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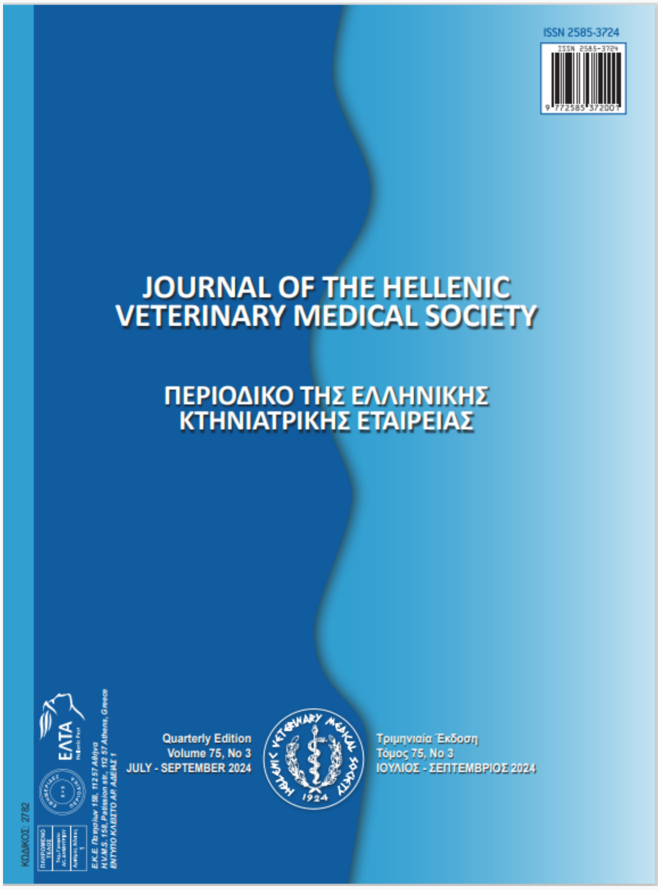

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Research article
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Ten-year comparison of the efficiency of legal regulations of foodborne pathogens in broilers in Türkiye

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ABSTRACT: Broiler meat are the primary source of foodborne pathogens, such as *Campylobacter jejuni* and *Salmonella* spp., and *E. coli* O157.The illegal use of antibiotics for preventive purposes or the habit of using broad-spectrum antibiotics in farms causes antibiotic resistance in these pathogens. This study compared *C. jejuni*, *Salmonella* spp. and *E. coli* O157 and their antibiotic resistance patterns in broiler meats in 2011-2012 and 2021-2022. The research we carried out in 2011-2012 to determine the risks posed by illegal and uncontrolled use of antibiotics was repeated with a similar sample set in 2021-2022 in order to observe the effectiveness of the legal regulations for the control of pathogens and monitoring of antibiotic use, which came into force in Turkey in 2014 and 2018. The results showed that the prevalence of *C. jejuni*, *Salmonella* spp., and *E. coli* O157 in 2011-2012 and 2021-2022 was 68.0%, 30.4%, 0.8% and 10.08%, 7.77%, 0.21%, respectively.

The antimicrobial susceptibility tests against gentamicin, streptomycin, erythromycin, ciprofloxacin, enrofloxacin, tetracycline, oxytetracycline, ampicillin and piperacillin were performed by agar disk diffusion method. According to results in 2011-2012, all *C. jejuni* and *Salmonella* spp. were resistant to at least one tested antibiotic, with 31.57% of *Salmonella* spp. and 16.47% of *C. jejuni* being multidrug-resistant. In addition, one of the *E. coli* O157 strains was resistant to one antibiotic, and the other was resistant to two antibiotics. In 2021-2022, 81.3% of *C. jejuni*, 46% of *Salmonella* spp. and only one *E. coli* O157 were found resistant to at least one antibiotic tested,8.33% of *C. jejuni* and 21.62% of *Salmonella* species were detected in multi-drug resistance.

In the past, antibiotics were used for preventative purposes, as indicated by the survey results. Today, it is evident that these rates have decreased to low levels as a result of legal regulations and monitoring.

Keywords: Antibiotic resistance; Broiler meat; *Campylobacter jejuni; E. coli* O157:H:7; *Salmonella* spp

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INTRODUCTION

The poultry industry is one of the leading food production branches globally. According to 2018 data, 36.3% of the world's meat production consists of poultry meat (Koç, 2020). FAO estimates poultry meat, which was promoted to the most produced meat position in 2015, is expected to have a share of 37.8% in 2025 (OECD/FAO, 2017). The poultry meat industry is also important in the Turkish food sector. Poultry meat production was 2.23 million tons in 2018, recorded as 2.16 million tons of chicken meat and 0.7 million tons of turkey meat. The share of Türkiye in world production is 2.3%, and it is in the eighth place in the world ranking in terms of production size (Koç, 2020). There are 21 integrated slaughterhouses spread across the country, and approximately 5-5.5 million chickens are slaughtered daily. As a production model, Türkiye's integrated facilities are among the most modern enterprises in the world (Koç, 2020).

Broiler meat, a valuable and economical protein source whose consumption is increasing day by day, still has some risks despite the producers' legal regulations and awareness-raising activities (Chouliara et al., 2007; Koç, 2020).Chicken is one of the primary sources of foodborne pathogens, such as *Campylobacter jejuni* (*C. jejuni*), *Salmonella* spp., and Verotoxigenic *Escherichia coli* (*E. coli*) (EFSA, 2012; Saei & Zavarshani 2018). These bacteria can cause severe infections and death (EFSA, 2012).

While the muscles are sterile in healthy chickens, the digestive system, lungs, skin, and feathers are loaded with bacteria. Surfaces, air and liquids in slaughterhouses, personnel hands and equipment can also be a significant source of contamination. Therefore, chickens may be secondary and cross-contaminated with different pathogenic bacteria after slaughter (Rouger et al., 2017). Poultry meat is considered the most crucial source of human Campylobacteriosis and Salmonellosis (Jay et al., 2005; Van Gerwe, 2012; Antunes et al., 2016).There are many studies about the incidence of *Campylobacter* spp. and *Salmonella* spp. in poultry meat from Türkiye (Yazıcıoglu et al., 2005; Goncagul et al., 2005; Savaşçı 2005; Pamuk, 2006; Bostan et al., 2009; Koluman, 2010). Some recent studies have also revealed that broiler meat can be a source of *E. coli* O157 (Akkaya et al., 2006; Dinçoğlu et al., 2016; Mustafa and Inanc, 2018).

In addition to the presence of these bacteria, the uncontrolled and unprocedural use of antimicrobials causes the emergence, selection and spread of antimicrobial-resistant bacteria, creating a severe threat to animal and public health (Neu, 1992; Witte, 1998; Koluman et al., 2009; Unlü et al., 2011; Koluman et al., 2011; Aydin et al., 2011; Jabbar & Rehman, 2013; Chen et al., 2019). Three categories of antimicrobials in human and veterinary medicine were defined: Critically important, highly important and important (Collignon et al., 2009). Antibiotic resistance of *C. jejuni* and *Salmonella* spp. has been subject to a community-based study that was published in the Official Journal of the European Union as "Commission Decision of 19 July 2007 concerning a financial contribution from the Community towards a survey on the prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler flocks and on the prevalence of *Campylobacter* spp. and *Salmonella* spp. in broiler carcasses" to be carried out in the Member States (Anonymous, 2007).

The use of antibiotics in Türkiye is limited by legislation that came into force in 2006. The use of antibiotics as growth promoters were prohibited from complying with EU regulations. Antibiotics were only allowed for diseases associated with a high rate of bird mortality from respiratory and other ailments treated by veterinarians with antibiotics (ISO10272: 2006). However, the inadequacies in follow-up and inspection made it necessary to make some new regulations over time. In 2014, a regulation was introduced for the detection and appropriate and effective control of all relevant stages of primary production, processing, and distribution, including feed, to reduce the risks and incidence of *Salmonella* spp. and other foodborne zoonotic agents to the public health and this regulation has been in effect for broilers since 2015 (Anonymous, 2014). The drug tracking system, which aims to "ensure the traceability" of veterinary medicinal products from production to consumption, was implemented in 2018(GKGM 2018). The regulation was an important step in reducing the risk. It is considered whether the Salmonella serotypes *Salmonella* Enteritidis or *Salmonella* Typhimurium, which are the most common causes of human salmonellosis, demonstrate increasing virulence issues, such as resistance or spreading capacity, based on data obtained by monitoring systems (Anonymous, 2014).

This study aims to determine the prevalence of *C. jejuni*, *Salmonella* spp., and *E. coli* O157 in broiler meats at the beginning and the end of a 10-year period, as well as the antibiotic resistance of the isolates and the efficacy of the legal regulations.

MATERIAL AND METHOD

Sampling

Between the years 2011-2012, 250 chicken breast samples were collected from different markets in seven locations in Türkiye: Ankara (n:68), İstanbul (n:52), Tunceli (n:21), Elazig (n:23), Kars (n:37), Samsun (n:25), and Aydin (n:24). In 2021-2022, 476 chicken breast samples were collected from different markets in seven cities in Türkiye: Ankara (n:76), İstanbul (n:187), Tunceli (n:37), Elazig (n:51), Kars (n:49), Samsun (n:41), and Aydin (n:35). The sampling locations are depicted on the map in Figure 1. All samples were transferred to the laboratory refrigerated (4-6°C) and analysed within 8-12 hours.

Enrichment of samples

A whole breast skin sample was homogenised using a blender, and this homogenised sample was separated into three sections. All pieces were weighed 25 g and placed into sterile stomacher bags with enrichment for each bacterium.

The first section was diluted with 225 mL of Buffered Peptone Water (BPW, Merck, Darmstadt, Germany), the second was diluted with 225 mL of modified Tryptone Soy Broth (mTSB, Merck) and the third section was diluted with 225 mL of Campy Food Broth (CFB, Biomerioux Marcy-l'Etoile, France).

All bags were homogenised using a Stomacher 400 (Seward Medical Ltd., London, UK) for 2 min. BPW bags were incubated at 37ºC for 24 hours, mTSB bags at 42ºC for 24 hours, and CFB bags in jars supplemented with gas packs (CN0025, Oxoid, Basingstoke, UK) at 42ºC under microaerophilic conditions.

Identification of C. jejuni, Salmonella spp. *and E. coli O157*

C. jejuni, Salmonella spp. and *E. coli* O157 were determined by ELFA (Enzyme-Linked Immunofluorescence Assay). VIDAS (VITEK Immunodiagnostic Assay System) provides an automated enzyme-linked immunofluorescence detection of *C. jejuni, Salmonella* spp. and *E. coli* O157. In this context, all samples were transferred to VIDAS strips after the enrichment step.

For *C. jejuni,* 500 µL of CFB enrichment was transferred to CAM strips, heat-treated with Heat and Go at 131ºC for 10 min., and warmed for 10 min. To identify *E. coli* O157, 500 µL of mTSB enrichment was transferred to UP ECO strips, heat-treated with Heat and Go at 131 ºC for 10 min., and warmed for 10 min. The strips were placed in VIDAS, and results were obtained within the same day. *Salmonella* spp. analyses were held in two steps. One of these steps was immune concentration, for which 800 µL of BPW enrichment was transferred to ICS strips and run in VIDAS, and 500 µL from this concentration

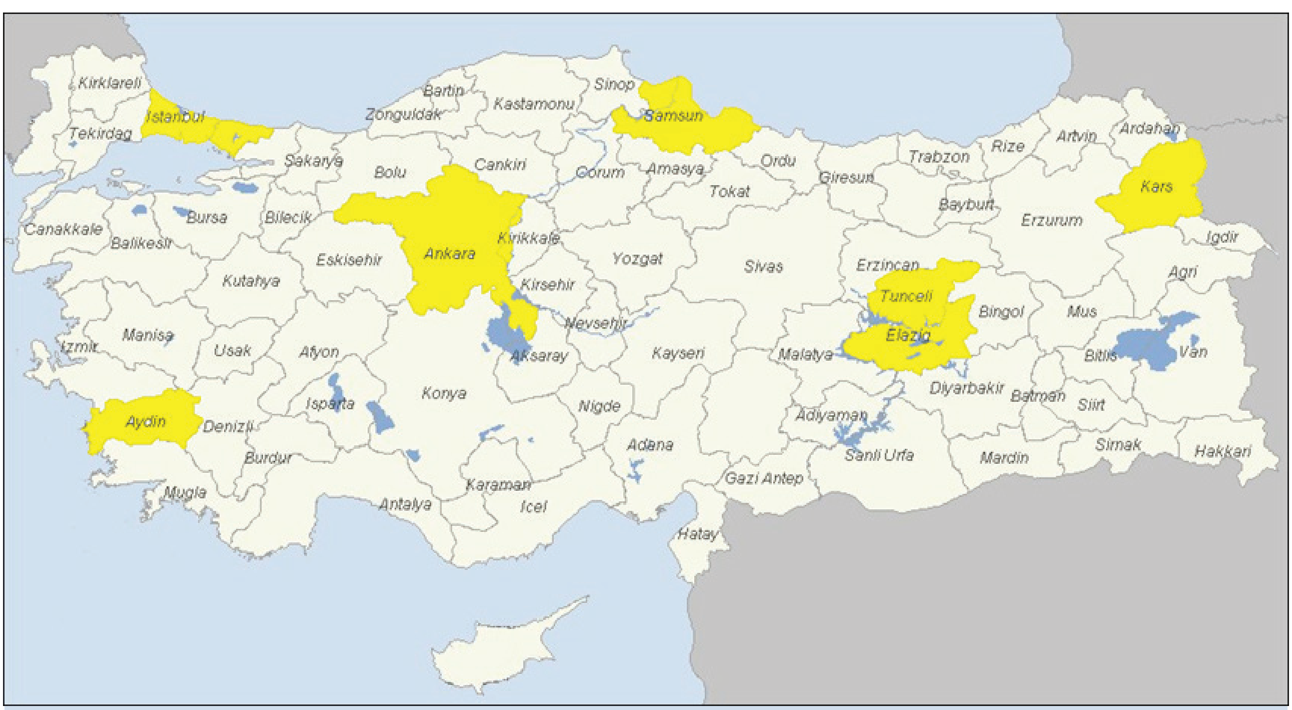

Figure 1. The cities in Türkiye that broiler meat samples were collected (2011-2012/2021-2022).

was transferred to ICS broth and incubated for 5 hours at 42ºC. At the end of the incubation period, 500 μL of enrichment was transferred into an SLM strip and heat-treated with Heat and Go at 100ºC for 10 min. and warmed for 10 min. The strips were placed in VIDAS, and results were obtained within the same day (AOAC, 2004).

All positive results obtained from VIDAS were confirmed with conventional cultural techniques using ISO methods. For *Salmonella* spp. ISO 6579/A1 (02-2006), ISO 6579-1:2017, for *C. jejuni* ISO10272: 2006, ISO10272: 2017 and for *E. coli* O157 ISO 16654:2001, 16654:2001/Amd1:2017were used. All isolation and identification protocols, including biochemical and serological identification of the three pathogens, are summarised in Figure 2.

Antibiotic susceptibility testing

The tested antibiotics are represented in two main categories of antibiotic classification (Collignon et al., 2009). Antimicrobial agents (or classes) we tested were assigned to 1 of the 2 categories of importance on the basis of 2 criteria: (1) the agent or class is the sole therapy or one of few alternatives to treat serious human disease, and (2) the antimicrobial agent or class is used to treat diseases caused by organisms that may be transmitted via non-human sources or diseases caused by organisms that may acquire resistance genes from non-human sources. Critically important antimicrobials (aminoglycosides (gentamicin and streptomycin), macrolides (erythromycin), quinolones (ciprofloxacin and enrofloxacin), β-lactam antibiotics (ampicillin, piperacillin) are those that meet both criteria. Highly important antimicrobials, tetracyclines (tetracyclines (tetracycline and oxytetracycline) are those that meet 1 of 2 criteria. The concentrations of antimicrobials were as follows: 10 µg of gentamicin (CN, Oxoid CT024B), 10 µg streptomycin (STR, Oxoid CT0047), 15 µg erythromycin (E, Oxoid CT020B), 1 µg ciprofloxacin (CIP, Oxoid CT0425), 5 µg enrofloxacin (ENR, Oxoid CT639B),

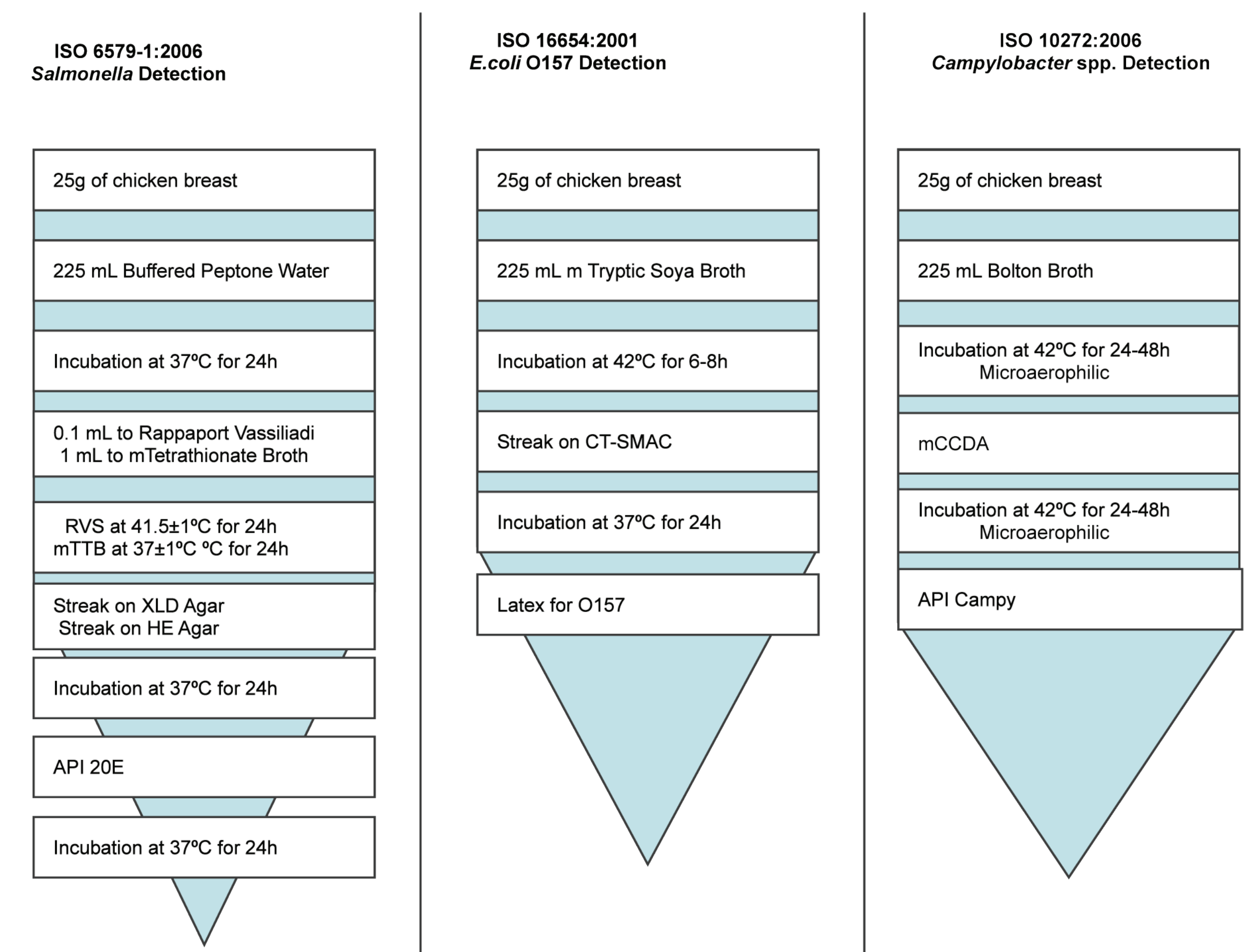

Figure 2. Isolation and identification protocols of the *Salmonella* spp., *E.coli* O157, *Campylobacter* spp.

30 µg tetracycline (TE, Oxoid CT054B), 30 µg oxytetracycline (OT, Oxoid CT041B), 10 µg of ampicillin (CT, Oxoid CT0003B) and 100 µg of piperacillin (CT, Oxoid CT0199B).

Antibiotic susceptibility testing was performed by agar disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI). In 2011-2012 CLSI 2006, and in 2021-2022 CLSI 2019 was used. All strains were inoculated in BHI broth. *Salmonella* spp. and *E. coli* O157 were incubated at 37°C, and *C. jejuni* at 42°C for 18-24h. 100 µL of these overnight cultures were streaked on Mueller Hinton Agar (MHA, Oxoid CM0337) for *E. coli* O157 and *Salmonella* spp., and MHA with 5% sheep blood for *C. jejuni*. Then, antibiotic discs were applied on the surface using an antibiotic disc dispenser (Oxoid ST8090). MHA plates were incubated at 37 °C and evaluated after 24 h of inoculation. *E. coli* ATCC 43889 and *E. coli* ATCC 8739 strains were used as positive control. On the other hand, *S. aureus* ATCC 25923 was used for negative control as quality control. The inhibition zone diameters were evaluated according to CLSI 2006 and 2019. For *Campylobacter* spp. isolates, the inhibition zones proposed by the CLSI 2006 and 2019 for *Enterobacteriaceae* were used since there are no currently available breakpoints specific for measured *Campylobacter*. Multi-drug resistance was defined as resistance to at least three antimicrobial categories.

Survey

The surveys were designed to determine antimicrobial usage in broiler chickens during their rearing period. This design was adopted from the study held by Ünlü et al. (2011) with modification and adaptation of a survey of veterinarians working in broiler chicken rearing farms. There were 5 questions, 4 of which were yes-no, and the 5th was a multiple-choice question.

The questions were as follows:

Q1. Are additions of synthetic or organic antibiotics obligatory for efficiency in the rearing period of broilers?

Q2. Are residues of the antibiotics used in the broiler rearing period minimised until the slaughter process?

Q3. Have you ever detected *Campylobacter* spp. in your flocks?

Q4. Do you apply preventative antibiotics to your chicks in the first three days of the rearing period?

Q5. Would you please tick the antibiotics you have preferred mainly to treat diseases in the rearing period?

The questionnaires were distributed to 50 veterinarians in 2011 and to 50 veterinarians in 2021 years, working in broiler chicken rearing farms. These surveys were distributed and evaluated under the maintenance of İstanbul University-Cerrahpaşa. (Faculty of Veterinary Medicine, Department of Food Hygiene and Technology). Descriptive statistics were used to summarize the characteristics and distribution of the data set by SPSS 21.0. All survey results were given in percentages.

RESULTS

Prevalence of *C. jejuni*, *Salmonella* spp. and *E. coli* O157

Due to the heterogeneous sampling, the city distribution of *C. jejuni*, *Salmonella* spp., and *E. coli* O157 could not be determined. The results for the years 2011-2012 showed that 170 of 250 (68%) samples were contaminated with *C. jejuni*. The distribution of *Salmonella* spp. and *E. coli* O157 in 250 samples were 76 (30.4%) and 2 (0.8%), respectively. The results for the years 2021-2022 showed that 48 of 476(10.08%) samples were contaminated with *C. jejuni*. The distributions of *Salmonella* spp. and *E. coli* O157 in 476 samples were 37 (7.77%) and 1 (0.21%), respectively.

Antibiotic resistance

The antibiotic resistance rates of *C. jejuni, E. coli* O157, and *Salmonella* spp. to selected antibiotics in the years 2011-2012 and 2021-2022 are provided in Table 1. According to the data in 2011 and 2012, *C. jejuni, E. coli* O157, and *Salmonella* spp were resistant to tetracycline, oxytetracycline, and streptomycin. *C. jejuni* strains were mainly resistant to tetracycline (56.47%), followed by enrofloxacin, piperacillin, ciprofloxacin, oxytetracycline, ampicillin, erythromycin, streptomycin and lastly, gentamicin (0.58%). Both *E. coli* O157 strains were resistant to oxytetracycline, but only one was resistant to tetracycline, and one was resistant to streptomycin. *Salmonella* spp. strains were most resistant to streptomycin (39.47%), followed by ampicillin, piperacillin, ciprofloxacin, gentamycin, enrofloxacin, oxytetracycline, erythromycin, and tetracycline (Table 1).

The resistance rates of *C. jejuni* isolates to piperacillin (36.47% to 0%), ciprofloxacin (32.94% to 12.50%), enrofloxacin (34.70% to 14.58%), ampicillin (12.94% to 8.33%) and the resistance rates of *Salmonella* spp. isolates to ampicillin (35.53% to 18.92%), enrofloxacin (10.53% to 5.41%) show a significant decrease from 2011-2012 to 2021-2022. On the other hand, the resistance rates of *Salmonella* spp. isolates to erythromycin (3.95% to 16.22%), oxytetracycline (6.58% to 27.02%), and tetracycline (1.32% to 27.02%) increased dramatically from 2011-2012 to 2021-2022.

Multi-drug resistance (MDR) of strains was evaluated, and resistance to equal or more than three antibiotic categories was accepted as MDR. The results of antibiotic resistance are provided in Table 2. In 2011-2012, 31.57% of *Salmonella* spp. and 16.47% of *C. jejuni* were resistant to multiple antibiotics, as provided in Table 2. These rates decreased to 21.62% and 8.33%, respectively, in 2021-2022.

Survey

The results of the survey showed differences between farms. Answers to the first four questions are summarised in Table 4. The antibiotics that veterinarians working in broiler chicken farms use most in infectious diseases are given in Figure 3. During 2011-2012, enrofloxacin was the most preferred antibiotic use by veterinarians against bacterial infections in poultry houses. It was observed that 88% of the veterinarians participating in the study used enrofloxacin for treatment during the breeding period. Amoxicillin and doxycycline were the next most used antibiotics, with 76% and 52% usage rates, respectively. Data for 2021-2022 showed that tylosin tartrate (52%) was the first choice for veterinarians to administer treatment during the breeding season. Oxytetracycline (48%) and doxycycline (24%) are the second and third choices.

Table 1. Antibiotic resistance rates of *C. jejuni*, *Salmonella* spp. and*E. coli* O157 strains obtained from the study.

		Critically Important														Highly Important			
		Gentamicin n/N (%)		Streptomycin n/N (%)		Erythromycin n/N (%)		Ciprofloxacin n/N (%)		Enrofloxacin n/N (%)		Ampicillin n/N (%)		Piperacillin n/N (%)		Tetracycline n/N (%)		Oxytetracycline n/N (%)	
		2011 2012	2021 2022	2011 2012	2021 2022	2011 2012	2021 2022	2011 2012	2021 2022	2011 2012	2021 2022	2011 2012	2021 2022	2011 2012	2021 2022	2011 2012	2021 2022	2011 2012	2021 2022
C. jejuni	R	1/170 0.58%	-	5/170 2.94%	1/48 2.08%	8/170 4.70%	2/48 4.17%	56/170 32.94%	6/48 12.50%	59/170 34.70%	7/48 14.58%	22/170 12.94%	4/48 8.33%	62/170 36.47%	-	96/170 56.47%	25/48 52.08%	43/170 25.30%	10/48 20.83%
	I	-	-	-	-	1/170 0.59%	-	4/170 2.35%	-	2/170 1.18%	-	73/170 42.94%	20/48 41.67%	51/170 30%	19/48 39.58%	7/170 4.12%	-	4/170 2.35%	-
	S	169/170 99.42%	48/48 100%	165/170 97.06%	47/48 97.92%	161/170 94.71%	46/48 95.83%	110/170 64.71%	42/48 87.50%	109/170 64.12%	41/48 85.42%	75/170 44.12%	24/48 50%	57/170 33.53%	29/48 60.42%	67/170 39.41%	23/48 47.92%	123/170 72.35%	38/48 79.17%
Salmonella spp.	R	14/76 18.42%	8/37 21.62%	30/76 39.47%	14/37 37.84%	3/76 3.95%	6/37 16.22%	17/76 22.36%	7/37 18.92%	8/76 10.53%	2/37 5.41%	27/76 35.53%	7/37 18.92%	25/76 32.80%	11/37 29.73%	1/76 1.32%	10/37 27.02%	5/76 6.58%	10/37 27.02%
	I	2/76 2.63%	2/37 5.41%	-	-	-	9/37 24.32%	1/76 1.32%	1/37 2.70%	-	1/37 2.70%	23/76 30.26%	10/37 27.03%	37/76 48.68%	9/37 24.32%	-	1/37 2.70%	-	-
	S	60/76 78.95%	27/37 72.97%	46/76 60.53%	23/37 62.16%	73/76 96.05%	22/37 59.46%	58/76 76.32%	29/37 78.38%	68/76 89.47%	34/37 91.89%	26/76 34.21%	20/37 54.05%	14/76 18.42%	17/37 45.95%	75/76 98.68%	26/37 70.27%	71/76 93.42%	27/37 72.97%
E. coli O157	R	-	-	1/2 50%	-	-	-	-	-	-	-	-	-	-	-	1/2 50%	1/1 100%	2/2 100%	1/1 100%
	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	S	2/2 100%	1/1 100%	1/2 50%	1/1 100%	2/2 100%	1/1 100%	2/2 100%	1/1 100%	2/2 100%	1/1 100%	2/2 100%	1/1 100%	2/2 100%	1/1 100%	1/2 50%	-	-	-

N: number of isolates resistant to antibiotic, N: all isolates, R: Resistant, I: Intermitans, S: Susceptible

Table 2. Multiple drug resistance rates of *C. jejuni*, *Salmonella* spp. and *E. coli* O157 strains.

Strains	Resistance					
	1 Antibiotic n/N (%)		2 Antibiotics n/N (%)		3≤ Antibiotics n/N (%)	
	2011-2012	2021-2022	2011-2012	2021-2022	2011-2012	2021-2022
C. jejuni	104/170 61.17%	39/48 81.25%	38/170 22.35%	5/48 10.42%	28/170 16.47%	4/48 8.33%
***Salmonella* spp.**	33/76 43.42%	17/37 45.95%	19/76 25%	12/37 32.43%	24/76 31.57%	8/37 21.62%
***E. coli* O157**	1/2 50%	1/1 100%	1/2 50%	-	-	-

n: number of isolates resistant to the antibiotic, N: all isolates

Table 3. Distribution of answers to the survey's first four questions.

Question Number	Questions	Number of veterinarians saying (%)			
		Yes		No	
		2011-2012	2021-2022	2011-2012	2021-2022
Q1	Are additions of synthetic or organic antibiotics obligatory for efficiency in the rearing period of broilers?	100	100	-	-
Q2	Are residues of the antibiotics used in the broiler rearing period minimised until the slaughter process?	100	100	-	-
Q3	Have you ever detected *Campylobacter* spp. in your flocks?	100	86	-	14
Q4	Do you apply preventative antibiotics to your chicks in the first three days of the rearing period?	40	28	60	72

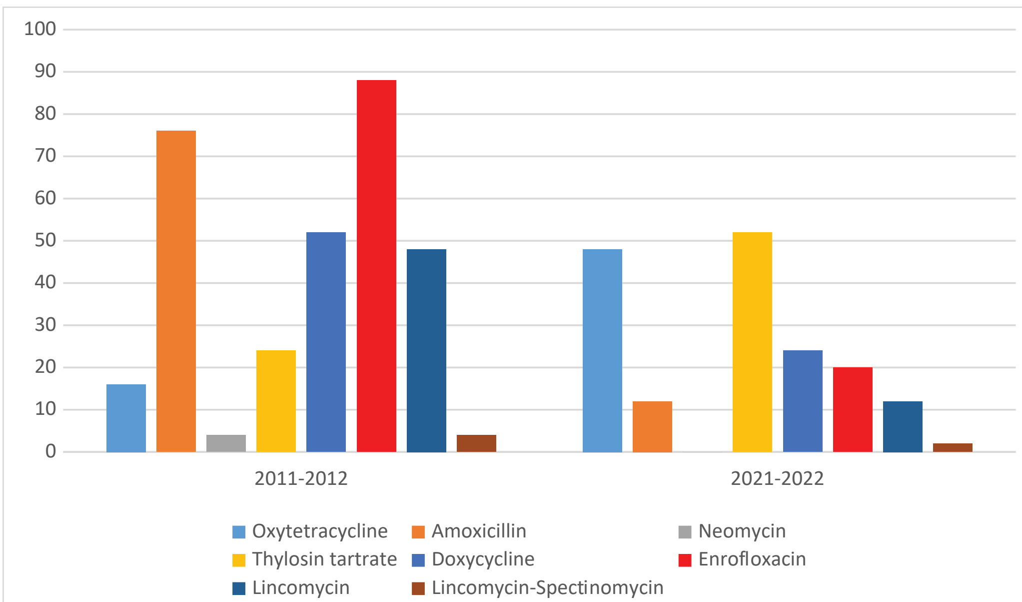

Figure 3. Results obtained from survey's fifth question*

*Would you please tick the antibiotics you have preferred mainly to treat diseases in the rearing period?

DISCUSSION

In this study, we assessed the prevalence and antimicrobial resistance of *C. jejuni*, *Salmonella* spp., and *E. coli* O157 isolated from fresh retail broiler breasts purchased from hypermarkets in seven provinces in Türkiye before and after the implementation of legal amendments. In our study, broiler breast samples were preferred, mainly because they are commonly served in school canteens and cafeteria menus with various presentation styles. In addition, since it is more practical to prepare, broiler breast is the main ingredient in chicken doner, and schnitzel sold at kiosks around schools is chicken breast.

We report a significantly lower prevalence of *C. jejuni*, *Salmonella* spp. and *E. coli* O157 contamination in 2021-2022 compared to 2011-2012 among retail broiler meat samples. In this study, 2011-2012 years results showed that 68% of samples were contaminated with *C. jejuni;* 30.4% with *Salmonella* spp. and 0.8% with *E. coli* O157; on the other hand, our 2021-2022 years results showed that 10.08% of samples were contaminated with *C. jejuni;* 7.77% with *Salmonella* spp. and 0.21% with *E. coli* O157.

The reasons for the positive developments in the broiler production sector in Türkiye between these two periods are believed to be due to legal regulations and the more effective operation of control and audit processes. Intensifying competition with the increasing number of subsidiaries caused an increase in company production capacities. Better quality, modern and systematic production chains: high-tech housing and management systems equipped with automatic systems for feeding, drinking, heating, cooling, air-conditioning and various sensors for data collection began to be obtained (Pereira et al., 2018; Yayli & Kilic, 2023).

Improving the conditions of the poultry house where the animals are raised, reducing stocking density, arranging and periodically planning the cleaning processes in the henhouses, giving importance to the water used by the animals and the hygiene of the water used for cleaning are seen as important factors in reaching the current level of the prevalence of pathogens (Pereira et al., 2018; Oladeinde et al., 2023; Shynkaruk et al., 2023). Raising awareness of chicken producers, increasing biosecurity measures, adequate technical information sources from the state, as well as the use of quality feed, vaccines and controlled antibiotics contributed to this positive picture (Ramukhithi et al., 2023).

Our study evaluated *C. jejuni*, *Salmonella* spp. and *E. coli* O157 strains for antibiotic resistance. In 2011-2012, resistance to only one antibiotic in *C. jejuni, E. coli* O157 and *Salmonella* spp. strains were 61.17, 50 and 43.42%, respectively. The highest resistance against tetracycline (56.47%) was recorded in *C. jejuni* strains. It was determined that the antimicrobial agent to which *Salmonella* isolates showed the highest resistance was streptomycin (39.47%). Two *E. coli* O157 strains were resistant 100% to oxytetracycline, 50% to tetracycline and streptomycin. In 2021-22, resistance to one antibiotic in *C. jejuni, E. coli* O157 and *Salmonella* spp. strains were 81.25, 100 and 45.95%, respectively. Although the values are seen to be high proportionally, the low values detected in the 2021-22 prevalences of the mentioned bacteria can be considered as a sign of the success of legal regulations, implementations and sanctions. The highest resistance against tetracycline (52.08%) was recorded in *C. jejuni* strains. The antimicrobial agent that *Salmonella* spp. showed the highest resistance was determined as streptomycin (37.84%). Only a *E. coli* O157 strains were resistant to oxytetracycline, and tetracycline.

C. jejuni is the leading cause of human bacterial gastroenteritis and is incriminated in systemic infections in the developed world. It is estimated that there are more than half a million cases and 80,000 general practitioner consultations annually in the UK (Bhunia, 2018; Jorgensen et al., 2019). *C. jejuni* was isolated from half of the caecal specimens collected from commercial broilers and layer hens immediately after slaughter from four registered chicken slaughterhouses in South Africa (Bester and Essack2008). While almost all of these isolates were resistant to tetracycline and ceftriaxone, high susceptibility was found to ciprofloxacin (91% in broilers and 76% in layers) and gentamicin (98% in broilers and 81% in layers). The susceptibility rate detected to antibiotics for the broilers and layers was 50% and 57% for erythromycin, 45% and 24% for clarithromycin, 68% and 43% for ampicillin and 64% and 48% for nalidixic acid, respectively. MDR was detected in 23% and 43% of the isolates from broiler and layer chickens, respectively.

In 2008, a large-scale survey on *Campylobacter* in broilers was performed in 26 EU Member States, including Norway and Switzerland. Samples of 10,132 batches of broilers were collected from slaughterhouses. *C. jejuni* prevalence was 51.0% (EFSA, 2010). During a monitoring plan in France throughout 2009, *C. jejuni* was detected in 46.5% of broiler meat products collected in retail outlets. While all isolates analysed were susceptible to erythromycin, chloramphenicol and gentamicin, streptomycin resistance was detected in only one isolate (Guyard-Nicodème et al., 2015).

Torralbo et al. (2015) from Southern Spain reported 30.29% (83/274) of *C. jejuni* in samples collected from the carcass surfaces and quartered carcasses in 2012. They also informed that 35.4% of quartered broiler carcass with packing were contaminated with *C. jejuni*. They reported the highest resistance to tetracycline (100%) and ciprofloxacin (100%) for *C. jejuni*. Moreover, 11 from 30 (36.7%) *C. jejuni* isolates were detected as MDR. The antimicrobial agent with the lowest percentage of resistance was erythromycin (10%).

In a study conducted between 2014 and 2018 in slaughterhouses in Poland, *C. jejuni* was identified in 525 (22.18%) chicken carcasses among 2,367 samples. *C. jejuni* strains presented resistance rates to ciprofloxacin 92.0%, nalidixic acid 90.3%; tetracycline 64.8%, streptomycin 20.2%, gentamicin 1.3%, and erythromycin 1.1%. Among a total of 525 tested strains of *C. jejuni*, 108 isolates (20.6%) were MDR, mainly to ciprofloxacin, nalidixic acid, streptomycin, and tetracycline (94/525; 17.9%) (Wieczorek et al., 2020).

As mentioned in the EU antimicrobial resistance report published by EFSA & ECDC (2019), the resistance rates to ciprofloxacin and nalidixic acid were reported as 63.4% and 61.9 respectively.

A total of 328 *C. jejuni* isolates from 392 retail chicken samples were tested for antimicrobial resistance by Public Health England (Jorgensen et al., 2019). 52.13% of the *C. jejuni* isolates were resistant to ciprofloxacin and tetracycline; 50.61% to nalidixic acid; 1.52% to streptomycin; but only two *C. jejuni* (0.6%) isolates were resistant to erythromycin. On the other hand, all isolates were sensitive to gentamicin. MDR was in 1.52% of *C. jejuni* isolates (tetracycline, nalidixic acid and/or ciprofloxacin, streptomycin 1.22%; tetracycline, nalidixicacid and/or ciprofloxacin, erythromycin 0.30%).

In another study accomplished in Pakistan in 2021, 182 (14%) strains of *C. jejuni* were isolated in the analysis of 1260poultry meat samples ready for commercialisation (Khan et al., 2021). Of these *C. jejuni* isolates, 82% were resistant to tetracycline, 50% to streptomycin, 44% to gentamicin, and 25% to ciprofloxacin. MDR was detected in 90% of these *C. jejuni* strains. On the other hand, antibiotic resistances of *C. jejuni* strains show variability between different countries. Despite a large number of specific data on the resistance of campylobacteria in different regions of the world, the high resistance to widely used antimicrobial drugs, tetracyclines and increased resistance to fluoroquinolones pose a significant risk and indicate that their use should be reviewed and reassessed (Urumova et al., 2014). Our study recorded the highest resistance against tetracycline in *C. jejuni* strains similarly. After the updated legislation, a decrease was observed in the number of *C. jejuni* and the antimicrobial resistance percentages of these isolates in 2021-2022.

Poultry products are one of the most significant sources of non-typhoidal *Salmonella*, a leading bacterial cause of acute gastroenteritis worldwide, with around a 0.0076% death rate in humans (Guran et al., 2020). Antimicrobial-resistant *Salmonella* spp. constitutes a global concern. Specifically, the WHO has classified fluoroquinolone-resistant *Salmonella* as a high-priority target for new drug development (WHO, 2017).

In studies conducted to investigate the presence of *Salmonella* spp. in broiler meat in Türkiye, Goncagül et al. (2005) reported that isolated *Salmonella* spp. from 8.9% of broiler wing skins, Ünlü (2011) 13.8% of broiler carcasses, Ceylan (2012) 44% of broiler breast samples, 52% of chicken thigh samples, and Guran et al. (2020) 6.3% of organic frozen chicken samples. In the study conducted by Ünlü (2011), all of the *Salmonella* isolates were resistant to vancomycin, methicillin, and novobiocin, 85% of the isolates were resistant to tetracycline, 50% were resistant to gentamicin, 28% were resistant to amoxicillin+clavulanic acid, 23% were resistant to ampicillin, and 15% were resistant to enrofloxacin. Guran et al. (2020) reported that all *Salmonella* isolates were resistant to ciprofloxacin (100%). The percentages of resistance to other antibiotics tested were: 86.3% to amikacin, 86.3% to gentamicin, and 81.8% to netilmicin. One isolate was resistant to colistin. On the other hand, all isolates were pan susceptible to aztreonam, cefepime, ceftazidime, ceftriaxone, imipenem and meropenem. Interestingly, all *Salmonella* isolates (100%) exposed resistance to at least two antibiotics and 86.3% of the isolates were defined as MDR.

In studies conducted to investigate the presence of *Salmonella* spp. in broiler meat in Iran, India, Indonesia, and Egypt, Soltan Dallal et al. (2014) reported that isolated *Salmonella* spp. from 45.26% in packed and unpacked broiler samples, Saravanan et al. (2015) 1.52% in poultry product samples, Yulistiani et al. (2019) 85% in broiler meat samples collected from the slaughterhouse in a traditional market, Shaltout et al. (2020) 11% in broiler samples collected from an automatic poultry dressing plant, respectively. Saravanan et al. (2015) found that all *Salmonella* spp. isolates were resistant to oxytetracycline, used as a routine growth promoter poultry feed additive in India. In contrast, these isolates showed higher sensitivity to less commonly used antibiotics such as ciprofloxacin, enrofloxacin and norfloxacin.

Rivera et al. (2021) reported that *Salmonella* spp. prevalence in the free-range broiler samples was 1.37% in Chile. They reported that 23.33% of *Salmonella* spp. isolates were single-drug resistant, 13.33% were MDR. One-third of the strains were resistant to tetracycline, 23.81% to ampicillin, 23.81% to streptomycin, 14.29% to amoxicillin, and 4.76% to chloramphenicol. All *Salmonella* spp. were susceptible to gentamicin and kanamycin.

Siddiky et al. (2021) reported that the overall prevalence of *Salmonella* spp. was 8.62% in broilers, 6.89% in sonali (crossbreed), and 3.1% in native chicken in Bangladesh. They reported that the MDR *Salmonella* spp. was 84%, 75%, and 44.4% in broilers, sonali, and native chickens, respectively.

According to our results, the antibiotic resistance of *Salmonella* spp. strains showed the highest distribution to streptomycin. These findings are also supported in different studies (Lestari et al., 2009; Dallal et al., 2010; Yildirim et al., 2011) and point out the widespread use of streptomycin in animals in many countries.

In our study, antibiotic resistance of *Salmonella* spp. isolates (2011-2012 years) show similarity with the studies held in Vietnam, Iran, South Korea, USA, and Türkiye (Dallal et al., 2010; Rayamajhi et al., 2010; Lestari et al., 2009; Yildirim et al., 2011), and our results showed differences with the results obtained from Brazil (Cardosa et al., 2006).

In line with the data obtained in our study, the number of *Salmonella* spp. isolated has decreased significantly in recent years. This is due to the National *Salmonella* Monitoring Program implemented under the new regulations. On the other hand, although tetracycline group antibiotics are prohibited from being added to feeds as growth factors due to the intensive use of broad-spectrum antibiotics such as oxytetracycline and tetracycline in the treatment of humans and animals, the resistance rate against these antibiotics has increased in *Salmonella* spp.

*E. coli*O157, whose main reservoir is cattle, has started to be detected in chicken meat in recent years. Since 1982, the year it was first described, O157 has been responsible for 85-95% of haemolytic uremic syndrome cases, which causes diseases ranging from sporadic to large outbreaks (Ferens & Hovde, 2011; Amalia et al., 2020). *E. coli* O157 prevalence was between 0-10.3 %in the studies conducted in poultry meat samples (Esteban et al., 2008; Chinen et al., 2009; Minami et al., 2010; Karadal et al., 2013; Guran et al., 2017; Ahmady, 2021).

A study conducted in Iran found that 7.3% of broiler samples were contaminated of *E. coli* O157:H7 (Momtaz and Jamshidi, 2013). It has been reported that 4% *E. coli* O157:H7 was isolated in breast meat samples of chickens slaughtered under hygienic conditions in northern Iraq (Mustafa and Inanc, 2018). According to bacteriological and serotyping study results from raw chicken samples in Egypt, 4% of the strains were identified as *E. coli* O157:H7 (Shaltout et al., 2020).

Mustafa and Inanc (2018) reported that the *E. coli* O157 strains were sensitive to gentamicin, norfloxacin, and neomycin but resistant to tetracycline, amoxicillin, doxycycline, ciprofloxacin, chloramphenicol, and ampicillin.

In our study in 2011-12, it was determined that two *E. coli* O157 strains isolated from the samples were resistant to oxytetracycline (100%), and one *E. coli* O157 strain was found to be resistant to tetracycline and streptomycin 50%. In 2021-22, an isolated *E. coli* O157 strain was found to be resistant to oxytetracycline and tetracycline (100%). Antimicrobial resistance is prevalent in *E. coli* O157 and other VTEC serotypes, including multiple-drug resistance to streptomycin, sulfamethoxazole, and tetracycline (Kim et al., 1994; Mora et al., 2005), and there is some evidence that resistance may be increasing over time (Kim et al., 1994; White et al., 2002). Although cattle-isolated *E. coli* O157 strains have a higher antimicrobial resistance ratio than human-induced strains, there is insufficient data to adapt this hypothesis to chickens(Meng et al., 1998; Mora et al., 2005). When comparing the results, the antimicrobial resistance profiles for isolates and positive samples obtained from chickens sampled at various locations, such as slaughterhouses, retailers, etc., may differ.

The antibiotic resistance results of *E. coli* O157 obtained from our study and the other studies in Ireland (Scott et al., 2009) were similar. The results of Mora et al. (2005), Klein and Bulte (2003) and Dontorou et al. (2003) are different from our results.

A partial similarity was observed between the rates of antibiotics that veterinarians preferred to use in diseases occurring during the broiler breeding period between 2011-12 and 2021-22, determined in our survey results, and the antibiotic resistance rates of the bacteria isolated in our study. A study held by Unlu et al. (2011) shows that the antibiotics applied at the farm can be found as a resistance pattern in pathogenic bacteria isolated from final products. This study also supports the same idea. The antibiotic application at the farm is thought to trigger antibiotic resistance in *C. jejuni, E. coli* O157 and *Salmonella* spp. strains.

Enrofloxacin (88%) was marked as the most preferred antibiotic by veterinarians surveyed in 2011-12. Enrofloxacin is an antibacterial agent of the quinolone class, with high activity against both Gram-negative and Gram-positive bacteria. It is widely used in veterinary medicine due to its effective results in respiratory tract diseases (Trouchon & Lefebvre, 2016; Grabowski et al., 2022).

According to the survey results in 2021-22, the use of enrofloxacin decreased to 20%. While 34.70% of *C. jejuni* strains and 10.53% of *Salmonella* spp. strains isolated in 2011-2012 were resistant to enrofloxacin, in 2021-2022 the rate of enrofloxacin-resistant *C. jejuni* strains increased to 14.58%, enrofloxacin-resistant *Salmonella* spp. strains decreased to 5.41%. Similarly, while 32.94% of *C. jejuni* strains and 22.36% of *Salmonella* spp. strains isolated in 2011-12 were resistant to ciprofloxacin; in 2021-22, the rate of ciprofloxacin-resistant *C. jejuni* strains has fallen to 12.50% and the rate of ciprofloxacin-resistant *Salmonella* spp. strains to 18.92%.

Amoxicillin (76%) was one of the most frequently used antibiotics by the veterinarians who participated in the survey in 2011-12. This antibiotic, which is an analogue of Ampicillin, is a broad-spectrum penicillin (beta-lactam family) effective against Gram-positive and Gram-negative microorganisms. It has found use in respiratory tract infections and lung infections (Akan, 2012). According to the survey results in 2021-22, the rate of use of amoxicillin decreased to 12%.

In 2011-12, isolated 12.94% of *C. jejuni* strains and 35.53% of *Salmonella* spp. were found to be ampicillin-resistant and isolated 36.47% of *C. jejuni* strains and 32.80% of *Salmonella* spp. were found to be piperacillin-resistant; in 2021-22, the rate of ampicillin-resistant *C. jejuni* strains decreased to 8.33%, *Salmonella* spp. strains decreased to 18.92% and piperacillin-resistant *C. jejuni* strain couldn't be detected, *Salmonella* spp. strains decreased to 29.73%.

Lincomycin is generally used for the prevention and treatment of infections caused by gram-positive pathogenic organisms in poultry. It was used in the past as growth promoters and prophylactic agents in broiler feeds and waters (Jamal et al., 2017). Lincomycin has found use due to its bacteriostatic effect on respiratory and septicemic strains of sensitive *E. coli* to maintain the indication for respiratory *E. coli*. Spectinomycin can reduce the environmental spread of *E. coli*, which can contribute to herd outbreaks. Therefore, a lincomycin-spectinomycin combination is used for this type of indication (Baptiste et al., 2016). Neomycin is an aminoglycoside antibiotic with bactericidal activity against gram-negative aerobic bacilli and some anaerobic bacilli that have not yet developed resistance. It is generally ineffective against gram-positive bacilli and anaerobic gram-negative bacilli. In the past, it was generally used to increase fattening performance by adding to medicated poultry feeds (Williams, 1985).

In the comparison of the survey data, 48% preferred lincomycin and 4% preferred lincomycin-spectinomycin combination in 2011-12; in 2021-22, it was seen that they were preferred by the rate of 12% and 2%, respectively.

In the survey data, it was seen that the use of neomycin, which was preferred by 4% in 2011-12, was not preferred in 2021-22. It was determined that the gentamicin and streptomycin resistance rates of isolated *C. jejuni* and *Salmonella* strains were close to each other in both periods (2011-12/2021-22).

Tylosin is a macrolide antibiotic and bacteriostatic feed additive used in veterinary medicine. It has a broad spectrum against Gram-positive organisms and a limited spectrum against Gram-negative organisms. It has also been used as a growth accelerator (Giguère, 2007). Tylosin, which was preferred by 24% in 2011-12, increased to 52% in 2021-22.

Against erythromycin, a macrolide, the resistance rate of *C. jejuni* strains isolated in 2011-2012 (4.7%) were similar to that of *C. jejuni* strains isolated in 2021-22 (4.17%). The erythromycin-resistance rate (16.22%) of *Salmonella* strains isolated in 2021-22 was proportionally higher than the resistance rate (3.95%) of *Salmonella* strains isolated in 2011-2012. When evaluated in intermittent strains against erythromycin, this proportional difference can be seen as an indicator of the increase in the use of erythromycin in recent years.

Doxycycline, oxytetracycline, and tetracycline are broad-spectrum antibiotics of the tetracycline class used to treat infections caused by bacteria and some parasites. The product is especially used for gastrointestinal infections and respiratory tract infections in poultry (Akan, 2012; Gutiérrez et al., 2017; Pokrant et al., 2021). Doxycycline was preferred by 52% and oxytetracycline by 16% in 2011-12 by surveyed veterinarians. In 2021-22, these rates were 24% and 48%, respectively.

Tetracycline and oxytetracycline resistance rates (56.47% and 25.30%) of *C. jejuni* strains isolated in 2011-12 were close to each other, with the resistance rates (52.08% and 20.83%) of *C. jejuni* isolated in 2021-22.

It was observed that *Salmonella* strains isolated in 2011-12 were resistant to tetracycline by 1.32% and oxytetracycline by 6.58%, while the resistance rates of *Salmonella* strains isolated in 2021-22 to these antibiotics were 27.02% and 27.02%, respectively.

Although the isolation of *E. coli* O157 from broiler breast meats in our study is an important finding for public health, these isolates could not be detected at a rate that could make detailed comments about their antibiotic resistance profiles.

The usage of antibiotics is the most important factor promoting the emergence, selection and spread of antibiotic-resistant microorganisms in both veterinary and human medicine (Witte1998; Van den Bogaard et al. 2001). The use of antibiotics not only select for resistance in pathogenic bacteria but also in the endogenous flora of exposed individuals or populations.(Van den Bogaard & Stobberingh1999). The industrialisation of poultry production and the widespread use of antimicrobial growth promoters have increased the risk of the emergence of antibiotic-resistant strains (Gilchrist et al. 2007). Antimicrobial agents can be fed continuously to food animals like broilers as antimicrobial growth promoters. Therefore, antibiotic selection pressure for resistance in poultry bacteria is high, and as a result their fecal flora contains a relatively high proportion of resistant bacteria. Since most of the commonly used antimicrobial growth promoters are effective mainly against Gram-positive bacteria, *E. coli*, *Salmonella* spp. and *Campylobacter* spp. resistance in the feces of most food-grade animals is against veterinary prescription antibiotics (Van den Bogaard et al., 2001). Although banned in the European Union in 2006, Türkiye had used antibiotics sub-therapeutically as growth promoters and as prophylaxis until 2014, usually as food/water supplements (Bester and Essack, 2008; Anonymous, 2014). As a result, decreased efficacy of various antibiotics to treat infections in humans and livestock has become a significant problem. Our study's first period (2011-12) results also reflected this scenario. Second-period antibiotic resistance rates reveal the effectiveness of the legal regulations and drug tracking systems created. It is thought that low antibiotic resistance rates are associated with the effective strategies and legal regulations and the limitation of antibiotic use. The fact that all kinds of antibiotic sales are subject to prescription and that free antibiotic circulation is not allowed in the market has been an important cornerstone. It is thought that this positive picture is shaped by the follow-up of antibiotics through a monitoring system at every stage, from the production or importation of antibiotics in pharmaceutical factories to their shipment to pharmaceutical warehouses and from there to pharmacies, and even their use by veterinarians and medical doctors.

It is thought that factors such as sample size, whether the samples are skinned or skinless, the methodology used for identification, the use of packaged or unpackaged products, hygienic deficiencies such as cross-contamination and breaking the cold chain, geographical changes, seasonal differences may contribute to this wide distribution in the results of the studies.

CONCLUSION

C. jejuni, *Salmonella* spp. and Verotoxigenic *E. coli* are significant food pathogens globally. The perception in evaluating the risk of these pathogens has changed. Currently, scientific interest is increasing in the antibiotic resistance of these food-borne pathogens.

Evidence shows that the massive use of antibiotics at the farm level has triggered significant increases in antibiotic resistance of these three pathogens. Antibiotic-resistant bacteria are a substantial hazard to public health.

The resistance rates of isolates obtained from broiler chicken breast samples, which have been tried to be associated with the choice of antibiotics for the treatment of infections occurring during the broiler breeding period, are also triggered by other factors. In the past, lincosamides, tetracycline, trimethoprim, sulfonamides, macrolides, aminoglycosides, beta lactams and pleuromutilins, which were approved by the official authorities, were widely used in animal feeds to increase feed utilisation and performance (Akan, 2012, Jamal et al., 2017). Inadequate dose, inadequate duration and inappropriate use of antibiotics are bad practices that can cause resistance to antibiotics in bacteria. Mutation of the bacterial gene on the chromosome, use of alternative metabolic pathways, enzymatic inactivation, changes in cell membrane permeability with mutations, and excretion of the drug by active pumping are important mechanisms that cause bacterial resistance (Yüce, 2001; Öztürk, 2002; Lambert, 2005; Coyne et al., 2011). Factors such as the ability of a bacterial species that has lost its sensitivity to one type of antibiotic to gain cross-resistance to another antibacterial drug with a similar chemical structure or similar mechanism of action are thought to be the cause of unrelated differences in the resistance rates of isolates (Ekici & Yarsan, 2008).

Various antibiotic resistance mechanisms of *C. jejuni*, *Salmonella* spp., and VTEC have been determined. It is now more visible that this trio can be resistant to different antibiotics, which is caused by the administration of varying antibiotics at the farm level. The Ministry of Agriculture and Forestry in Türkiye holds strict control and regulates antibiotic usage in animal farms. Even legislative measures are in place for controlling antibiotic applications during husbandry.

Rising awareness among producers and other people must be managed for additional value to public health. The results of this study showed that a widespread survey must be undertaken in Türkiye to determine the bias of veterinarians in the administration of antibiotics. Also, the strains should be analysed with molecular tools for a deeper comprehension of antibiotic resistance.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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