamethoxazole	4	100,00%	3	60,00%	1	100,00%	1	100,00%	0	0,00%	8	66,67%	3	75,00%	5	83,33%
Trimethoprim	4	100,00%	3	60,00%	1	100,00%	1	100,00%	0	0,00%	8	66,67%	4	100,00%	4	66,67%
Chloramphenicol	0	0,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	4	66,67%
Tetracycline	0	0,00%	0	0,00%	1	100,00%	0	0,00%	0	0,00%	1	8,33%	3	75,00%	6	100,00%
Colistin	0	0,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	1	25,00%	1	16,67%
Cefotaxime	0	0,00%	0	0,00%	0	0,00%	0	0,00%	1	100,00%	1	8,33%	0	0,00%	0	0,00%
Ceftazidime	1	25,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	1	8,33%	0	0,00%	0	0,00%
Cefoxitin	2	50,00%	1	20,00%	0	0,00%	1	100,00%	0	0,00%	3	25,00%	1	25,00%	6	100,00%
Cefepime	1	25,00%	0	0,00%	0	0,00%	0	0,00%	0	0,00%	1	8,33%	0	0,00%	0	0,00%
Meropenem	2	50,00%	1	20,00%	0	0,00%	0	0,00%	0	0,00%	3	25,00%	4	100,00%	2	33,33%

(2014) and Zarei et al., (2013) have reported similar isolation rates to our study with 2.6 and 2.8%, respectively.

There were no chicken and cheese samples contaminated with *E. coli* O157 among the collected samples. In a similar study, Manguiat & Fang (2013) and Jo et al., (2004) have reported that cheese samples were uncontaminated with *E. coli* O157. Also, Arslan & Özdemir, (2008) and Gonzalez et al., (2000) reported the same negative contamination results for chicken samples.

There were only two (1.66%) bovine samples contaminated with *L. monocytogenes*. Similar results have been reported from studies conducted in Turkey and other countries Büyükkunal et al., (2015), Kahraman T & Aydın, (2009), Iannetti et al., (2016). A study conducted by Vitas & Garcia-Jalon, (2004) reported that 34.9% of the meat samples were contaminated with *L. monocytogenes*.

The chicken samples in our study were not contaminated with *L. monocytogenes*. Similar results were reported in studies of Yerlikaya (2015), Sağlam (2011). Vitas & Garcia-Jalon, (2004) isolated *L. monocytogenes* from 36.1% of the chicken samples, reporting a very high number similar to the result with bovine samples. However, Mena (2004) have reported that 60% of the chicken samples were contaminated with *L. monocytogenes*, expressing an extreme rate.

Among the cheese samples, *L. monocytogenes* was isolated from three (2.5%) white and one (0.83%) kosher cheese. The researches conducted by Aygun & Pehlivanlar (2006), and Çiftçioğlu & Uğur (1991), analyzing white cheese, indicate comparable findings with 2.85% and 2.9%. In the study of Ekici et al. (2018), 3% and Kahraman et al. (2010) 1.7% of the kosher cheese samples were contaminated with *L. monocytogenes*, similar to our findings.

There might be various reasons for explaining the different isolation rates in the studies. There could be too many probable contamination points to consider, beginning from feeding

the animals to the cleaning and disinfection procedures of the packaging and the shelves the products were kept. Also, sampling methods should be considered. Therefore the isolation results might be evaluated regionally or even locally to reach reliable interpretations.

The findings of the antibiotic resistance of the strains showed a variety of results. Streptomycin was the only antimicrobial to which all the microorganisms showed resistance.

A study conducted by Şahan et al. (2016) showed that among *S. Infantis* isolates, resistance to nalidixic acid, sulfonamides, tetracycline, and trimethoprim rates were 92.7%, 92.3%, 88.3%, and 78.6%, respectively. The resistance rates in *S. Typhimurium* isolates for sulfonamides, ampicillin, and trimethoprim were 65%, 47%, and 35%, respectively. The nalidixic acid and sulfonamide resistance in the *S. Enteritidis* strains were 75% and 92%, respectively. The research by Bozkurt (2018) showed that all of the *Salmonella* sp. were resistant to penicillin and 66% to ampicillin and erythromycin. Similar high rates for *Salmonella* sp. were reported in a study by Yang et al. (2020) with 72.3% to nalidixic acid, 55.3% to ampicillin and 48.7% to streptomycin.

The antibiotic resistance analysis of *E. coli* conducted by Dursun (2008) showed that the resistance rates for cefalotin, chloramphenicol, sulphamethoxazole-trimethoprim, erythromycin, and amoxicillin-clavulanic acid were 100%, 87.5%, 81.25%, 81.25%, and 62.5%, respectively. Various studies highlighted *E. coli* O157 showing resistance to streptomycin, cefalotin, tetracyclines, amikacin, cefotaxime, erythromycin, chloramphenicol, nalidixic acid, neomycin, ofloxacin (Çadircı et al., 2017; Elafify et al., 2020).

Alonso-Hernando et al. (2012) have reported an increase in the rate of one or more antibiotic-resistant *L. monocytogenes* isolates from 37.2 % to 96% in 13 years. Interestingly, in both studies conducted in 1993 and 2006, the nalidixic acid resistance rate was 100%. In the study, the multi-drug resistance among *L.*

monocytogenes isolates was reported to increase from 18.6% to 84% in the time frame. Regarding resistance to enrofloxacin and ciprofloxacin, the rates were increased from 23.3% and 25.6% to 68% and 52%. Alonso-Hernando et al. (2012) indicate that in addition to the number of antibiotics *L. monocytogenes* isolates were resistant to, the percentage of the strains with resistance also increased. A similar study conducted by Maung et al. (2019) presented a five-year difference in the *L. monocytogenes* strains' resistance to fosfomycin and oxacillin between 2012 and 2017. The resistance to fosfomycin and oxacillin were increased from 57.3% and 72% to 95.7% and 82%, respectively. The multi-drug resistance rate of the *L. monocytogenes* strains rose from 46.7% to 82.62%.

The previous studies show similar results to our findings. In recent years, reports highlight the increase in the strength and variety of antibiotic resistance of the pathogenic bacteria.

The study results for the Tekirdağ region might include low isolate numbers. Nevertheless, the findings indicate a significant risk to public health and should be in consideration. Also, the increased rate and variety in antibiotic resistance results boost the amount at public health risk.

At this point, the careful prescription of antibiotics to animals with performing antibiogram tests, avoiding misuse and repetition of the same molecule, and following gold-standard treatment methods might decelerate antibiotic resistance rates. Moreover, following HACCP processes in the production of animal foods such as adequate sanitation conditions and sufficient thermal procedures might help eliminate pathogenic bacteria at the source.

Conflict of Interest

The authors declare no conflicts of interest.

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