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Determination of Virulence Factors and Antibiotic Resistance Profile of *E. coli* O157:H7 in some Marine and Freshwater Fish from Samsun, Türkiye

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Abstract

In this study, *Escherichia coli* O157:H7 contamination was investigated in *Engraulis encrasicolus*, *Merlangius euxmus*, *Trachurus mediterraneus*, *Mullus barbutus*, *Tinca tinca*, *Cyprinus carpio* and *Oncorchynhus mykiss* (total of 300 samples). The presence of certain virulence genes such as shiga toxin 1 (*stx1*), shiga toxin 2 (*stx2*), intimin (*eaeA*), and hemolysin (*hlyA*) was determined. The E-test method was employed to determine the antibiotic resistance profile and minimum inhibitory concentration (MIC) values of the isolates. Out of a total 141 freshwater fish samples, *E. coli* O157:H7 was detected in 5 of them (3.5%). In 159 marine fish, 2 of them (1.25%) were *E. coli* O157 positive. In terms of virulence characteristics, the *E. coli* O157:H7 isolate obtained from the *Oncorhynchus mykiss* sample had the *stx1, eaeA*, and *hlyA* genes. Both *eaeA* and *hlyA* genes were determined as positive in the *E. coli* O157 isolate identified from the *Merlangius euxmus* sample. Resistance to tetracycline, cefotaxime, cefepime, ciprofloxacin, gentamicin, ertapenem and cefoxitin was demonstrated in both samples. In this study, the presence of *E. coli* O157 in fish, detection of virulence genes and antibiotic resistance profile indicate the potential risk of infection from contaminated seafood.

Introduction

Fish meat, being rich in protein and containing various macro, micro, and other essential nutrients, forms the cornerstone of a healthy and balanced diet (Liasota et al., 2023). Nowadays, fish for human consumption is provided both from their natural habitats and through aquaculture. In many communities in the world, acces to fish is easier and cheaper than other animal products (Boyd et al., 2022).

In the last 50 years, significant progress has been made in the aquaculture sector worldwide, and issues related to the export, fishing, and farming of aquatic products hold a significant place in the economy. In FAO (2022) report, the global quantity of aquatic products obtained through fishing is declared as 90.265.933 tons worldwide; with China (13.266.203 tons), Indonesia (6.925.352 tons), and Peru (5.626.542 tons), among others, ranking high in production. In Türkiye, this figure is reported as 364.400 tons. Asia accountes for 52% of global fisheries. Türkiye, divided into 7 geographical regions, surrounded by seas on three sides, and possessing numerous inland waters (25 million hectares of sea and 1.5 million hectares of inland waters), enjoys a geographical advantage for both fishing and aquaculture (Akdeniz et al., 2023). The annual quantity

of fish caught in Türkiye is 335.002 tons, with 254.535 tons coming from the sea and 33.256 tons from inland waters. Additionally, the quantity of farmed aquatic products is 514.805 tons, with 368.742 tons from the seas and 146.063 tons from inland waters (TUIK, 2023). In Türkiye, 77% of the aquatic product demand obtained through fishing is met by the Black Sea region. The Samsun province (41˚ 17 25 N-36˚ 20 01 E), where fish materials for this study were obtained, is an important region in the fishing industry (TEPGE, 2022).

Fish are creatures with high microbial contamination depending on living conditions and fishing technologies. This depends on many factors such as the season, the proximity of the environment where they are caught, the bacterial load of the water, the amount of salt and temperature, or insufficient attention to hygiene rules during the processing procedures. The deterioration period starts depending on the microorganism load (Parlapani et al., 2023). According to CDC (2023), the number of people affected by foodborne diseases is 48 million, with 3.000 death each year (WHO, 2023; FAO, 2023). In studies conducted in the USA, it was reported that approximately 260 thousand people were affected by fish-borne contamination (Barrett et al., 2017).

Vibrio spp., *Salmonella* spp., *Listeria monocytogenes*, *Clostridium botulinum*, *Staphylococcus aureus*, and *Escherichia coli* are some of the most important contamination agents for public health detected in market fish. In fish consumed, Emerging pathogens such as *Arcobacter* spp., *Cronobacter* spp. and enteric viruses, which were not previously thought to be foodborne, are now threatening public health (Sankar, 2023). The main reservoir of *Escherichia coli* (*E. coli*) is the intestines of humans and animals, and unhygienic environmental factors. Personnel in contact with fish and inappropriate storage conditions play an important role in the contamination of fish (Franco-Duarte et al., 2023). *E. coli*, which is normally found commensally in the human and animal intestine, can infect hosts with impaired intestinal mucosal integrity or weakened immune systems (Tenaillon et al., 2010). *E. coli*, which can cause gastrointestinal diseases in humans, has 6 different species: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAgEC), enteroinvasive *E. coli* (EIEC), diffuse-adhering *E. coli* (DAEC), and enterohemorrhagic *E. coli* (EHEC). All these pathogenic *E. coli* species cause disease in humans. However, *E. coli* O157:H7 (EHEC), which can produce shiga toxin, can cause diarrhoea, haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) in children and the elderly. If left untreated, it can cause death. (Gambushe et al., 2022; Liu et al., 2022; Franco-Duarte et al., 2023). *E. coli* O157:H7 pathogenicity is characterised by a number of virulence factors, the most important of which are shiga toxin 1 (*stx1*), which causes haemorrhagic diarrhoea by invading epithelial cells; shiga toxin 2 (*stx2*), which causes haemolytic and uremic syndrome by invading the kidneys through the bloodstream; intimin (*eaeA*), which is an adhesion protein located on the chromosome; enterohemolysin (*hlyA*), which helps the bacteria to take up iron by lysing erythrocytes and other cells (Binandeh et al., 2020; Sallam et al., 2023; Xue et al., 2023).

Antibiotic resistance in bacteria is a global problem at the moment. The first antibiotic was penicillin, which was discovered in 1928 and used in human medicine in 1940. The uncontrolled use of antibiotics in the past and today has caused resistance to many antibiotics in bacteria (Davies and Davies 2010). When human and animal faeces containing bacteria resistant to antibiotics are mixed into the sea and fresh waters, the agents can contaminate the environment and pass to aquatic organisms, and antibiotic-resistant bacteria can cause infections in humans by consuming these fish (Leonard, et al., 2022). It is estimated that antibiotic-resistant bacteria will contribute to approximately 300 million premature deaths by the year 2050 and result in economic losses of up to 100 trillion dollars in the global economy (Munita & Arias, 2016; Stephen et al., 2023). Antibiotic resistance is one of the most important factors that enhance the pathogenicity of *E. coli* O157 and *E. coli* O157:H7. Many of the resistance genes are mobile, capable of being carried by plasmids, transposons, and integrons (such as bla_{TEM1}, *aadA1*-like, *strA/B*, *tet(A)* and *tet(B)*, *sul1*, *sul2*, *dfrA1*-like, *dfrA14*, and bla_{OXA1}), which gives them genetic flexibility (Bonnet et al., 2009; Guerra et al., 2003; Szmolka et al., 2012). Furthermore, protein structures that lead to a decrease in membrane permeability (*acrAB-TolC*) have also been reported to contribute to multi-drug resistance. This can make the treatment of infections more challenging (Iyer et al., 2013; Szmolka & Nagy, 2013).

Many countries have implemented guidelines for the identification of *E. coli* O157:H7 in food as part of their public health efforts, and monitoring *E. coli* O157:H7 has become a mandatory requirement in the field of food safety (Bazsefidpar et al., 2023). In recent years, various studies related to *E. coli* contamination in different seafood products have been conducted in different countries (Islam et al., 2022; Marijani, 2022; Schar et al., 2021; Vidic et al., 2023; Westgate et al., 2022), but there have been limited studies conducted in Türkiye (Onmaz et al., 2020). In this study, possible contamination of *Escherichia coli* O157:H7 was investigated in the most commonly preferred fish species for consumption, including anchovy (*Engraulis encrasicolus*), whiting (*Merlangius euxmus*), horse mackerel (*Trachurus mediterraneus*), red mullet (*Mullus barbutus*), tench (*Tinca tinca*), carp (*Cyprinus carpio*), and rainbow trout (*Oncorchynhus mykiss*), from different dates and sales points. Additionally, the presence of certain virulence genes (*stx1*, *stx2*, *eaeA*, and *hlyA*) was investigated in the isolates, and antibiotic resistance in virulence-characterized isolates was determined using the E-test method.

Materials and Methods

The samples used in the study were collected from various fish sales points in Samsun province between September 2022 and June 2023, covering different seasons and months (Table 1). A total of 300 samples, including freshwater (river, lake, dam) and marine fish, caught and offered for sale, were used in the research.

E. coli O157:H7 Isolation and Identification

The fish samples were delivered to the laboratory as soon as possible under cold chain and samples for microbiological analyses were taken according to ISO (International Organization for Standardization) 6887-3. In this study, samples were taken from the dorsal and tail muscle surfaces of the fish in compliance with the guidlines of ISO 6887-3. For this purpose, 25 grams of each sample was taken for pre-enrichment and 225 mL of Modified Tryptone Soy Broth with Novobiocin (mTSB-Merck-1.09205.0500,USA) was added, homogenised with stomacher and incubated at 37˚C for 18-24 hours (Doyle and Schoeni, 1987, De Boer and Heuvelink, 2000). At the end of the period, 1 ml of the selective homogenisation was taken and Immuno Magnetic Separation (IMS) was performed according to the manufacturer's (Dyneabeads- anti *E. coli* O157-710.04, Dynal) procedure (Nou et al., 2006; O'Brien et al., 2005). Following IMS, 50 µl of the beads-bacteria resuspension was transferred to Sorbitol Mac Conkey Agar (CT-SMAC-Sorbitol Mac Conkey Agar CM0813-UK-Cefixime Tellürite Selective Supplement- Oxoid SR0172E,UK) and incubated at 37˚C for 24-48 hours. In CT-SMAC, suspicious colonies with transparent and smooth edges were selected and transferred to MUG-SMAC Agar (Sorbitol Mac Conkey Agar CM0813,UK-MUG supplement, Oxoid BR0071E,UK) and incubated at 37˚C for 24 hours. Colonies that did not give green or blue fluorescence under UV light (366 nm wavelength) were considered as MUG negative and accepted as suspected *E. coli* O157:H7, then switched to Trypticase Soy Agar (TSA-Merck-5458.0500,USA) and incubated at 37˚C for 24-48 hours (Dontorou et al., 2003).

Identification of Suspected *E. coli* O157:H7 Isolates and Determination of Virulence Genes

Suspected isolates were identified as *E. coli* O157 using primers specific for *rfbO157*, and for H7 serotype, primers targeting the *fliCh7* gene region were used. Additionally, the presence of virulence factors defined as shiga toxin 1 (*stx1*), shiga toxin 2 (*stx2*), hemolysin (*hlyA*), and intimin (*eaeA*) gene regions was investigated in the identified isolates (Fratamico et al., 2000; Maurer et al., 1999). The nucleotide sequences of the primers used in the method (*E. coli* O157:H7 ATCC 43895 and *E. coli* O157:H7 ATCC 35150) are provided in Table 2.

DNA Extraction

The suspected isolates were transferred to TSA (Tryptic Soy Agar) and incubated at 37°C for 24 hours. After 24 hours, the colonies were collected and placed in eppendorf tubes containing 800 µl of buffered sterile distilled water. The tubes were then heated in a dry block heater at 100°C for 10 minutes. After heating, the tubes were centrifuged at 10.000 rpm for 5 minutes using a centrifuge machine. The supernatant was stored at -20°C to be used as template DNA for the PCR process (Lin et al., 1996).

mPCR and Electrophoresis

Amplification of *rfbO157*, *fliCh7*, *stx1*, *stx2*, *eaeA* and *hlyA* genes was performed in a Thermal Cycler. In the protocol, the method determined by Fratamico et al. (2000) (initial denaturation at 94˚C for 2 minutes, 35 cycles, denaturation at 94˚C for 20 seconds, primer

Table 1. The species and numbers of freshwater and marine fish analyzed seasonally

SEASONS	MONTHS	FISH SPECIES						
		FRESHWATER FISH			MARINE FISH			
		Tinca tinca	*Cyprinus carpio*	*Oncorhynchus mykiss*	*Engraulis encrasicolus*	*Merlangius euxmus*	*Trachurus mediterraneus*	*Mullus barbatus*
AUTUMN	SEPTEMBER	7	10	3	-	2	3	2
	OCTOBER	-	8	1	3	4	6	4
	NOVEMBER	-	12	12	11	-	9	-
WİNTER	DECEMBER	-	10	9	14	20	4	15
	JANUARY	-	10	5	6	2	2	6
	FEBRUARY	-	10	5	6	3	2	6
SPRİNG	MARCH	-	-	17	-	10	3	3
	APRIL	-	-	12	-	4	4	4
	MAY	-	-	10	-	1	-	-
TOTAL		7	60	74	40	46	33	40

binding at 54°C for 1 minute, primer extension at 72°C for 1 minute and final extension at 72°C for 10 minutes after 35 cycles) was used. The obtained PCR product amplicons were subjected to electrophoresis in a 1.2% agorose gel containing ethidium bromide (5 ug/ml) at 90 V for 60 min.

Antibiotic Resistance

In the study, the presence of antibiotic resistance in virulence-characterized isolates was determined using the disk diffusion method. For this purpose, the antibiotic resistance profile was investigated against the following antibiotics: amoxicillin-clavulanic acid, ampicillin, cefepime, cefoxitin, ciprofloxacin, ertapenem, gentamycin, imipenem, meropenem, tetracycline, trimethoprim-sulfamethoxazole, ceftriaxone, cefotaxime, and levofloxacin. Isolates stored at -20°C were transferred to TSA and incubated for 24 hours at 37°C. After incubation, the isolates were suspended in sterile tubes containing 0.9% FTS to achieve a 0.5 McFarland standard (10^8 CFU/ml). Then, they were streaked onto Mueller Hinton Agar (MHA), and antibiotic disks were placed on the agar. The plates were incubated at 37°C for 18-24 hours. After incubation, the zone diameters were measured, and the resistance status of the isolates was classified as resistant and susceptible according to EUCAST standards. The MIC (Minimum Inhibitory Concentration) values of the isolates were also determined and categorized (EUCAST, 2023).

Results

Between September 2022 and June 2023, a total of 300 samples were obtained from various fish sales points, collected monthly and seasonally, and from different dates. These samples included both freshwater (from rivers, lakes, and dams) and marine fish caught and offered for sale. Out of the total of 141 freshwater fish samples (*Tinca tinca*: 7, *Cyprinus carpio:* 60, and *Oncorhynchus mykiss:* 74), *E. coli* O157:H7 was detected in 5 of them (3.5%). Additionally, out of the total of 159 marine fish samples (*Engraulis encrasicolus:* 40, *Merlangius euxmus:* 46, *Trachurus mediterraneus:* 33, and *Mullus barbatus:* 40), *E. coli* O157 was found positive in 2 samples (1.25%). Regarding virulence characteristics, the *E. coli* O157:H7 isolate obtained from the *Oncorhynchus mykiss* sample (Isolate Code: S4-1) was found to have the *stx1*, *eaeA,* and *hlyA* genes. Similarly, in the *E. coli* O157 isolate identified from the *Merlangius euxmus* sample (Isolate Code: M24-1), the *eaeA* and *hlyA* genes were found to be positive. Information about the samples is provided in Table 3, and the mPCR image of the isolates is shown in Figure 1.

The phenotypic antibiotic resistance profile of the *E. coli* O157:H7 isolate with isolate code S4-1, isolated from the *Oncorhynchus mykiss* sample and possessing virulence genes, was determined using the disk diffusion method. The resistance profile was identified against tetracycline, cefotaxime, cefepime, ciprofloxacin, gentamycin, ertapenem, imipenem, and cefoxitin. Similarly, the *E. coli* O157 isolate with isolate code M24-1, identified from the *Merlangius euxmus* sample, showed resistance to tetracycline, cefotaxime, levofloxacin, cefepime, ciprofloxacin, gentamycin, ertapenem, cefoxitin, and meropenem. The resistance profiles of both isolates and the MIC (minimum inhibitory concentration) values for the antibiotics to which they were resistant are provided in Table 4.

Discussion

This study investigated the potential contamination of *Escherichia coli* O157:H7 in the most commonly consumed fish species (*Engraulis encrasicolus*, *Merlangius euxmus*, *Trachurus mediterraneus*, *Mullus barbutus*, *Tinca tinca*, *Cyprinus carpio*, *Oncorchynhus mykiss*) obtained from different dates and different sales points. In this research, the presence of some virulence genes (*stx1*, *stx2*, *eaeA*, and *hlyA*) was examined in isolates, and the presence of antibiotic resistance in isolates carrying virulence genes was determined using the E-test method.

Various studies conducted worldwide have obtained samples from different parts of fish, including

Table 2. Primer sequences used in *E. coli* O157:H7 and virulence genes (Fratamico et al., 2000; Maurer et al., 1999)

Target genes	Sequences (5'- 3')	Product size
rfbO157 F	CGTGATGATGTTGAGTTG	420 bp
rfbO157 R	AGATTGGTTGGCATTACTG	
fliCh7 F	GCGCTGTCGAGTTCTATCGAGC	625 bp
fliCh7 R	CAACGGTGACTTTATCGCCATTCC	
stx1 F	TGTAACTGGAAAGGTGGAGTATACA	210 bp
stx1 R	GCTATTCTGAGTCAACGAAAAATAAC	
stx2 F	GTTTTTCTTCGGTATCCTATTCC	484 bp
stx2 R	GATGCATCTCTGGTCATTGTATTAC	
eaeA F	ATTACCATCCACACAGACGGT	397 bp
eaeA R	ACAGCGTGGTTGGATCAACCT	
hlyA F	ACGATGTGGTTTATTCTGGA	166 bp
hlyA R	CTTCACGTCACCATACATAT	

Table 3: *E. coli* O157, *E. coli* O157:H7 and virulence genes positive in seasonally analysed fish

Marine Fish	Freshwater Fish	Isolate Code/Isolation Period	*E. coli* O157/ *E. coli* O157:H7 Positive sample		Virulence genes			
Positive sample/isolate	Positive sample/isolate		*E. coli* O157 (*rfbO157a*)	H7 *(fliCh7)*	*stx1*	*stx2*	*eaeA*	*hlyA*
	Oncorhynchus mykiss	A41-1 (Spring-March)	+	+	-	-	-	-
		S4-1 (Spring-April)	+	+	+	-	+	+
Merlangius euxmus		M24-1(Spring-March)	+	-	-	-	+	+
Trachurus mediterraneus		I27-1 (Spring-March)	+	-	-	-	-	-
	Cyprinus carpio	S28-2(Autumn-November)	+	+	-	-	-	-
		S59-1(Winter-February)	+	+	-	-	-	-
		S60-1(Winter-February)	+	+	-	-	-	-
TOTAL			**7**	**5**	**1**	**-**	**2**	**2**

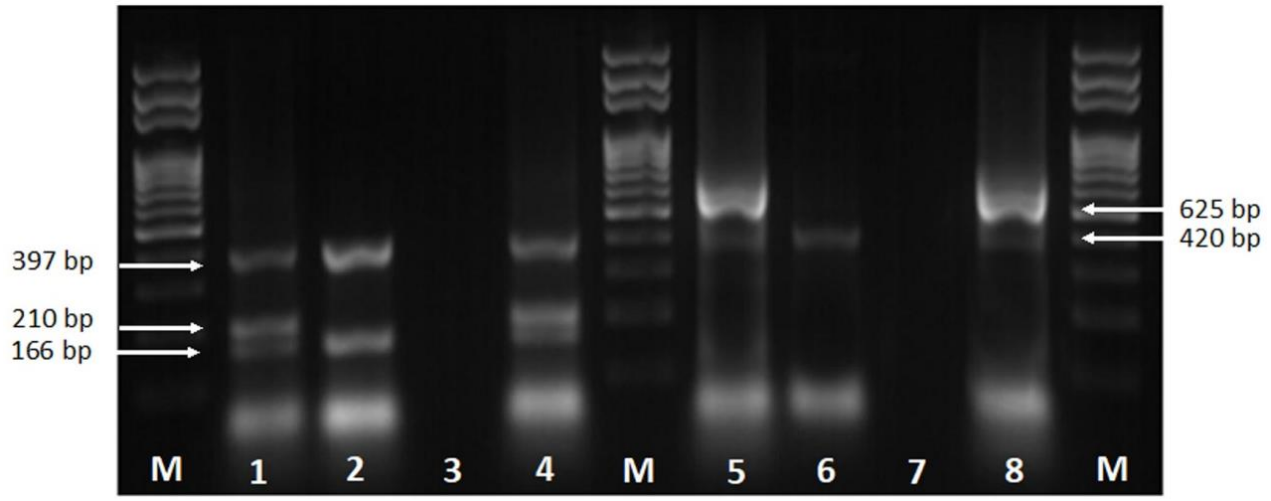

Figure 1. mPCR image of *E. coli* O157, *E. coli* O157:H7, *stx1*, *eaeA* and *hlyA* genes of isolates M: 100 bp DNA marker, **Column 1:** *eaeA*, *hlyA* and *stx1* genes of positive control isolate (*E. coli* O157:H7 ATCC 35150), **Column 2:** *Merlangius euxmus* (M24-1) isolate *eaeA* and *hlyA* positive isolate, **Column 3:** negative control (ionised water), **Column 4:** *Oncorhynchus mykiss* (S4-1) isolate *stx1*, *eaeA* and *hlyA* positive isolate, **Column 5:** *Oncorhynchus mykiss* (S4-1) isolate *E. coli* O157:H7 positive isolate, **Column 6:** *Merlangius euxmus* (M24-1) isolate *E. coli* O157 positive isolate, **Column 7**: Negative control (ionised water) **Column 8:** *E. coli* O157:H7 positive control isolate (*E. coli* O157:H7 ATCC 35150).

the gastrointestinal system (Ameer, 2016), as well as from their muscles, fins, and surfaces (Gupta et al., 2013; Marijani, 2022; Onmaz et al., 2020). In this study, samples were taken from the dorsal and tail muscle surfaces of the fish in compliance with the guidlines of ISO 6887-3.

While there have been various studies on the presence of *E. coli* in both marine and freshwater fish (Chibuike et al., 2021; Gupta et al., 2013; Marijani, 2022; Sekhar et al., 2017), there are relatively few studies that specifically investigate the *E. coli* O157 and *E. coli* O157:H7 serotypes (Ameer, 2016; Manna et al., 2008; Saad et al., 2018; Onmaz et al., 2020; Ribeiro et al., 2016; Sekhar et al., 2017).

In a study conducted by sampling both muscle and gastrointestinal tract of 140 fish samples in Türkiye (Onmaz et al. (2020), the prevelance of *E. coli* O157 was found to be 1.4% (2/140). Prakasan et al. (2022) serotyped *E. coli* in 41 fish samples in a study on fish and shrimps sold in India. *E. coli* O157 was found to be 3.44% (1/29) in 29 fish samples. Ribeiro et al. (2016) sampled muscle, surface, and gastrointestinal tract of 96 fish samples in Brazil and found 7.29% (7/96) *E. coli* O157:H7. Tilahun and Engdawork (2020) detected 2.3% (8/343) *E. coli* O157:H7 in 343 fish in Ethiopia.

In addition to these studies that detected *E. coli* O157 and *E. coli* O157:H7, there are also studies that did not detect any *E. coli* O157:H7. Saad et al. (2018) investigated the serotypes of *E. coli* in 100 fish randomly selected from sales points in Egypt, but could not detect any tract of *E. coli* O157 or *E. coli* O157:H7. Thampuran et al. (2005) collected 414 frozen and fresh fish samples for 5 years in India but did not find *E. coli* O157. Again, Manna et al. (2008) investigated the serotypes of *E. coli* in a study on 61 fish samples collected from sales points in India, but did not find any fish contaminated with *E. coli* O157. Thereupon, they stated that food contamination with *E. coli* O157 is not possible. However, in contrast to this view, Ameer (2016) collected 50 fish samples from different fish markets in

Table 4. Antibiotic resistance profile of *E. coli* O157:H7 isolate (S4-1) and *E. coli* O157 isolate with virulence gene feature and MIC values according to E-test analysis

PHENOTYPIC RESISTANCE		**Fish Species**	
Antibiotics, MIC Ranges and Resistance states		**Isolation Code/Type and Isolation Period (*E. coli* O157/H7)**	
		Oncorhynchus mykiss	***Merlangius euxmus***
(Resistant:R/Sensitive:S)		**S4-1 O157:H7 Spring- April**	**M24-1 O157 Spring- March**
Trimethoprim/sulfamethoxazole	R	-	-
MIC: 0.002-32 µg/ml	S	+	+
Tetracycline	R	MIC: 1 µg/ml	MIC: 25 µg/ml
MIC: 0.016-256 µg/ml	S	-	-
Ampicillin	R	-	-
MIC: 0.016-256 µg/ml	S	+	-
Cefotaxime	R	MIC: 2 µg/ml	MIC: 2 µg/ml
MIC: 0.002-32 µg/ml	S	-	-
Levofloxacin	R	-	MIC: 2 µg/ml
MIC: 0.002-32 µg/ml	S	+	-
cefepime	R	MIC: 8 µg/ml	MIC: 8 µg/ml
MIC:0.016-256 µg/ml	S	-	-
Ciprofloxacin	R	MIC: 0,6 µg/ml	MIC: 12 µg/ml
MIC: 0.002-32 µg/ml	S	-	-
Amoxicillin/Clavulanic acid	R	-	-
MIC: 0.016-256 µg/ml	S	+	+
Gentamicin	R	MIC: 25 µg/ml	MIC: 12 µg/ml
MIC: 0.016-256 µg/ml	S	-	-
Ertapenem	R	MIC: 1 µg/ml	MIC: 1 µg/ml
MIC: 0.002-32 µg/ml	S	-	-
İmipenem	R	MIC: 4 µg/ml	-
MIC: 0.002-32 µg/ml	S	-	+
Cefoxitin	R	MIC: 2 µg/ml	MIC: 2 µg/ml
MIC:0.016-256 µg/ml	S	-	-
ceftriaxone	R	-	-
MIC:0.016-256 µg/ml	S	+	+
Meropenem	R	-	MIC: 8 µg/ml
MIC: 0.002-32 µg/ml	S	+	-

Baghdad and found 60% (30/50) *E. coli* O157:H7 by sampling the intestines. In this study, it was aimed to investigate the effect of temperature by making a seasonal comparison, and 2.3% (7/300) *E. coli* O157 was found in the fish examined in spring, autumn and winter seasons, while 1.6% (5/300) *E. coli* O157:H7 was detected in spring and winter seasons. The data obtained as a result of this study contain similar results with most of the other studies. It is thought that such a difference between the studies varies depending on the seasonal temperatures, the proximity of the hunting environment to the settlements, the bacterial load of the water and the percentage of salt content or the lack of attention to hygiene rules in the process after the catch, the waiting time and temperature in the place where they are offered for sale.

The pathogenicity of *E. coli* O157:H7 is dependent on a number of virulence factors and studies have emphasised the 4 most important genes (*stx1*, *stx2*, *eaeA* and *hlyA*). Ribeiro et al. (2016) reported *eaeA* and *stx2* gene in 3 of the *E. coli* O157:H7 samples. Onmaz et al. (2020) reported that they found *eaeA* gene in one of the two *E. coli* O157 isolates and *stx1* gene in the other. In this study, these genes were also investigated and *eaeA* and *hlyA* genes were found in *E. coli* O157 isolate and *stx1*, *eaeA* and *hlyA* genes were found in *E. coli* O157:H7 isolate.

Antibiotic resistance has become a global issue, and research on this topic is being conducted worldwide. The consumption of contaminated foods with antibiotic-resistant bacteria can make treatment difficult and, in some cases, even impossible. In the selection of antibiotics to be investigated, preference is given to antibiotics highlighted by EUCAST as important. Ameer (2016) conducted sensitivity testing with antibiotic disks on samples in which they identified *E. coli* O157:H7. They reported high resistance to trimethoprim, moderate resistance to amikacin, erythromycin, and ceftriaxone, and susceptibility to chloramphenicol and ampicillin. Tilahun and Engdawork (2020) conducted antibiotic resistance tests on *E. coli* O157:H7 isolates and found that all samples were

susceptible to ciprofloxacin, gentamicin, trimethoprim, and sulfamethoxazole, while they showed resistance to ampicillin and cefoxitin. They also examined resistance to tetracycline, streptomycin, and doxycycline in the same samples and found isolates with varying degrees of resistance, intermediate resistance, and susceptibility. Onmaz et al. (2020) found susceptible isolates against amoxicillin/clavulanic acid, cefotaxime, gentamicin, meropenem, florfenicol, and tetracycline, susceptible isolates and resistant isolates against ciprofloxacin in the antibiotic resistance test performed on two *E. coli* O157 isolates obtained.

The antibiotics examined in our study for antibiotic resistance testing were selected based on EUCAST data and recent research findings. In this study, *E. coli* O157 (M24-1) and *E. coli* O157:H7 (S4-1) samples containing virulence genes were analysed and both isolates were resistant to tetracycline, cefotaxime, cefepime, ciprofloxacin, gentamicin, cefoxitin, and ertapenem; and sensitive to trimethoprim/sulfamethoxazole, amoxicillin/clavulanic acid, and ceftriaxone. The widespread and uncontrolled use of antibiotics in both veterinary and human medicine contributes to high antibiotic resistance rates in bacteria worldwide. Vieira et al. (2011) reported a significant relationship between antibiotic resistance properties (quinolones, fluoroquinolones, beta-lactams, and aminoglycosides) in *E. coli* isolates obtained from animal-derived foods and clinically ill humans in many European countries. The European Medicines Agency (EMA) recommends the classification of antibiotics into Category A (Avoid - groups not allowed in veterinary medicine in the European Union, such as fosfomycin or monobactams) and Category B (Restrict - groups that should be restricted in animals to reduce the risk to public health, such as quinolones, 3rd and 4th generation cephalosporins, and polymyxins) (EMA, 2020). According to this proposed classification, the high resistance rates detected in our study were found to be very important in terms of public health.

Conclusion

The presence of *E. coli* O157:H7, especially in the gastrointestinal flora of many animals, primarily ruminants, and humans, leads to its characterization as a hygiene indicator in food and environmental samples. Contamination of water sources with *E. coli* poses a water hygiene problem due to its potential to cause disease and its antibiotic resistance characteristics. The ability of *E. coli* O157 to survive in different environmental conditions and its low infectious dose are among the key factors contributing to the occurrence of water and foodborne diseases resulting from the consumption of aquatic organisms contaminated with the pathogen. Moreover, research has revealed the presence of antibiotic residues in aquatic environments, attributed to the increasing use of antibiotics in both human and veterinary medicine, which in turn contributes to the growing problem of antibiotic-resistant bacteria in the environment. Additionally, studies have reported the horizontal and vertical genetic transfer of antibiotic resistance among different bacterial species in various habitats. While the presence of *E. coli* O157 in fish has been found to be relatively low in this study and other similar studies, it highlights the potential contamination of fish by this pathogen. Furthermore, the detection of the pathogen in fish with both virulence factors and antibiotic resistance properties is of significant concern for public health.

Ethical Statement

There is no need for an ethics committee decision in this study.

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Author Contribution

Ayşegül BÖLÜKBAŞ: Writing -review and editing, Data Curation, Laboratory analysis, Material collection

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Conflict of Interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

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