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Presence and virulence characterization of *Listeria monocytogenes* from fish samples in the Black Sea, Türkiye

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Abstract: *Listeria monocytogenes,* characterized by a high mortality rate in humans, is a bacterium that causes listeriosis and is found in various aquatic products. The aim of this research was to investigate the presence, serotype distribution, virulence factor genes, and antibiotic susceptibility of *L. monocytogenes* strains isolated from a total of 500 fish samples of whiting (*Merlangius merlangus euxinus*) (n:243) and striped red mullet (*Mullus surmuletus*) (n:257) caught in the Black Sea between the years 2013-2014. Only one (0.2%) *L. monocytogenes* strain (striped red mullet) was isolated according to the cultural method (EN ISO 11290-1) and confirmed by PCR analysis. The *L. monocytogenes* strain was identified as serogroup 4b-4d-4e. Furthermore, the strain harboured *hlyA*, *inlA*, *inlC*, *inlJ*, *plcA*, *plcB*, *prfA*, *mpl*, *actA*, *monoA-B*, *flaA*, *lip 1-2a*, *fri, iap, and gtcA* genes except the *dltA* gene. On the other hand, *L. monocytogenes* strain susceptibility to ampicillin, meropenem, erythromycin, trimetophrim/sulfamethoxazole, and penicillin G was evaluated with the disc-diffusion method. According to the results, serogroup 4b-4d-4e isolated from striped red mullet was found to be unique to raw fish and susceptible to all tested antibiotics. In addition, it is considered that carrying out this research in different seas and with different fish species would be appropriate for determining the prevalence and virulence characteristics of *L. monocytogenes*.
Keywords: Antibiotic susceptibility, fish, *Listeria monocytogenes,* virulence genes.

Karadeniz kaynaklı balıklardan izole edilen *Listeria monocytogenes* suşlarının varlığı ve virülens özelliklerinin incelenmesi

Özet: *Listeria monocytogenes*, insanlarda yüksek mortalite oranı ile karakterize listeriozise neden olan bir bakteri olup, su ürünlerinde çeşitli düzeylerde bulunabilmektedir. Bu çalışmanın amacı, Karadeniz'den 2013-2014 yılları arasında avlanan toplam 500 adet Tekir (*Mullus surmuletus*) (n: 257) ve Mezgit (*Merlangius merlangus euxinus*) (n: 243) balığından izole edilen *L. monocytogenes* varlığı, serogrup dağılımı, virülens faktör genler ve antibiyotik duyarlılığını araştırmaktır. Bir adet (%0,2) *L. monocytogenes* suşu (Tekir balığı) standart kültür yöntemi (EN ISO 11290-1) ile izole edilmiş ve PCR analizi ile doğrulanmıştır. Söz konusu, *L. monocytogenes* suşu 4b-4d-4e serogrubu olarak tespit edilmiştir. Ayrıca *L. monocytogenes* suşunun *hlyA*, *inlA*, *inlC*, *inlJ*, *plcA*, *plcB*, *prfA*, *mpl*, *actA*, *monoA-B*, *flaA*, *lip 1-2a*, *fri, iap, gtcA* genlerini içerdiği, fakat *dltA* genini içermediği belirlenmiştir. Diğer taraftan, *L. monocytogenes* suşunun ampicillin, meropenem, erythromycin, trimetophrim/sulfamethoxazole ve penicillin G antibiyotiklerine karşı duyarlılığı disk diffüzyon testi ile ölçülmüştür. Elde edilen sonuçlara göre, Tekir balığından izole edilen 4b-4d-4e serogrubunun çiğ balığa özgü olduğu ve test edilen antibiyotiklerin hepsine duyarlı olduğu bulunmuştur. Ayrıca, bu araştırmanın farklı denizlerde ve farklı balık türleri ile yapılmasının *L. monocytogenes*'in prevalans ve virülens özelliklerinin belirlenmesi için uygun olacağı düşünülmektedir.
Anahtar sözcükler: Antibiyotik duyarlılığı, balık, *Listeria monocytogenes,* virülens genleri.

Introduction

Considering that the majority of fish such as anchovy, bonito, horse mackerel, whiting, striped red mullet, etc., which are mostly hunted in Türkiye, are caught in the Black Sea. It is reported that the Black Sea has a very important place in fishing. Especially in the period after 2000 and still, 70-80% of the total seafood fishing is provided from the Black Sea (43). In addition, whiting fish first level with 8,941 tons and striped red mullet fish with 2,342 tons in the production of demersal fish, which are the most hunted fish as of 2019 in Türkiye, reveals that these fish are among the fish species that are popularly consumed in Türkiye (18).

Aquaculture is an increasingly important source of food suitable for human consumption. The common seafood bacterial pathogens are *Vibrio* spp., *L. monocytogenes*, *Aeromonas hydrophila* and *Salmonella* spp. (31). During 2010-2017 in EU/EAA, the ranking of the food vehicles implicated in strong evidence of Foodborne Outbreak was as follows: first "mixed food" followed by "fish and fish products" and then "vegetables and juices" and other products such as crustaceans, shellfish, and molluscs (10). Although the contamination level tends to be low in raw fish, this has been changing in previous years (44). However, The prevalence of *Listeria* spp. was observed 30% in freshwater fish samples and 10.4% in marine fish samples in Türkiye (50). On the other hand, in aquaculture, improper and mostly use of antibiotics can increase the prevalence of antibiotic resistance of zoonotic pathogens (4).

L. monocytogenes, which causes listeriosis, is common in the environment. It has been isolated from soil, water, wastewater, faeces, food, agricultural environment, food processing plants (42). The genus *Listeria* has been recognized as having 17 species. Only two of these species, *L. monocytogenes* and *L. ivanovii,* are considered human pathogens (34). *L. monocytogenes* strains are classified into 13 serotypes. At least 95% of the strains isolated from foods and humans are serotypes 1/2a, 1/2b, 1/2c, 4b. Although human listeriosis in sporadic cases has been mostly caused by serotypes 4b, 4e, 1/2a, 1/2c; serotypes 4b, 4e commonly have been detected, lesser 1/2b (37). Further typing and characterization steps are requirement to precisely determine the virulence factors caused by *L. monocytogenes* strains.

The pathogenicity of *L. monocytogenes* depends on various virulence factors. There are many virulence genes identified in *L. monocytogenes*. Among these genes, *hlyA* is one of the essential pathogenic factors and is responsible for the escape from the phagosomes and invasion of host cells (9). The *actA* gene encodes the surface protein ActA and is associated with cell-to-cell spread (8). The internalin genes are involved in the internalization and adhesion of *L. monocytogenes* (2). The *prfA* gene regulates and controls the expression of a number of virulence genes. The pathogenicity of *L. monocytogenes* depends mainly on the *prfA* virulence gene cluster (9).

The aim of the study was to determine the occurrence, virulence properties (*hlyA*, *inlA*, *inlC*, *inlJ*, *plcA*, *plcB*, *prfA*, *mpl*, *actA*, *monoA-B*, *flaA*, *lip 1-2a*, *fri, gtcA*, *dltA*, *iap*), serotyping (*Imo0737, Imo1118, ORF2819, ORF2110)* and antimicrobial susceptibility of *L. monocytogenes* isolated from fishes caught in the Black Sea in Türkiye.

Materials and Methods

Sampling: A total of 500 fish samples were collected from December 2013 to May 2014 from Kumkapı Commercial fish collection area, Istanbul. These fishes originated from the selected areas of the Black Sea in Türkiye (Figure 1). The fishes were brought to the commercial fish collection area after hunting from the Black Sea. Tested fresh fish samples (n=500) were striped red mullet (257 samples), whiting (243 samples) (Table 1). Each sample consisted of one fish. All samples were transported to the laboratory in sterile plastic bags (one sample per bag) in a thermos box at 4°C and analysed immediately.

Table 1. Distribution of fish caught in coded coordinate regions of the Black Sea in 2013-2014 fishing season by months**.**

Years	**2013**		**2014**								
Months	**December**		**January**		**February**		**March**		**April**		**May**
Type of Fishes	**[a]SRM**	**[b]Wh**	**SRM**	**Wh**	**SRM**	**Wh**	**SRM**	**Wh**	**SRM**	**Wh**	**SRM**
Coordinate areas and numbers of collected fish samples	[c]K-1 (10)	K-1 (10)	K-1 (20)	K-1 (24)	K-1 (18)	K-1 (22)	K-1 (65)	K-1 (80)	K-1 (27)	K-1 (24)	K-3 (9)
	K-3 (3)	K-3 (3)	K-3 (2)	K-3 (6)	K-3 (4)	K-3 (4)	K-3 (18)	K-3 (17)	K-3 (4)	K-3 (7)	K-11 (9)
	K-5 (2)	K-5 (2)	K-11 (6)	K-11 (10)	[d]K-9 (3)	K-9 (3)	K-9 (8)	K-7 (4)	K-9 (8)	K-9 (4)	K-15 (10)
	K-9 (4)	K-11 (1)	K-15 (2)		K-11 (10)	K-11 (14)	K-11 (4)		K-11 (4)	K-11 (8)	K-25 (2)
	K-11 (1)										
	K-19 (4)										

[a.]SRM, Striped Red Mullet; [b.]Wh, Whiting; [c.]K, Coordinate Area; [d.]K-9, The area where the fish isolated from *Listeria monocytogenes* was hunted

Isolation and identification of L. monocytogenes: The samples were analysed for detection of *L. monocytogenes* according to the EN ISO 11290-1 (19). Twenty-five grams of each fish samples (skin, gill and intestine) was transferred to 225 ml of Half Fraser Broth (Oxoid CM 895). And the suspension was incubated at 30°C for 24 h. Then 100 µl of the culture was transferred to 10 ml of Fraser Broth (Oxoid CM 895) and performed at 37°C for 48 h. An aliquot of 10 µl from the Fraser Broth was spread on the surface of an ALOA agar (Oxoid CM 1084) plate using a sterile loop. The ALOA plates were incubated at 37°C for 24 – 48 h. A sample was considered positive if there were one or more typical colonies (blue-green with an opaque halo) on ALOA agar plates.

The single colony isolate was purified by streaking onto ALOA agar plates and incubated at 37°C for 48 h. Following this, a single colony was subcultured on Tryptic Soy Agar (Oxoid CM 131) plate and incubated at 37°C for 24 h. The culture was transferred with a loop in Tryptic Soy Broth (Oxoid CM 129) and incubated at 37°C for 18 h. This culture was frozen in 20% glycerol containing 1 ml of Tryptic Soy Broth first at -20°C and then at -80°C (stepwise to prevent shock).

***Confirmation of isolates as L. monocytogenes and molecular serotyping with PCR*:** DNA was prepared using the Liu et al. (25) method. *monoA* and *monoB* primers were used for the specific identification by conventional PCR of all serotypes of *L. monocytogenes* and the PCR program was set as follows: initial denaturation 1 cycle of 94°C for 5 min; annealing 35 cycles of 95°C for 1 min, 53°C for 45 s, and 72°C for 1 min; extending 72°C for 7 min and 10°C limitless (3).

The conventional PCR technique was used to determine the main *L. monocytogenes* serogroups (1/2a-3a; 1/2b-3b; 1/2c-3c; 4b-4d-4e) (7). Additionally, PCR was designed to identify the *Imo0737*, *Imo1118*, *ORF2819*, and *ORF2110* genes. The procedure was performed according to following conditions: initial denaturation 1 cycle of 94°C for 3 min; annealing 35 cycles of 94°C for 0.40 min, 53°C for 75 s and 72°C for 75 s; extending 72°C for 7 min and 10°C limitless.

Imo0737 (691bp) 5'- AGGGCTTCAAGGACTTA CCC-3' ve 5'-ACGATTTCTGCTTGCCATTC-3',

Imo1118 (906bp) 5'- AGGGGTCTTAAATCCTG GAA-3' ve 5'- CGGCTTGTTCGGCATACTTA-3',

ORF2819 (471bp) 5'-, AGCAAAATGCCAAAACT CGT-3' ve 5'- CATCACTAAAGCCTCCCATTG-3',

ORF2110 (597bp) 5'- AGTGGACAATTGATTGG TGAA-3' ve 5'- CATCCATCCCTTACTTTGGAC-3'

***Identification of virulence genes*:** PCR technique was designed to identify the presence of virulence genes in *L. monocytogenes.* The mixture contained: 5 µl DNA, 3 µl $MgCl_2$ (25 mM) (Thermo), 1 µl reverse primer (10 µM), 5 µl dNTP (Thermo), 5 µl 10X buffer KCL (Thermo), 1 U (0.24 µl) Taq DNA polymerase (Thermo), 1 µl forward primer (10 µM) and dH_2O. The total volume was 50 µl. PCR reactions were performed using obtained genomic DNA. The primer sequences and amplification conditions were presented in Table 2. The electrophoretic separation of PCR products was carried out in 1-1.5% agarose gel.

Table 2. Virulence genes and primers sequences.

Virulence genes (bp)	Primer sequences (5'-3')	References
prfA (571)	GGTATCACAAAGCTCACGAG CCCAAGTAGCAGGACATGCTAA	(33)
mpl (1473)	GGCTCATTTCACTATGACGG GCTTCCCAAGCTTCAGCAACT	(33)
plcA (129)	CGAGCAAAACAGCAACGATA CCGCGGACATCTTTTAATGT	(24)
plcB (261)	GGGAAATTTGACACAGCGTT ATTTTCGGGTAGTCCGCTTT	(47)
hlyA (234)	CGGAGGTTCCGCAAAAGATG CCTCCAGAGTGATCGATGTT	(30)
actA (268 or 385)	GACGAAAATCCCGAAGTGAA CTAGCGAAGGTGCTGTTTCC	(21)
inlA (800)	ACGAGTAACGGGACAAATGC CCCGACAGTGGTGCTAGATT	(26)
inlC (517)	AATTCCCACAGGACACAACC CGGGAATGCAATTTTTCACTA	(26)
inlJ (238)	TGTAACCCCGCTTACACAGTT AGCGGCTTGGCAGTCTAATA	(26)
Lip1-2a (274)	GATACAGAAACATCGGTTGGC GTGTAACTTGATGCCATCAGG	(5)
iap (453)	GGGCTTTATCCATAAAATA TTGGAAGAACCTTGATTA	(40)
gtcA (251)	TGGGTTACTACAAGAAGAG AGTACTGATGCGATAAAAGCA	(35)
dltA (1000)	AAGTAGTGCAGTTTAGGAGAGGA AGATTGTACCACCGGATGTC	(23)
fri (471)	ATGAAAACAATCAACTCAGT CTACTCTAATGGAGCTTTT	(40)
flaA (864)	ATGAAAGTAAATACTAATATC TTAGCTGTTAATTAATTGAGT	(40)

***Antibiotic susceptibility testing*:** The antibiotic susceptibility of isolated *L. monocytogenes* strains was performed by the disc-diffusion method (13). Suspension of isolated *L. monocytogenes* strains was prepared according to the optical density of 0.5 in MacFarland standard in 0.9% saline solution. The suspension was performed on the Mueller Hinton Agar (Oxoid CM 337) plate with 5% defibrinated horse blood and 20 mg/L β-Nicotinamide Adenine Dinucleotide (Biolab Inc., NAD10025) (14) and then ampicillin (CT0002B, 2 µg), penicillin G (CT0152B, IU), erythromycin (CT0020B, 15 µg), meropenem (CT0774B, 10 µg), trimethoprim-

sulfamethoxazole (co-trimoxazole) (CT0052B, 25 µg) antibiotic discs (Oxoid) were included. The plate was incubated at 35±1°C in 5% CO_2 in air for 16-20 h. *Streptococcus pneumoniae* ATCC 46919 was used as quality control strains. Then inhibition zones around antibiotic discs were analyzed according to EUCAST (2018) Version 8.0 (15). Minimum inhibitory concentrations were determined using E-test (BioDisc) and MICE strips (Thermo) (Figure 8).

Results

***Isolation of L. monocytogenes strain*:** One *L. monocytogenes* strain was isolated from only one (1/500, 0.2%) fish sample. The fish sample belonged to the striped red mullet (1/257, 0.3%) originated from the K-9 area of The Black Sea (Figure 1).

***Confirmation and Serotyping*:** The identification of *L. monocytogenes* was performed by PCR amplification of the *monoA-B* gene (3). The strain possessed *monoA-B* gene (Figure 2). According to the results of PCR, the strain was identified as *L. monocytogenes*.

The strain harboured *Imo0737* (Figure 3) and *Imo1118* genes (Figure 4), except *ORF2110* (Figure 5) and *ORF2819* genes (Figure 6). Therefore, the strain was classified as serogroup 4b-4d-4e.

***Presence of selected virulence genes*:** The strain possessed the *hlyA*, *inlA*, *inlC*, *inlJ*, *plcA*, *plcB*, *prfA*, *mpl*, *actA*, *flaA*, *lip 1-2a*, *fri, gtcA*, and *iap* genes (Figure 7). But it did not have the *dltA* gene.

***Antibiotic susceptibility*:** The *L. monocytogenes* isolate was all susceptible to ampicillin, penicillin G, erythromycin, meropenem, and trimethoprim-sulfamethoxazole.

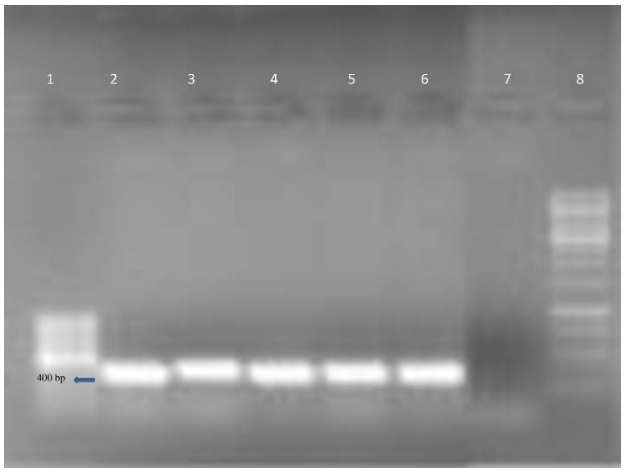

Figure 2. PCR gel vision of *monoA-B* (400 bp) gene.
From left to right: 1. 100 bp DNA Marker; 2. 186; 3. ATCC 7644; 4. ATCC 19111; 5. ATCC 19115; 6. ATCC 13932; 7. negative control (sterile ultra pure water); 8. 1 kb DNA Marker.

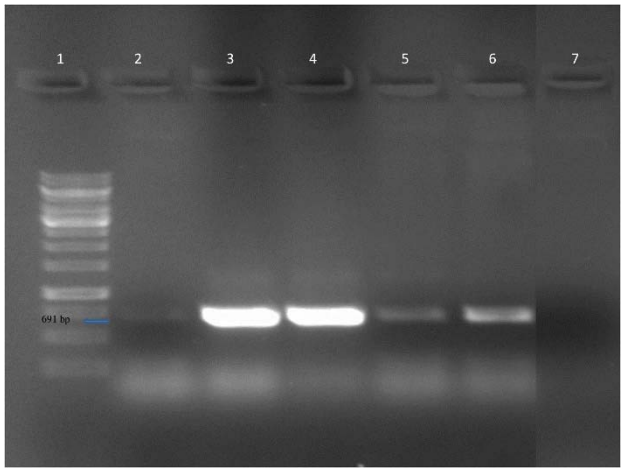

Figure 3. PCR gel vision of *Imo0737* (691 bp) gene.
From left to right: 1. 1 kb DNA Marker; 2. 186; 3. ATCC 7644; 4. ATCC 19111; 5. ATCC 19115; 6. ATCC 13932; 7. negative control (sterile ultra pure water).

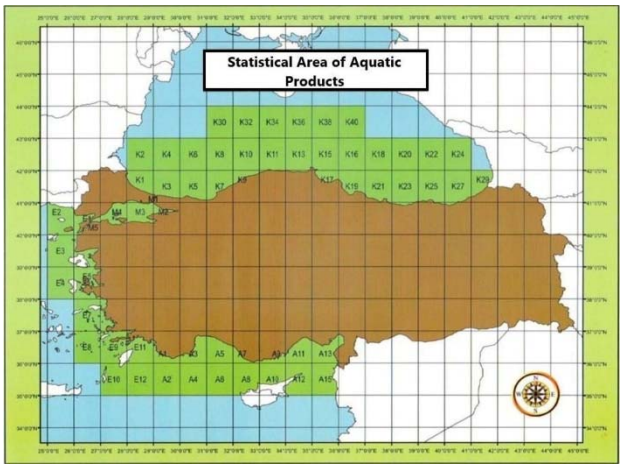

Figure 1. Statistical area of aquatic products (17).

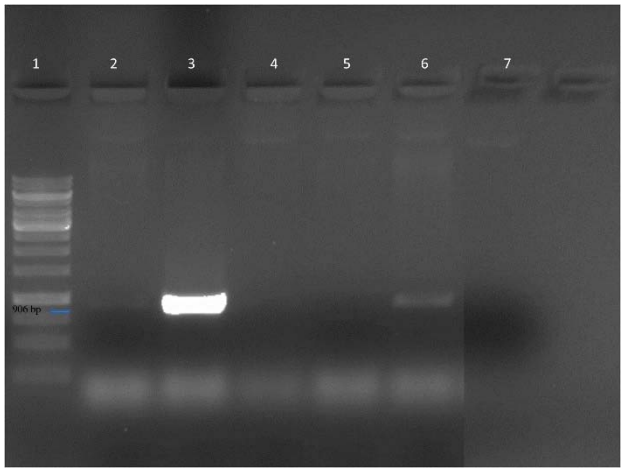

Figure 4. PCR gel vision of *Imo1118* (906 bp) gene.
From left to right: 1. 1 kb DNA Marker; 2. 186; 3. ATCC 7644; 4. ATCC 19111; 5. ATCC 19115; 6. ATCC 13932; 7. negative control (sterile ultra pure water).

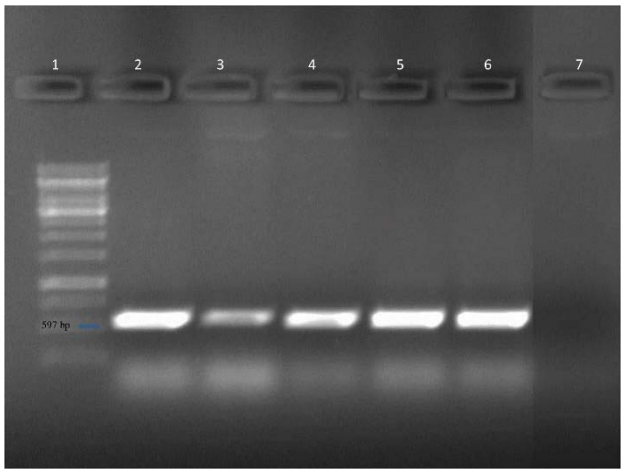

Figure 5. PCR gel vision of *ORF2110* (597 bp) gene.
From left to right: 1. 1 kb DNA Marker; 2. 186; 3. ATCC 7644; 4. ATCC 19111; 5. ATCC 19115; 6. ATCC 13932; 7. negative control (sterile ultra pure water).

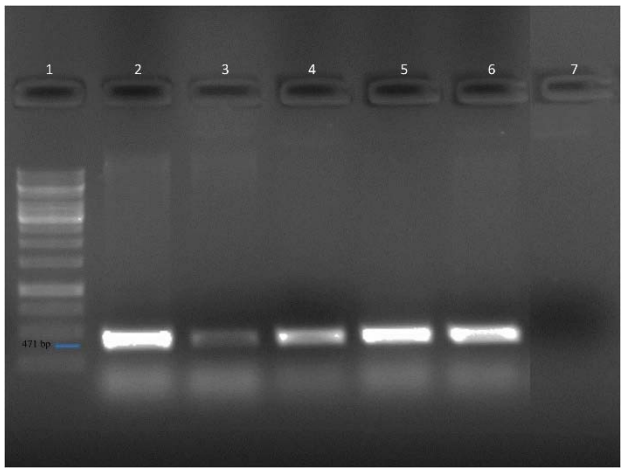

Figure 6. PCR gel vision of *ORF2819* (471 bp) gene.
From left to right: 1. 1 kb DNA Marker; 2. 186; 3. ATCC 7644; 4. ATCC 19111; 5. ATCC 19115; 6. ATCC 13932; 7. negative control (sterile ultra pure water).

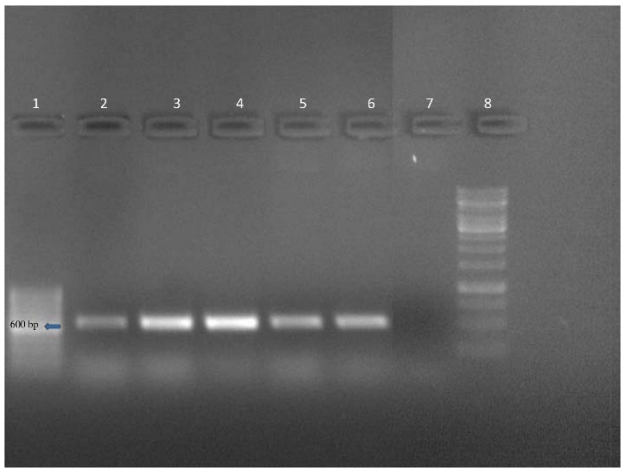

Figure 7. PCR gel vision of *prfA* (600 bp) gene.
From left to right: 1. 100 kb DNA Marker; 2. 186; 3. ATCC 7644; 4. ATCC 19111; 5. ATCC 19115; 6. ATCC 13932; 7. negative control (sterile ultra pure water); 8. 1 kb DNA Marker.

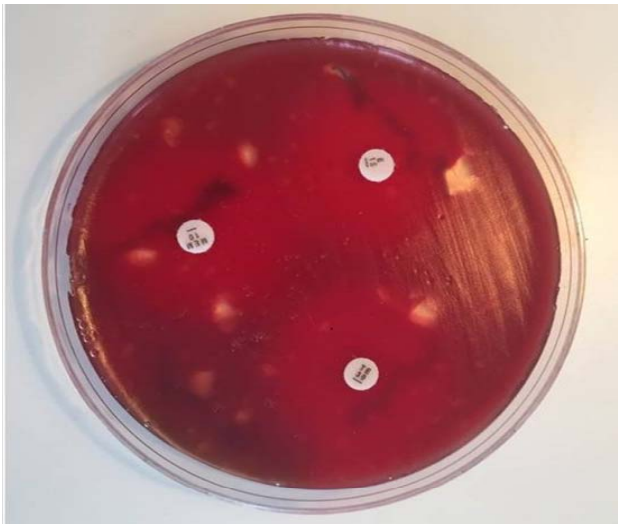

Figure 8. Antibiotic disc-diffusion test on Mueller-Hinton Agar with 5% defibrinated horse blood.

Discussion and Conclusion

L. monocytogenes is a ubiquitous bacterium isolated from freshwater and coastal water. In contrast, it is rarely isolated from deep seawater. Environmental conditions, such as waterfall, can affect the number of *Listeria* in the water, and as a result, *Listeria* may be present on the skin surface of fish and in aquaculture in the natural environment (44). In the study, *L. monocytogenes* was isolated from only one sample (0.2%) of the 500 fish samples. The strain belonged to the fish sample originated from the K-9 area in February 2014.

The quantities of *L. monocytogenes* in fish are highly determined by the amount of *L. monocytogenes* of water. Therefore, there are numerous studies conducted on seawater. In a study by El Marrakchi et al. (11) in seawater, it was shown that five out of 161 tested seawater samples were contaminated with *L. monocytogenes*. In Mexica, in an investigation of seawater 12 out of 144 samples were positive for these bacteria (36).

A high prevalence of *L. monocytogenes* in fresh fishes and sea fishes has been reported in previous studies. High percentage of contaminated fishes was detected, e.g. 8.8% (28 out of 317) in Estonia (22), 7.6% (37 out of 488) in Iran (20). In previous investigations in Türkiye, 6.6% of samples of freshwater fish (n=150) (12), 4% of samples of fresh fish (n=100) (1) were contaminated by these bacteria. Our results were consistent with other investigations (6, 32). Siriken et al. (38) isolated *L. monocytogenes* from one of 50 samples of raw anchovy (*Engraulis encrasicolus*) collected from the retailers and small-scale producers from the Black Sea region of Türkiye. In Greece, Soultos et al. (41) isolated *L. monocytogenes* from one of 120 marine fish samples. Thirty of these samples were whiting. Similarly, *L. monocytogenes* was not isolated from whiting samples examined in our study.

Marian et al (29) did not detect L. monocytogenes in fish samples. Similarly, Yucel and Balci (50) showed that none of the skin and gill samples of marine fish (n=48) was positive for *L. monocytogenes* in Türkiye.

In our study, the *L. monocytogenes* strain had *ORF1118* and *ORF2819* genes. The obtained results showed that the strain belonged to serogroup 4b-4d-4e. Serogroup 4b-4d-4e was isolated from a striped red mullet sample collected in February 2014. Striped red mullet is a fish that lives in demersal water in the Black Sea. *L. monocytogenes* determine lesser in fresh fishes that live in in-depth seas (44).

Ruminants faeces on surface waters flowing from agricultural and urban areas are identified as the potential source of *L. monocytogenes* (28). In our study, *L. monocytogenes* strain was isolated from the fish sample that originated from the K-9 area. This area contains Bartın Harbor and the place where the Bartın river flows into the Black Sea. The isolation of *L. monocytogenes* strain from a fish sample collected from this area can give information about the level of contamination of water in the area. In this context, there are publications about the pollution and microbial load of the Bartın River (45).

In the current study, the *L. monocytogenes* strain had *hlyA*, *inlA*, *inlC*, *inlJ*, *plcA*, *plcB*, *prfA*, *mpl*, *actA*, *monoA-B*, *flaA*, *Lip1-2a*, *fri, gtcA* and *iap* genes, except *dltA* gene. Wieczorek and Osek (49) and Skowron et al. (39) found virulence genes (*hlyA*, *plcA*, *plcB*, *iap*, *inlB*, *actA* and *prfA* genes) in *L. monocytogenes* strains obtained from the fish samples. These studies showed that *L. monocytogenes* strains obtained from fishes could be a threat to public health.

Most infections caused by *L. monocytogenes* are due to serotypes 1/2a, 4b, 1/2b. Whereas, serotype 1/2c is rarely found in clinical cases (27). On the other hand, in the present study, the serogroup of *L. monocytogenes* strain isolated from the fish sample was classified as 4b-4d-4e by PCR.

The serogroup 4b-4d-4e in different countries was observed in previous investigations of fish (46, 48). Whereas, serotype 1/2a was predominantly found in several countries (22, 39, 49). Fallah et al. (16) observed that serotype 4b was predominantly at cold conditions, although serotype 1/2a was predominantly at warm conditions. According to the reporting result, the growing ability of serotype 4b is better than 1/2a at cold conditions (16). Similarly, in our study, serogroup 4b-4d-4e was isolated from striped red mullet collected in winter (February 2014).

Jamali et al. (20) observed that 43 strains of *L. monocytogenes* were isolated from 488 raw fish samples and 374 swab samples. In this study, serotype 1/2a was predominant, but only serotype 4b was isolated from raw fish samples. Similarly, our study was carried out in raw fish samples and the strain was identified as serogroup 4b-4d-4e. In Türkiye, *L. monocytogenes* was isolated from eight out of 50 salted anchovies samples, 50 raw anchovies and 50 raw mussels collected from the retailers and small-scale producers from the Black Sea region. Serotype 1/2b (3b) was detected in six salted anchovies and a fish sample. On the other hand, Serotype 1/2b (3b) and 4b (4d or 4e) were obtained together from a raw mussel sample (38).

In most studies, isolated *L. monocytogenes* strains showed resistance to tested antibiotics at different rates (20, 49). In the Skowron et al. (39) study on fish and swab samples, serogroup 1/2a-3a among 70 *L. monocytogenes* strains was highly resistant to penicillin (44.4%). Furthermore, serogroup 1/2c-3c to ampicillin (30%), serogroup 1/2c-3c to erythromycin (60%), serogroup 1/2b-3b to trimethoprim/sulfamethoxazole (52.2%), serogroup 1/2c-3c to meropenem (40%) were highly resistant. Serogroup 4b-4d-4e was most susceptible among serogroups. Similarly, in our study serogroup 4b-4d-4e was susceptible to all tested (penicillin, ampicillin, meropenem, erythromycin, and trimethoprim-sulfamethoxazole) antibiotics.

In the present investigation, the low number of *L. monocytogenes* strains in tested fish samples is substantial for public health. The *L. monocytogenes* strain was isolated from a fish sample that originated from the K-9 area. This area contains Bartın Harbor and polluted Bartın river. In this context, the presence of *L. monocytogenes* in the area indicates that the microbiological quality of the water in the area should be low. On the other hand, it is consistent with our findings that *L. monocytogenes* serogroup 4b-4d-4e is mostly isolated from raw fish collected during the cold season. Furthermore, the present strain in our study was susceptible to all tested antibiotics, similar to other studies. This result obtained in terms of Public Health of Türkiye; showed that fish samples that were caught from the Black Sea and sampling directly from the boat were largely safe in terms of antibiotic resistant aquatic *L. monocytogenes* strains. However, to reliably determine antibiotic susceptibility and the presence of *L. monocytogenes* in fish, it is significant to carry out studies on different fish species in other seas in the future.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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