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RESEARCH ARTICLE

# Phenotypic, Genotypic Characterisation and Antimicrobial Susceptibility Determination of *Lactococcus garvieae* Strains [1]

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## Summary

In this study, phenotypic and genotypic features of 10 *L. garvieae* strains isolated from rainbow trout farms were examined with 3 reference strains (Spain, England and ATCC 43921) comperativly. Rapid 32 STREP and conventional microbiologic tests were used for determining phenotipic features of *L. garvieae* strains. Altough there are differences, in Rapid 32 STREP system, between strains in terms of β-Glucuronidase, D-ribose, sorbitol, lactose, raffinose, alanyl-phenylalanyl-proline arylamidase, pyrrolidonyl arylamidase, hippurate hydrolysis, urease tests, all strains have been confirmed as *L. garvieae* by API web. In RAPD PCR analysis, in which ERIC 2 primer is used, *L. garvieae* isolates were genotyped within 3 separate clusters according to similarity coefficient index of 70%, and it was detected that a vast majority of isolates with Turkey-origin (8 isolates) belongs to predominant type LG1 genotype. In addition to this, antimicrobial tests of *L. garvieae* strains shows that there are resistance against gentamycin, neomycin, lincomycin, sulfamethoxazole-trimethoprim, oxytetracycline, erythromycin, amoxicillin, florfenicol and doxycycline, which are frequently used on fish in our country.

***Keywords:*** *Oncorhynchus mykiss, Lactococcus garvieae, Rapid32 STREP, RAPD PCR, Antimicrobial sensitivity*

# *Lactococcus garvieae* Suşlarının Fenotipik, Genotipik Karakterizasyonu ve Antimikrobiyal Duyarlılıklarının Belirlenmesi

## Özet

Araştırmada, gökkuşağı alabalığı işletmelerinden izole edilmiş olan 10 adet *Lactococcus garvieae* suşunun 3 adet referans suşla (İspanya, İngiltere ve ATCC 43921) karşılaştırmalı olarak fenotipik ve genotipik özellikleri incelenmiştir. *L. garvieae* suşlarının fenotipik özelliklerinin belirlenmesinde konvansiyonel mikrobiyolojik ve Rapid 32 STREP testleri kullanılmıştır. Rapid 32 STREP sistemde suşlar arasında β-Glucuronidase, D-ribose, sorbitol, lactose, raffinose, alanyl-phenylalanyl-proline arylamidase, pyrrolidonyl arylamidase, hippurate hydrolysis, urease testleri yönüyle farklılıklar olmasına rağmen API Web'de tüm suşlar *L. garvieae* olarak doğrulanmıştır. ERIC2 primerinin kullanıldığı RAPD PCR analizinde *L. garveae* izolatları %70 benzerlik katsayısına göre 3 farklı genotipe ayrılmış ve Türkiye kökenli izolatların büyük bir bölümü (8 izolat) predominant tip olan LG1 genotipine dahil olduğu belirlenmiştir. Ayrıca bu çalışmada *L. garvieae* suşlarının antibiyotik duyarlılık testlerinde ülkemizde balıklarda sıklıkla kullanılan gentamisin, neomycin, lincomycin, sulphamethoxazole-trimetoprim, oksitetrasiklin, eritromycin, amoxycillin, florfenikol ve doksisiklin'e karşı direnç geliştirmiş oldukları belirlenmiştir.

***Anahtar sözcükler:*** *Oncorhynchus mykiss, Lactococcus garvieae, Rapid32 STREP, RAPD PCR, Antimikrobiyal duyarlılık*

## INTRODUCTION

*Lactococcus garvieae* is the etiological agent of lactococcosis, causes significant economic losses both in marine and freshwater aquaculture all over the world [1]. The first isolation of agent from fish was originally made

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in Japan in 1991 and referred to *Enterococcus seriolicida* [2]. From that time to today, Lactococcosis was progressively spread in aquatic organisms and the agent has been isolated from most areas of the world such as Bulgaria, Brazil, Greece, England, France, Italy, Israel, Portugal and Spain [3-10]. In Turkey first isolation was performed in 2001 from farmed rainbow trout [11]. Since then, such infections have been reoccurred, especially during the warm summer months. Therefore, *L. garvieae* is now considered one of the most important pathogens in the rainbow trout industry in Turkey [11,12].

The identification of *L. garvieae* can be performed by conventionally microbiological methods and API diagnostic kits. However, in these methods clindamycin sensitivity test and PCR method are recommended for the exact identification of the agent, because different results are obtained depending on the culture mediums used, and a great majority of phenotypic characteristics of *L. garvieae* and *L. lactis* subs. l*actis* which is the most associated species, is similar [1,13,14]. In addition, it is stated that the presence of clindamycin resistant *L. lactis* strains may lead to wrong diagnosis. Therefore, PCR technique in identification of *L. garvieae* strains comes to the forefront [13].

Molecular characterization of isolates plays a very important role in detecting transmission ways, as well as in describing genetic relationships among strains isolated from different ecologic regions [6]. Ribotyping, pulsed-field gel electrophoresis, random amplified polymorphic DNA (RAPD) PCR, sau-PCR and amplified fragment length polymorphism methods have been used for detecting epidemiologic features of *L. garvieae* isolates so far. Numerous studies, while there was very low genetic relations among isolates isolated from different hosts or environmental sources, there was genetic variety among fish isolates isolated from different countries [6,15,16].

The aim of the present study was to determine morphologic, physiologic, biochemical features and anti-microbial sensitivity profiles of *L. garvieae* strains isolated from rainbow trout farms in Turkey. In addition to these, it is aimed to create RAPD patterns of *L. garvieae* strains isolated from different geographic regions in Turkey by PCR and to determine genetic relationship among isolates, and thus to introduce an epidemiological data source for our country.

## MATERIAL and METHODS

### *Bacterial Isolates and Growth Conditions*

We examined 12 *L. garvieae* isolates, comprising 10 isolates from different cities of Turkey, one isolate from Spain, and one from England. The type strain *L. garvieae* ATCC 43921 was included in analyses for comparative purposes *(Table 1)*. For all experiments, the strains were routinely grown on trypticase soy agar (TSA) and Columbia blood agar (CA) plates and incubated aerobically at 22°C for 24-48 h.

***Table 1.*** *L. garvieae strains investigated in the study*
***Table 1.*** *Çalışmada kullanılan L. garvieae suşları*

| Isolate No | Origin | Source |
|---|---|---|
| 1 | Muğla/Turkey | Rainbow trout |
| 2 | Muğla/Turkey | Rainbow trout |
| 3 | England | Rainbow trout |
| 4 | Spain | Rainbow trout |
| 5 | Antalya/Turkey | Rainbow trout |
| 6 | Kütahya/Turkey | Rainbow trout |
| 7 | Bilecik/Turkey | Rainbow trout |
| 8 | Isparta/Turkey | Rainbow trout |
| 9 | Bursa/Turkey | Rainbow trout |
| 10 | Samsun/Turkey | Rainbow trout |
| 11 | Samsun/Turkey | Rainbow trout |
| 12 | Samsun/Turkey | Rainbow trout |
| 13 | ATCC 43921 | Bovine mastitis |

### *Phenotypic Characterization*

All isolates were characterized using the following classical phenotypic tests: Gram staining reaction, motility, oxidation-fermentation (O/F), oxidase, catalase, gelatinase, Simmons citrate, indole production, methyl red (MR), reduction of nitrate, starch hydrolysis, O/129, $H_2S$, growth in MacConkey agar (MA), Bile esculin azide agar, eosin methylene blue (EMB) agar, nutrient agar (NA), TSA and brain heart infusion agar (BHIA), hemolysis in 5% sheep blood agar, ability to growth in pH 9.6, 1%, 2.5%, 6.5% and 8% NaCl [17,18]. These isolates were further characterized biochemically by using the rapid ID 32 STREP (Biomerieux) according to the manufacturer's instructions, except for the temperature of incubation which was set at 35°C 4 h, and results were compared with the manufacturer database.

### *Molecular Confirmation by PCR*

PCR reaction with *L. garvieae* specific primer pairs (pLG-1/pLG-2) was performed for genetic confirmation of isolates as described by Zlotkin et al.[13]. Isolates giving a predicted amplification product of 1100 bp were identified as *L. garvieae*.

### *Genotyping by RAPD Analysis*

The RAPD technique was performed to investigate clonal relatedness among 13 *L. garvieae* isolates, using the universal primer ERIC 2 primer (5'-AAGTAAGTGACTGG GGTGAGCG-3') as described with some modification [19]. PCR amplifications were performed in a total reaction volume of 25 µl. The reaction mixture contained, 200 µM of each dNTP, 2.5 mM $MgCl_2$, 25 pmol primer, 2.5 unit of Taq DNA polymerase, 5 µl of template DNA. The amplification program was one cycle at 95°C for 1 min; 30 cycles at 94°C for 1 min, at 40°C for 1 min, at 72°C for 1 min; followed by one cycle at 72°C for 5 min. The PCR products were separated on

1.5% agarose gel stained with 2 mg/ml of ethidium bromide.

The DNA profiles were analyzed with CHEF-DR® III, Quantity One® software (Bio-Rad Laboratories, Hercules, CA). Dendrogram was constructed using the unweighted-pair group method (UPGMA). To determine reproducibility of RAPD analysis, 5 isolates were selected randomly and RAPD analysis was repeated 3 times subsequently.

### *Antimicrobial Susceptibility Patterns*

*L. garvieae* isolates were tested for antimicrobial susceptibility by the disc diffusion method on Mueller-Hinton agar. The antimicrobial agents (Oxoid) were tested including neomycin (10 µg), gentamycin (120 µg), oxytetracycline (30 µg), florfenicol (30 µg), erythromycin (15 µg), sulfamethoxazole + trimethoprim (1.25 µg/ 23.75 µg), doxycycline (30 µg), lincomycin (2 µg) and amoxicillin (25 µg). Both at 24$^{th}$ and 48$^{th}$ h of the incubation, incubation zone diameters were measured and evaluated. The isolates were classified as sensitive (S), intermediary sensitive (I), or resistant (R), on the basis of the size of the zone of bacterial growth inhibition, according to the Clinical and Laboratory Standards Institute [20].

## RESULTS

### *Phenotypic Characterization*

There were phenotypic differences among *L. garvieae* isolates in β-Glucuronidase, D-ribose, sorbitol, lactose, raffinose, voges proskauer (VP), alanyl-phenylalanyl-proline arylamidase (APPA), pyrrolidonyl arylamidase (PyrA) and

***Table 2.*** *Phenotypic characteristic of L. garvieae strain with convansionally tests*

***Tablo 2.*** *L. garvieae suşlarında konvansiyonel testler kullanılarak belirlenen fenotipik özellikleri*

| **Phenotypic Characters** | **Strain No** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** |
| Colony morphology | S* | S* | S* | S* | S* | S* | S* | S* | S* | S* | S* | S* | S* |
| Gram staining | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Motility | - | - | - | - | - | - | - | - | - | - | - | - | - |
| O-F | F | F | F | F | F | F | F | F | F | F | F | F | F |
| Oxidase | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Catalase | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Gelatin hydrolysis | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Citrate utilization | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Indole production | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Methyl red | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Nitrate Reduction | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Starch hydrolysis | - | - | - | - | - | - | - | - | - | - | - | - | - |
| O/129 (10 µg) | S | S | S | S | S | S | S | S | S | S | S | S | S |
| $H_2S$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Growth on** | | | | | | | | | | | | | |
| MacConkey agar | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Bile esculine azide agar | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Eozin methylen blue agar | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Nutrient agar | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Trypticase soy agar | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Brain Heart infusion agar | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hemolysis in blood agar | α | α | α | α | α | α | α | α | α | α | α | α | A |
| **Growth in** | | | | | | | | | | | | | |
| pH 9.6 | + | + | + | + | + | + | + | + | + | + | + | + | + |
| %1 NaCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| %2.5 NaCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| %6.5 NaCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| %8 NaCl | - | - | - | - | - | - | - | - | - | - | - | - | - |

***S***: Smooth colony, **S:** Sensitive*

urease tests. Only reference strain in 13 isolates hydrolyized hippurate. Despite differences among isolates, all isolates were identified as *L. garvieae* in API web. Results obtained from conventional microbiologic tests and Rapid ID 32 STREP test kits are given in *Table 2* and *3*.

### *Molecular Identification by PCR*

All isolates used in the study were confirmed by specific PCR, giving the expected amplification product of 1100 bp belonging to the 16S rRNA gene *(Fig. 1)*.

### *Genotyping*

In RAPD method, *L. garvieae* isolates were grouped within three seperate genotype clusters according to 70% similarity coefficient index. It was detected that 8 of the isolates (66.6%) belong to predominant type LG 1 genotype, 3 other isolates (25%) to LG3 genotype, and one isolate (8.3%) to LG2 *(Fig. 2)*. Furthermore, it was detected that isolates belonging to LG2 and LG3 genotypes have high similarity. It was found that reproducibility of RAPD PCR, in which M13 primer was used, is 100%.

***Table 3.*** *Phenotypic characteristic of L. garvieae strains with Rapid 32 Strep tests*
***Tablo 3.*** *L. garvieae suşlarında Rapid 32 Strep testi kullanılarak belirlenen fenotipik özellikler*

| Phenotypic Characters | Strain No | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Adh | + | + | + | + | + | + | + | + | + | + | + | + | + |
| β-glucosidase | + | + | + | + | + | + | + | + | + | + | + | + | + |
| β-galactosidase | - | - | - | - | - | - | - | - | - | - | - | - | - |
| β-glucuronidase | + | + | - | - | - | - | + | + | + | + | + | + | - |
| α-galactosidase | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Pal | - | - | - | - | - | - | - | - | - | - | - | - | - |
| D-ribose | - | - | - | - | - | - | + | - | - | - | - | - | - |
| Mannitol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sorbitol | - | - | - | - | - | - | + | - | - | - | - | - | - |
| Lactose | - | - | - | - | - | - | + | - | - | - | - | - | - |
| Trehalose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Raffinose | - | - | - | - | - | - | + | - | - | - | - | - | - |
| Voges proskauer | - | + | - | - | - | - | - | + | - | + | + | + | - |
| Appa | + | + | - | - | - | - | + | + | + | + | + | + | + |
| β-galactosidase | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Pyra | + | + | - | - | - | - | - | + | + | + | + | + | + |
| β –Nag | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Gta | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Hip | - | - | - | - | - | - | - | - | - | - | - | - | + |
| Glycogen | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Pullulan | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Maltose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Melibiose | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Melezitose | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Saccharose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L-arabinose | - | - | - | - | - | - | - | - | - | - | - | - | - |
| D-arabitol | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M-βdg | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Tagatose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| β-mannosidase | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Cyclodextrin | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Urease | + | - | + | + | + | - | + | + | + | - | - | + | + |

***Adh:*** *arginine dihydrolase,* ***Pal:*** *alkaline phosphatase,* ***Appa:*** *alanyl-phenylalanyl-proline arylamidase,* ***Pyra:*** *pyrrolidonyl arylamidase,* ***ß-Nag:*** *N-acetyl-β-glucosaminidase,* ***Gta:*** *glycyl-tryptophan-arylamidase,* ***Hip:*** *hippurate hydrolysis,* ***M-βdg:*** *methyl-β-D-glucopyranoside acidification*

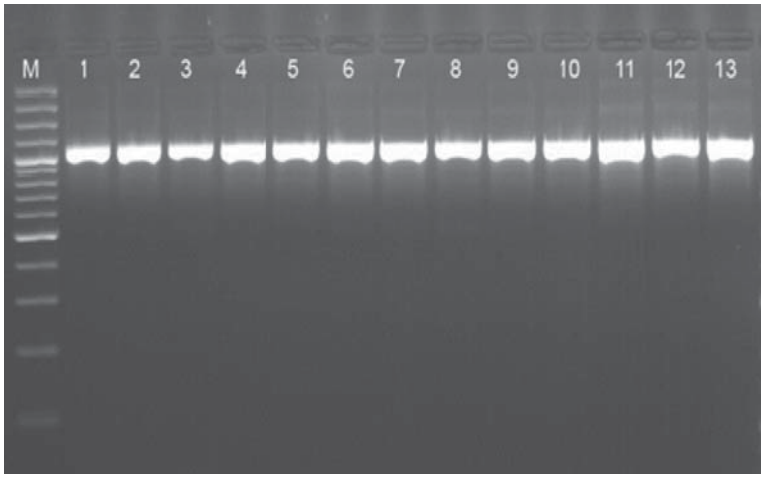


**Fig 1.** *L. garvieae* specific PCR, 1100 bp. molecular weight standard (100-3000 bp), 1-13; *L. garvieae* field ve referance strains (see *Table 1*).

**Şekil 1.** *L. garvieae* spesifik PCR, 1100 bp. M; 3000 bp moleküler marker 1-13; *L. garvieae* saha ve referans suşları (bknz. *Tablo 1*)

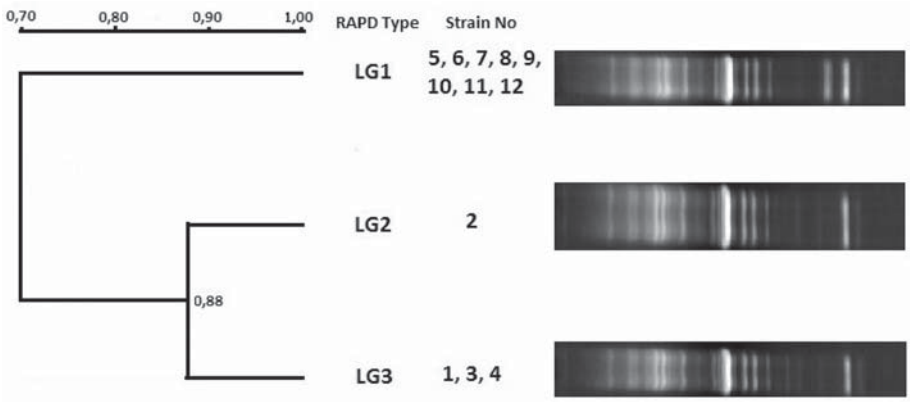


**Fig 2.** RAPD-PCR profiles of *L. garvieae* strains

**Şekil 2.** *L. garvieae* suşlarının RAPD PCR profilleri

***Table 4.*** *Antimicrobial susceptibility profiles of L. garvieae strains*
***Tablo 4.*** *L. garvieae suşlarının antibiyotik duyarlılık profilleri*

| **Antimicrobial Disc** | **Strain No** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** |
| Gentamycin | R | R | R | R | R | R | R | R | R | R | R | R | R |
| Neomycin | R | R | R | R | R | R | R | R | R | R | R | R | R |
| Lincomycin | R | R | R | R | R | R | R | R | R | R | R | R | R |
| Oxytetracycline | R | R | R | R | R | I | R | R | R | S | R | R | R |
| Amoxicillin | R | R | R | I | R | R | R | R | R | I | R | I | R |
| Florfenicol | R | R | R | R | R | R | R | I | I | I | I | I | I |
| Erythromycin | R | R | R | R | R | I | R | R | R | R | R | R | R |
| Doxycycline | R | R | R | R | R | I | I | I | R | I | R | I | R |
| Sulphamethoxazole- trimethoprim | R | R | R | R | R | R | R | R | R | R | R | R | R |

***R:*** *Resistant,* ***I:*** *Intermediate,* ***S:*** *Sensitive*

### *Antimicrobial Susceptibility Patterns*

Thirteen *L. garvieae* isolates were examined with nine different antimicrobial in terms of antimicrobial sensitivity and results were given in *Table 4*. All *L. garvieae* isolates used in the study were resistant to neomycin, lincomycin, sulfamethoxazole-trimethoprim, oxytetracycline (except for 6$^{th}$ isolate) and erythromycin (except for 6$^{th}$ isolate); besides, 10, 7 and 8 isolates were resistant to amoxicillin, florfenicol and doxycycline, respectively.

## DISCUSSION

Lactococcosis has become increasingly widespread after epizooties occurred in Turkey, as is the case all around the world, causing significant economic losses in trout farms in particular [21]. Knowing genotypic and phenotypic features of the agent is very important to understand epidemiology of infections and to reduce economic losses caused by infection.

Miniaturizing systems, as well as conventional tests, are used for identification of *L. garvieae* isolates and for determination of phenotypic features [1,7]. In phenotypic characterization studies which are done with conventional methods and miniaturizing systems such as API 20 strep, API 50CH, *L. garvieae* strains isolated different regions without caring host range have a lot of common biochemical features and phenotypic homogeneity [4,15,22]. At the same time some studies shows that there may be different results in miniaturizing systems [16]. It is suggested that there is huge diversity among *L. garvieae* isolates in characterization studies carried out with miniaturizing systems (Rapid ID 32 Strep and API 50CH). API 50 CH and Rapid ID 32 strep systems give different results about ribose acidification; thus, this test must be considered in routine identification with Rapid ID 32 Strep of clinic isolates of *L. garvieae* [14]. Conventional methods and miniaturizing systems were evaluated comparatively and *L. garvieae* isolates used in this study exhibited very high level of phenotypic similarity regardless of geographic origins and sources thereof. Researchers got different results among strains about utilization of hip, ß-alactosidase, ß-Nag, ß-mannosidase and D-arabitol, melezitose and pululan (acid from). In our study, it has been established with conventional microbiologic tests that strains isolated from different geographic regions *(Table 1)* (ATCC 43921) have common phenotypic features. There are differences among strains in β-glucuronidase (8 positive), D-ribose (1 positive), sorbitol (1 positive), lactose (1 positive), raffinose (1 positive), VP (5 positive), Appa (9 positive), Pyra (8 positive) and urease (9 positive) tests, when Rapid ID 32 Strep system is applied. While some of our findings obtained from Rapid 32 Strep system are consistent with those of Vela et al.[16] (acification of pullatan, M-βdg and β-mannosidase and presence of enzyme Gta), some other results are not (presence of enzyme Appa and urease and acidification of cyclodextrin). Results of biochemical parameters in our study are not consistent with those of Ravelo et al.[14]. This difference is most probably linked to the incubation temperature and duration used. In our experiments we have employed the instructions of manufacturer (35°C for 4 h) where the others incubated at 25°C or 30°C for 24 h (Ravelo et al.[14], Vela et al.[16]).

RAPD PCR method, which has been commonly used for detecting genetic relationships of bacterial fish pathogens recently [22-28], is a simple, sensitive, reproducible, and easy to apply technique with high differentiating rate [6,29,30]. However, this method is not so common in molecular typing of *L .garvieae* isolates, being limited to only a few studies [6,10,29]. Ravelo et al.[10] used RAPD PCR method to determine genetic similarity of strains isolated from different geographic regions and fish type. Same authors; Random primers used in test were determined comparatively and P5 and P6 primers gave reproducible pattern and isolates were divided into 3 genogroups according to analysis of similarity among different profiles. Spain, Portugal, England and Turkey isolates isolated from rainbow trouts were grouped into group 1, France and Italy isolates isolated from rainbow trouts were grouped into group 2, and Japan isolates isolated from yellowtails and *L. garvieae* NCDO 2155-reference strain were grouped into group 3. In another study, P5 primers were used and Spain, England and Turkey trout isolates were grouped into same genotype. Isolates isolated from Israel has unique RAPD profile. Foschino et al.[6] used RAPD method, in which M13 and P5 primers are used, and it was suggested that M13 primers had the best differentiating rate. Eighty one isolates collected from Italian fish and dairy samples were divided into 52 RAPD genotype in 5 group. Isolates isolated from fish were classified into three different groups. In conclusion, researchers suggested that there was a low genetic relation between dairy and fish isolates, and that Spanish and English isolates were generally included in the same group in terms of geographic origin. ERIC 2 was used as a random primer in our study and *L. garvieae* gave different band patterns with this primer, thereby suggesting that it can be used in epidemiologic studies. *L. garvieae* isolates were grouped into group which had 3 different genotypes with ERIC 2 primer and some of the isolates isolated from our country (isolates 3 and 4) had high similarity with Spain and England isolates, consistent with the study of Ravelo et al.[10]. According to RAPD PCR results, isolates 5-12 belong to predominant LG1 genotype by %66.6 and these isolates shows genetic heterogeneity with Spain and England isolates and isolates isolated from outbreak in 2001. Accordingly, isolates in LG2 and LG3 genotypes have very close relationship. Altun et al.[21] reported that *L. garvieae* strains isolated from Turkey, Spain and England with a different method (16s rDNA sequence analysis) were genetically similar 99-100%. However, *L. garvieae* strains isolated from Turkey in 2001 (isolates 1 and 2) are not related to the other strains in our study. That local isolates are included in dominant group suggests that *L. garvieae* isolates obtained from rainbow trout farms, except for those in Muğla, are associated with a single epidemiologic strain, in Turkey. Researches show that strains originated from the same root can be infective for a long time [31].

The disease can be treated with chemotherapeutics, but choosing sensitive antimicrobial agents is very important due to the resistant strains. *L. garvieae* isolates in our study are totally resistant to gentamycin, neomycin, lincomycin, sulfamethoxazole-trimethoprim, which are very common in our country; moreover, resistance is developed against oxytetracycline, erythromycin, amoxicillin, florfenicol and doxycycline to a great extent. Although, in previous studies [11,20,32,33] carried out in our country on this topic, *L. garvieae* was sensitive to oxytetracycline, erythromycin, amoxicillin, florfenicol and doxycycline, strains tested in this study developed resistance to these antimicrobial agents.

In conclusion, it is required that isolation of agent and antimicrobial sensitivity tests in fish diseases treatment should be performed sooner, that the treatment should be started with sensitive antimicrobial agents from the onset

of diseases, and that the use of any antimicrobial to develop resistance against drugs should be prevented. This situation suggests that bacteriostatic and bactericidal effect of antimicrobial drugs to be used in diseases either for protective, or therapeutic purposes against specific disease agent should be carefully determined, and that sensitive antimicrobial agents should be selected and administered in enough dosage and for sufficient time. RAPD PCR method, in which ERIC primer 2 is used, can be used to determine genetic differences among *L. garvieae* strains and it provides rapid results with high differentiating rate. In addition to this, knowing distribution to different geographic regions of genetic groups of *L. garvieae* can help to prepare effective vaccine formulation and take protective measures against lactococcosis.

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