

## The Isolation of *Lactococcus garvieae* from Eyes of Diseased Rainbow Trout (*Oncorhynchus mykiss*) with Exophthalmia

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**Abstract:** In this study, the diseased fish samples were collected from fish farms in the nine different dam lakes during the summer months between July 2014 and September 2018. It is isolated twenty-two bacteria from diseased rainbow trout (*Oncorhynchus mykiss*) eyes showing exophthalmos symptoms. All isolates identification was carried out by using conventional biochemical tests, API 20 Strep test kit and PCR test. ATCC 43921 reference strain of the *Lactococcus garvieae* was used as a positive control. The PCR test was confirmed by using the pLG-1 and pLG-2 reference genes (16S rRNA) specific for the *L. garvieae* species. As a result of this study, all of the twenty-two isolates isolated from the eyes of diseased rainbow trout were confirmed as *L. garvieae* by PCR test. According to the results of antibiotic susceptibility test, all of the *L. garvieae* isolates were resistant to streptomycin, sulfamethoxazole, and sulfamethoxazole/trimethoprim. The same isolates have been determined to be resistant to ampicillin (94.2%), oxytetracycline (72.5%), erythromycin (55.07%), oxolinic acid (43.5%), enrofloxacin (26.09%), doxycycline (20.3%), amoxicillin and florfenicol (8,7%) as well. Thus, florfenicol, amoxicillin, doxycycline, and enrofloxacin were found as the most effective antibiotics.

**Anahtar sözcükler:** *Lactococcus garvieae*, API 20 strep, 16S rRNA, PCR, antimicrobial agents.

## Ekzoftalmuslu Gökkuşuğu Alabalıklar (*Oncorhynchus mykiss*)'ın Gözlerinden *Lactococcus garvieae*'nin İzolasyonu

**Öz:** Bu çalışmada, Temmuz 2014-Eylül 2018 yaz ayları süresince baraj göllerindeki dokuz farklı balık çiftliğinden hastalıklı balık örnekleri toplanmıştır. Ekzoftalmus semptomları gösteren hastalıklı gökkuşuğu alabalığının (*Oncorhynchus mykiss*) gözlerinden yirmi dört bakteri izole edilmiştir. Tüm izolat tanımlamaları, geleneksel biyokimyasal testler, API 20 strep test kiti ve PZR testi kullanılarak yapıldı. *Lactococcus garvieae*'nin ATCC 43921 referans suşu pozitif kontrol olarak kullanılmıştır. PZR testi, *L. garvieae* türleri için spesifik pLG-1 ve pLG-2 referans genleri (16S rRNA) kullanılarak doğrulandı. Bu çalışma sonucunda, hastalıklı gökkuşuğu alabalığının gözlerinden izole edilen 22 izolatin tamamı PZR testi ile *Lactococcus garvieae* olarak doğrulanmıştır. Antibiyotik duyarlılık testi sonuçlarına göre, tüm *L. garvieae* izolatlarının streptomisin, sulfametoksazol ve sulfametoksazol/trimetoprim'e dirençli olduğu tespit edilmiştir. Aynı izolatların ampisiline %94,2, oksitetrasikline %72,5, eritromisine %55,07, oksolinik asite %43,5, enrofloksasine %26,09, doksisisiklin %20,3, amoksisilin ve florfenikole %8,7 oranında dirençli oldukları belirlenmiştir. Bu çalışmanın sonuçlarına göre en etkili antibiyotikler olarak florfenikol, amoksisilin, doksisisiklin ve enrofloksasin bulunmuştur.

**Keywords:** *Lactococcus garvieae*, API 20 strep, 16S rRNA, PCR, antimikrobiyal ajanlar.

## INTRODUCTION

*Lactococcus garvieae* is a species of the genus *Lactococcus*, which is of clinical importance in humans, vertebrates and fish. *L. garvieae* is responsible for mastitis in cows and buffalos, and it has been isolated from clinical specimens of human blood, urine and skin ulcer (Vale et al., 2000).

Lactococcosis is a worldwide septicemic fish disease characterized by bilateral exophthalmia. It has been reported that *Lactococcus garvieae* was a causative agent of disease of the yellowtail (*Seriola quinqueradiata*), eels (*Anguilla anguilla*), rainbow trout (*Oncorhynchus mykiss*), tilapia (*Oreochromis* sp.), kingfish (*Seriola lalandi*), mullet (*Mugil cephalus*) and giant freshwater prawn (*Macrobrachium rosenbergii*) (Austin & Austin, 1999; Kusuda & Salati, 1999). *L. garvieae* has been reported from other homoeothermic and poikilothermic animals such as cows, buffalos, cats and dogs. *L. garvieae* is also a zoonotic pathogen, occurring rarely and with a low virulence in human infections. In summer, when the water temperature increased, above 20°C, the infections occurred in fish farms (Tsai et al., 2013).

There appears to be pronounced variation in disease signs, including exophthalmia and distended abdomen (these are also common features with bacterial kidney disease), hemorrhaging in the eye (this is a characteristic of enteric red mouth), hemorrhaging at the opercula, fins, surface (this could be confused with vibriosis), and darkening of the skin. Moribund fish swim erratically just below the surface of the water. It has been reported that lactococcosis is a hyperacute systemic disease that occurs suddenly in rainbow trout and causes bleeding in the eyes (Austin & Austin, 1999; 2016) and bilateral exophthalmos (Ture & Cimagil, 2018). Marine fish were harmed in internal organs (liver, kidney, spleen and intestine), and there was full a content of ascetic fluid in the intraperitoneal region. Marine fish is present a pronounced enteritis, pale livers, and blood in the peritoneal cavity (Austin and Austin, 1999; 2007; 2016).

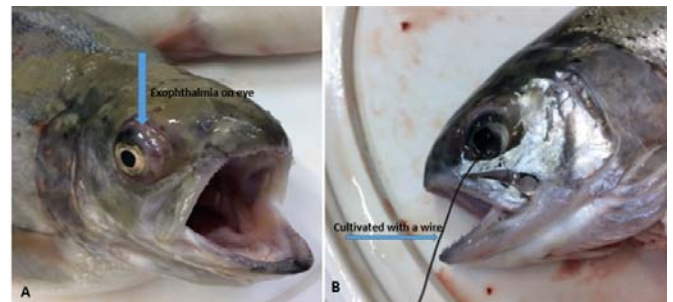
In previous studies, it was reported that *Lactococcus garvieae* was isolated from the internal organs (kidney, spleen, liver and heart) of infected fish (Cagırgan & Tanrikul, 1995; Diler et al., 2002; Didinen et al., 2014; Ture & Cimagil, 2018), but there has not been any report that was isolated from infected rainbow trout' eyes and symptom of exophthalmos. Thus, the aim of this study is to determine the phenotypic, biochemical and genetic characterization of the disease agents showing only the symptoms of exophthalmos, which cause deaths between 10% and 80% in trout farms in the region between 2014 and 2018.

## MATERIAL and METHOD

### Bacterial isolation from eyes of the rainbow trout:

Fish samples were collected from the disease cases during the summer months when the water temperature was high in the dam lakes in the Eastern Black Sea Region. This study was

carried out on nine different rainbow trout farms between July 2014 and September 2018. *L. garvieae* isolates were collected from diseased fish showing exophthalmos symptoms from trout farms in different geographic areas (Artvin, Bayburt, Erzurum, Gümüşhane, Sivas). Bacteria were isolated on tryptic soy agar (TSA, Merck) and tryptic soy broth (TSB Merck) from eyes and kidney of the diseased rainbow trout with exophthalmia symptoms (Figure 1). Twenty-two Gram positive (Table 1) and two Gram negative bacteria were isolated from eyes of the diseased rainbow trout. All isolates were stored in 20% glycerol containing TSB, at -80°C. For analyses, they were inoculated on TSA and incubated at 25°C for 24 h.



**Figure 1.** Cultivated with a wire on TSA for bacterial isolation from with exophthalmia eyes of the rainbow trout (A, B).

**Table 1.** Data on the *L. garvieae* isolates from rainbow trout eyes analyzed in this study.

No	Location	Dates	No	Location	Dates
1	Artvin/Borçka Dam	2014	12	Sivas/Suşehri Dam	2016
2	Gümüşhane/Kürtün Dam	2014	13	Gümüşhane/Kürtün Dam	2016
3	Gümüşhane/Torul Dam	2014	14	Sivas/Suşehri Dam	2016
4	Sivas/Suşehri Dam	2014	15	Artvin/Borçka Dam	2016
5	Artvin/Borçka Dam	2014	16	Artvin/Borçka Dam	2017
6	Erzurum/Kuzgun Dam	2015	17	Gümüşhane/Kürtün Dam	2017
7	Gümüşhane/Torul Dam	2015	18	Artvin/Borçka Dam	2017
8	Gümüşhane/Kürtün Dam	2015	19	Sivas/Suşehri Dam	2017
9	Sivas/Suşehri Dam	2015	20	Artvin/Borçka Dam	2018
10	Artvin/Borçka Dam	2015	21	Erzurum/Kuzgun Dam	2018
11	Artvin/Borçka Dam	2015	22	Bayburt/Demirözü Dam	2018

**Bacterial identification:** Biochemical tests were performed with conventional tests and Gram positive bacteria with the rapid API 20 strep (bioMe'rieux, France) and Gram negative bacteria with API 20 NE kit systems. The api 20 strep test kits were incubated at 25°C for 48h. The API 20 strep wells were dropped test reagents after incubation 24h. In this study, *Lactococcus garvieae* ATCC 43921 isolate was used as the reference strain. *L. garvieae* ATCC 43921 was also kindly provided by A. Kubilay (Süleyman Demirel University, Isparta, Turkey). Specific primers to identify *L. garvieae*, pLG-1 (5'-CATAACAATGAGAATCGC-3') and pLG-2 (5'-GCACCCTCGGGTTG-3') were used (Collins et al. 1989).

**PCR protocol:** PCR amplification of *L. garvieae* was performed according to Zlotkin et al., (1998). The DNA of *L. garvieae* was acquired from the cells which were produced in 25°C for 16 hours by passaging to the TSB from pure colonies on the blood agar. The DNA of the bacteria

was removed according to the procedure using the Promega mini genomic DNA purification kit. The PCR cycling conditions were as follows; a denaturation step at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 43°C for 45 sec and extension at 72°C for 1 min, ending with a 10 min final extension step at 72°C. All PCR products were run on electrophoresis in 1% agarose gel containing ethidium bromide and visualized by UV trans illuminator. *L. garvieae* ATCC 43921 and *Aeromonas hydrophila* were used as a positive and negative controls, respectively. The PCR product anticipated size of the DNA obtained from *L. garvieae* was 1100 bp.

**Antimicrobial sensitivity test:** Antimicrobial susceptibility tests of the *L. garvieae* isolates were determined by the standard disk diffusion method on Müller-Hilton agar (Merck) plates, by using the eleven antibiotics. The plates were incubated at 25°C for 20 h. The following antibiotic disks (Oxoid) were used: oxytetracycline, doxycycline, oxolinic acid, sulfamethoxazole, ampicillin, amoxiclin, florfenicol, streptomycin, enrofloxacin, erythromycin and sulfamethoxazole/trimethoprim. The antibiotic disks were placed on the Müller-Hilton agar by using a disc dispenser. Reference strain *Staphylococcus aureus* was used as quality a control in the antimicrobial susceptibility tests in Table 2 (CLSI 2008, 2013).

**Table 2.** Antimicrobial susceptibility test breakpoints used in the study.

Disc Concentration	Zone Diameter Interpretive Standards (mm)			References
	R	I	S	
T-30 µg	≤ 14	15-18	≥ 19	CLSI, 2008
OA-2 µg	≤ 10	11-12	≥ 13	CLSI, 2008
SMZ 100 µg	≤ 12	13-16	≥ 17	CLSI, 2013
AM-10 µg	≤ 13	14-16	≥ 17	CLSI, 2013
FFC-30 µg	≤ 14	15-18	≥ 19	CLSI, 2008
S-10 µg	≤ 11	12-14	≥ 15	CLSI, 2013
ENR-5 µg	≤ 16	17-20	≥ 21	CLSI, 2008
E-15 µg	≤ 13	14-22	≥ 23	CLSI, 2008
D-30 µg	≤ 10	11-13	≥ 14	CLSI, 2013
AX-10 µg	≤ 13	14-17	≥ 18	CLSI, 2013
SXT-25 µg	≤ 10	11-15	≥ 16	CLSI, 2008

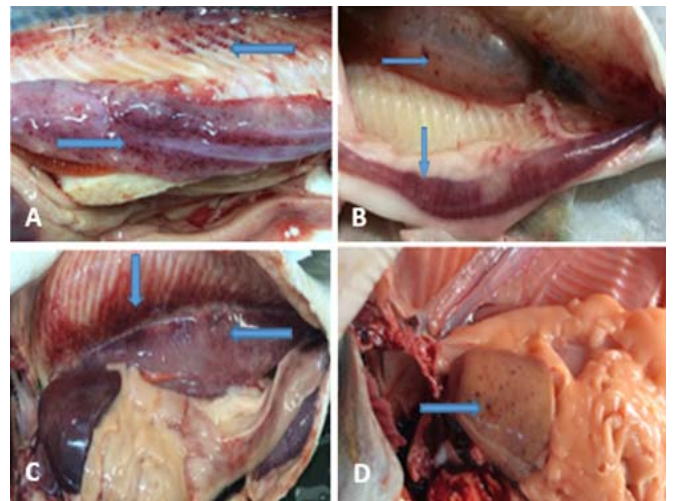
T: Oxytetracycline, OA: Oxolinic acid, SMZ: Sulfamethoxazole, AM: Ampicillin, FFC: Florfenicol, S: Streptomycin, ENR: Enrofloxacin, E: Erythromycin, DO: Doxycycline, AX: Amoxicillin, SXT: Sulfamethoxazole-Trimethoprim.

## RESULTS

**Clinical symptoms:** Fish presented clinical symptoms such as lethargy, anorexia, darkening of the skin, erratic swimming, pronounced mono or bilateral exophthalmia (Figure 2), hemorrhages in the ocular zone and fallen eyeball, perianal area, fins, petechial hemorrhaging in the air bladder, petechial hemorrhages and congestion on the liver and pale liver, and congestion of the intestine, a characteristic hemorrhagic enteritis, and anal prolapses (Figure 3). Liver and spleen were enlarged. Petechial and focal hemorrhages were seen in liver, adipose tissue, pyloric cecum and muscle.



**Figure 2.** Darkening on the skin (A, B), pronounced exophthalmos (A,B,C) and bleeding in the eyes (A, D) (Original).



**Figure 3.** The petechial hemorrhaging in the air bladder and congestion on the peritoneum (A, C), The congestion in the gut and petechial hemorrhaging on the air bladder (B), The petechial hemorrhaging on the liver and pale liver (D) (Original).

**Biochemical, cultural and physiological characteristics of *L. garvieae*:** *L. garvieae* is a bacterium occurring gram positive cocci. *L. garvieae* isolates were usually seen in the microscopic field individually, in the form of ovoid pairs or short chains (Figure 4). All of the strains in this study was detected to be produce  $\alpha$ -hemolysis on blood agar (BA). According to the results of the API 20 strep test, the differences were determined to be in the sugar test. The obtained results of the API 20 strep and API 20NE tests were analyzed using apiweb<sup>TM</sup> resources (<https://apiweb.biomerieux.com/servlet/Authenticate>). API 20 strep profile of the all our isolates showed three different profiles such as 7143115, 7143515 and 7143555, while reference *L. garvieae* (ATCC 43921) isolate showed 7143115. The results of the API 20 strep profiles were given on Figure 5 and Figure 6.



Figure 4. The appearance of binary or short chains of the gram staining of *Lactococcus garvieae* cells.



Figure 5. Evaluation of positive and negative results API 20 strep strip for identification of *L. garvieae* reference isolate (ATCC43921). Before dropping into the API 20 strep test kit reagents (A) and after dropping (B) (Original).



Figure 6. Evaluation of positive and negative results API 20 strep for identification of *L. garvieae* wild type isolates. Before dropping into the API 20 strep test kit reagents (A) and after dropping (B) (Original).

Table 1. Characteristics of *Lactococcus garvieae* wild type isolates and reference isolate (ATCC43921)

Character	The number of the bacteria isolates isolated from rainbow trout																					
	C <sup>1</sup>	C <sup>2</sup>	C <sup>3</sup>	C <sup>4</sup>	C <sup>5</sup>	C <sup>6</sup>	C <sup>7</sup>	C <sup>8</sup>	C <sup>9</sup>	C <sup>10</sup>	C <sup>11</sup>	C <sup>12</sup>	C <sup>13</sup>	C <sup>14</sup>	C <sup>15</sup>	C <sup>16</sup>	C <sup>17</sup>	C <sup>18</sup>	C <sup>19</sup>	C <sup>20</sup>	C <sup>21</sup>	C <sup>22</sup>
Morphology	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gram	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O/F metabolism	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
H <sub>2</sub> S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lysine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ornithine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simon citrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aesculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemolysis	α	α	α	α	α	α	α	α	α	α	α	α	α	α	α	α	α	α	α	α	α	α
0% NaCl growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6,5% NaCl growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10°C on growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20°C on growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37°C on growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45°C on growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2. The test results API 20 strep for identification of *L. garvieae*

Isolates no	<i>Lactococcus garvieae</i> isolates API 20 strep																				
	VP	HIP	ESC	PYRA	αGAL	βGUR	βGAL	PAL	LAP	ADH	RIB	ARA	MAN	SOR	LAC	TRE	INU	RAF	AMD	GLYG	HEM
R	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
1	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
2	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
3	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
4	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
5	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
6	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
7	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
8	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
9	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
10	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
11	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
12	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
13	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
14	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
15	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
16	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
17	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
18	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
19	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
20	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
21	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α
22	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	α

VP: Pyruvate, HIP: Hippurate hydrolysis, ESC: Esculin, PYRA: Pyrrolidonyl arylamidase, αGAL: α-galactosidase, βGUR: β-glucuronidase, βGAL: Galactosidase, PAL: Alkaline phosphatase, LAP: Leucine arylamidase, DH: Arginine dihydrolase, RIB: Ribose, ARA: L-Arabinose, MAN: Mannitol, SOR: Sorbito, LAC: Lactose, TRE: Trehalose, INU: Inulin, RAF: Raffinose, AMD: Starch, GLYG: Glycogen, HEM: Hemolysis.

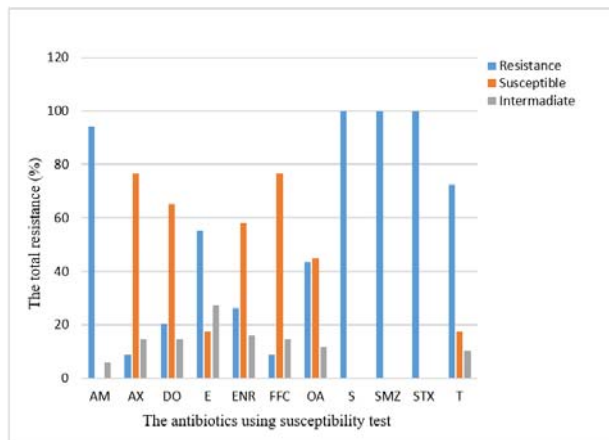
**PCR test results:** In PCR, the amplification of a single DNA band of 1,100 bp, specific for *L. garvieae*, was obtained from the samples of naturally diseased fish (Figure 8). The PCR results of the all wild type isolates and reference isolate (ATCC43921) have confirmed the preliminary biochemical and API 20 strep tests for identification.



**Figure 8.** PCR test results of *L. garvieae* isolates.

M; Marker (100-1500bp), Negative control (Distilled water; 17 and C), *L. garvieae* positive control (ATCC 43921; 1. and 25.) specific PCR (1100 bp), *L. garvieae* wild type isolates (2-24) specific PCR, (1100 bp), Negative control (*Aeromonas hydrophila*; A)

**The results of antimicrobial sensitivity test:** In this study, the highest incidence of resistance was to sulfamethoxazole, streptomycin and sulfamethoxazole-trimethoprim (100%), ampicillin (94.2), oxytetracycline (72.5%), followed by erythromycin (55.07%), oxolinic acid (43.5%), enrofloxacin (26.09%), doxycycline (20.3), but all isolates were less resistant to amoxicillin and florfenicol (8.7%). The most effective antibiotic was found to be florfenicol, amoxicillin, doxycycline and enrofloxacin. The results of antimicrobial sensitivity test were showed on Figure 9.



**Figure 9.** Antibiotic susceptibility profiles of *L. garvieae* isolates.

T: Oxytetracycline, OA: Oxolinic acid, SMZ: Sulfamethoxazole, AM: Ampicillin, FFC: Florfenicol, ENR: Enrofloxacin, STX: Trimethoprim-Sulfamethoxazole, E: Erythromycin, S: Streptomycin, DO: Doxycycline, AX: Amoxicillin.

## DISCUSSION

Lactococcosis causes significant economic losses in the rainbow trout farms that are cultured in the dam lakes in the eastern Black Sea Region. In Turkey, Lactococcosis has recorded in rainbow trout farms since 1995 in summer months (Cagirgan & Tanrikul, 1995), but it has appeared in the trout farms in our region after 2005 (unpublished). In this study, in the summer months when the water temperature increased above 20°C, it was observed that the fish deaths in some rainbow trout farms reached 80%. It has been reported

that Lactococcosis caused hemorrhagic septicemia, enteritis, ascites, bilateral exophthalmos with hemorrhage, (Austin & Austin 2016; Ture & Cimakil, 2018) darkening of the skin, congestion of the intestine, liver, kidney, spleen, and hemorrhagic enteritis in diseased rainbow trout (Cagirgan & Tanrikul, 1995; Diler et al., 2002). The similar clinical findings in this study have been reported by different researchers (Cagirgan & Tanrikul, 1995; Eldar & Ghittino, 1999; Diler et al., 2002; Cagirgan, 2004; Vendrell et al., 2006; Avci et al., 2010; Ozturk et al., 2013). In this research, described the clinical signs of lactococcosis in rainbow trout are similar to those reported in rainbow trout by Eldar & Ghittino (1999) with congestion and hemorrhage of the internal organs. Moreover, the petechial hemorrhages on the air bladder and peritoneum were determined.

In this study, the isolated bacteria from rainbow trout eyes were identified as *L. garvieae* by using the API 20 Strep kits (25°C, 48h). The biochemical properties of *L. garvieae* isolated from rainbow trout eyes in this study are very similar to those described in other studies (Austin & Austin, 1999; Diler et al., 2002; Çağırkan, 2004; Avci et al., 2010;)

PCR is a widely used detection method for various fish pathogens. PCR-based assays for *L. garvieae* include amplification of the 16S ribosomal RNA gene (Zlotkin et al. 1998). Zlotkin et al. (1998) reported that PCR assay resulted in the amplification of a band of 1100 bp in all 22 of the isolates tested with *L. garvieae* (ATCC 43921) reference strains. In this study, the twenty-two strains isolated from rainbow trout eyes, and *L. garvieae* ATCC 43921 were homologous by PCR assay.

Our results showed that florfenicol and amoxicillin antibiotics were most effective for the treatment of lactococcosis in fish, followed by doxycycline, and that sulfamethoxazole, streptomycin and sulfamethoxazole-trimethoprim were not effective (Figure 9). Sulfamethoxazole and trimethoprim/sulfamethoxazole are the most commonly used antibiotics to treatment of yersinosis in Turkey, because it is cheaper than other antibiotics (Balta, 2016). Therefore, all of the isolates are resistant to sulfamethoxazole and sulfamethoxazole-trimethoprim. Florfenicol has mostly used as an antibiotic in aquaculture for the last ten years (Kayis et al., 2009). Our results indicated the florfenicol can be efficiently used for the treatment of *L. garvieae* in fish in Turkey. In this study, the results of susceptibility tests indicated that all the *L. garvieae* isolates were susceptible to broad spectrum antibiotics like florfenicol, amoxicillin and doxycycline, by flowed enrofloxacin, erythromycin, oxytetracycline. In a previous study, the first isolated *Lactococcus garvieae* from rainbow trout has been reported to be susceptible to other antibiotics (erythromycin, tetracycline, ofloxacin, ampicillin and chloramphenicol) while are resistant to penicillin, clindamycin and ceftriaxon (Diler et al., 2002) Most of the *L.*

*garvieae* isolates have acquired resistance to the most commonly employed antibiotics (e.g., streptomycin, sulfamethoxazole, trimethoprim/sulfamethoxazole, ampicillin and oxytetracycline) in rainbow trout rearing. Antimicrobial test results were found to similar previous studies (Kubilay et al., 2005; Öztürk et al., 2013; Didinen et al., 2014, Ture & Cimagil, 2018). It was reported that ampicillin was the most active agent against *L. garvieae* strains (Ture & Boran, 2015), but our results showed that *L. garvieae* was resistant to ampicillin. In another study, all of the isolates were reported to be resistant to gentamycin, lincomycin, neomycin, sulfamethoxazole-trimethoprim, besides some strains were resistant to amoxicillin, doxycycline and florfenicol (Altun et al., 2013). Consequently, random application of these antimicrobials has led to the generation of resistant strains of *L. garvieae*. For the last twenty years, the different antibiotics have been used as an effective method to control lactococcus infections in fish. Although some of these substances have been demonstrated activity in vitro against *L. garvieae*, they have been usually reported to be ineffective when used under field conditions. It is probably because of rapid anorexia in the animals and the appearance of resistant strains (Vendrell et al., 2006). This research was also determined to similar result because of rapid anorexia during disease fish and used irregular of the drugs.

In conclusion, this research has made some valuable contribution to our knowledge about known Lactococcosis from the Eastern Black Sea region of the Turkey by providing data for isolation and identification (phenotypic, biochemical and PCR) of the *L. garvieae* isolated from rainbow trout eyes. According to result of this study, increased resistance to antibiotics in some strains of *L. garvieae* has been observed. it is thought that need to be carry out on new vaccine studies by using wild isolates to protect from lactococcosis (*L. garvieae*) that cause serious economic losses from rainbow trout farms in the future years.

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