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Phenotypic and genotypic antimicrobial resistance of *Lactococcus* sp. strains isolated from rainbow trout (*Oncorhynchus mykiss*)

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Abstract

A current profile of antimicrobial resistance and plasmid of 29 *Lactococcus garvieae* and one *Lactococcus lactis* strains isolated from rainbow trouts (*Oncorhynchus mykiss*) from farms throughout Turkey were investigated. All isolates were sensitive to penicillin G (90%), ampicillin (86.7%), florfenicol (83.3%), amoxicillin (80.1%), and tetracycline (73.4%), and resistant to trimethoprim+sulfamethoxazole (86.6%) and gentamycin (46.6%) by disc diffusion method. Twenty-eight (93%) isolates had two to seven antibiotic resistance genes (ARGs) determined by PCR. The most prevalent ARGs were tetracycline (*tetB*), erythromycin (*ereB*), and β-lactam (bla_{TEM}). Bacterial strains were also screened for plasmid DNA by agarose gel electrophoresis and two strains harboured plasmids, with sizes ranging from 3 to 9 kb.

Keywords: fish, *Lactococcus garvieae*, antibiotic resistance, resistance gene, plasmid.

Introduction

Lactococcus garvieae (junior synonym - *Enterococcus seriolicida*), the bacterial pathogen causing lactococcosis, is a zoonotic pathogen, which has been isolated from various sources such as fish, human, terrestrial animals, and food products. It causes great economic losses in the aquaculture industry worldwide (5, 26). *L. garvieae* is also isolated from the milk of cows, buffalos with mastitis infections, and from clinical specimens of human such as blood, urine, and skin (5, 8). Therefore, this bacterium has an increasing clinical significance in the fields of fishery, as well as veterinary and human medicine. *L. garvieae* has been isolated in yellowtail (*Seriola quinqueradiata*) in Japan (15) and has subsequently been found in many areas, including Turkey (9).

The aquaculture production is considerably large in Turkey. Rainbow trout (*Oncorhynchus mykiss*), Black Sea trout (*Salmo trutta labrax*), sea bass (*Dicentrarchus labrax*), and sea bream (*Sparus aurata*) are the most extensively cultured fish species. The development of fish production engenders the problems of diseases and, consequently, economic losses. Only several kinds of antimicrobial agents, including florfenicol, enrofloxacin, and oxytetracycline, were successfully approved for the treatment of fish diseases in Turkey (12).

There is an increasing international concern regarding the potential transfer of the antimicrobial resistant bacteria between animals and humans. Antibiotic resistance genes (ARGs) in plasmids can also spread between bacterial strains of animals and humans in aquatic environment (1). Generally, transference of resistance determinants occurs by mobilising genetic elements including plasmids, transposons, integron, and gene cassettes, and these elements are important factors that can contribute to an increase in multiresistant bacteria. The prophylactic and therapeutic use of antibiotic drugs also results in the occurrence of drug resistant bacteria and ARGs in the aquaculture environment. Thus, antibiotic treatments often fail in fish farms (4, 18).

The literature on the studies on ARGs of *Lactococcus* isolated from fish is limited. In a previous study, a total of 146 *L. garvieae* strains isolated from fish were tested for antibiotic susceptibility and screened for the presence of transferable R-plasmids using a conjugation experiment and Southern hybridisation (17). Therefore, it is important to

investigate bacterial susceptibility to antimicrobial agents and to detect the presence of ARGs in aquatic organisms. In the present study, we aimed to determine the antibiotic resistance and the presence of related ARGs, including tetracycline (*tetB, tetS*), erythromycine (*ereA, ereB*), sulphonamide (*sulI, sulII*), β-lactam (*ampC, bla$_{TEM}$, bla$_{PSE}$*), trimetophrim (*dhfr1*), aminoglycoside (*aadA*), and florfenicol (*floR*), in the *Lactococcus* isolates. All isolates were also screened for plasmid DNA by agarose gel electrophoresis.

Material and Methods

Bacterial isolates and culture conditions. A total of 29 isolates of *L. garvieae* and 1 isolate of *L. lactis* were analysed. Seventeen of *L. garvieae* strains were isolated from local rainbow trout farms between 2007 and 2011. The Ilhan Altinok, KTU, Faculty of Marine Science, Department of Fisheries Technology Engineering provided 6 Spanish, 4 Italian, and 1 French *L. garvieae* strains isolated from rainbow trout. The reference *L. garvieae ATCC49156* strain was purchased from the American Type Culture Collection. A strain of *L. lactis* was isolated in another study in Turkey. All the isolates were stored at -80°C in 15% to 20% glycerol containing tryptic soy broth (TSB, Merck). For the analyses, they were inoculated on tryptic soy agar (TSA, Merck) and incubated at 30°C for 18 h.

Antibiotic susceptibility tests. Susceptibility of all bacteria was determined by the agar diffusion method using 6 mm diameter commercial discs (Oxoid, U.K.) containing the following antibiotics (μg/disc): florfenicol (30), enrofloxacin (5), erythromycin (15), amoxicillin (10), oxytetracycline (30), tetracycline (30), penicillin G (10 U), gentamycin (10), trimethoprim +sulfamethoxazole (25), and ampicillin (10). Antimicrobial tests were performed on Muller Hinton Agar (MHA, Oxoid, U.K.) plates. The bacteria were incubated overnight at 30°C and bacterial optical density was adjusted to be approximately equal to 0.5 of McFarland Opacity. The bacterial suspension (0.1 mL) was poured on MHA plates and spread with sterile swab. Antibiotic discs were dispensed on the surface of the plates with sterile pliers, and the plates were incubated aerobically at 30°C for 48 h. After incubation, the zone diameters were measured with a ruler. The susceptibility of the isolates was determined according to Performance Standards for Antimicrobial Susceptibility Testing: Twenty-First Informational Supplement, Zone Diameter Standards for *Enterococcus* spp. (7).

Genomic and plasmid DNA preparation and PCR assays for detection of ARGs. At first, all *L. garvieae* isolates were confirmed by PCR assay as described by Altinok (2). Briefly, a forward primer LgF (5'-CCA ACT TCC GTG GTG TGA CG-3') and a reverse primer LgR (5'-AGT GGC TCA ACC ATT GTG TGC-3') for PCR amplification of a less conserved region of the small subunit (16S) ribosomal RNA (rRNA) gene sequence of *L. garvieae* were synthesised by Life Technologies (Grand Island, USA). The predicted size of the amplified product was 857 base pairs (bp), based on the DNA sequence (GenBank accession number FJ915634). Genomic DNA of all isolates was extracted as template for PCR assay by QIAamp DNA mini kit (Qiagen, Germany), according to the manufacturer's instructions. To increase the DNA yield of Gram-positive bacteria, the manufacturer's instructions were modified and the lysozyme treatment was extended to 1 h. The RNA/DNA calculator (Spectrophotometer, Biorad, USA) was used to measure optical density at 260 and 280 nm, and then the quality of the DNA was analysed in agarose gel (1.2%).

Primer sequences used for PCR amplification of 12 ARGs (*tetB, tetS, ereA, ereB, sulI, sulII, ampC, floR, dhfr1, bla$_{TEM}$, bla$_{PSE}$*, and *aadA*) were selected based on the published articles (Table 1) and confirmed by Genbank. These ARGs have been generally used in the studies on aquatic organisms.

The presence of ARGs, including erythromycin (*ereA, ereB*), tetracycline, oxytetracycline (*tet(B), tet(S)*), sulphonamide (*sulI, sulII*), β-lactam (*ampC, bla$_{TEM}$, bla$_{PSE}$*), trimetophrim (*dhfrA1*), gentamycine (*aadA*), and florfenicol (*floR*), was analysed in 30 *Lactococcus* strains by PCR. Each PCR reaction mix (25 μL total) included 100 ng of sample DNA, 100 ng of each primer, 12.5 μL of 2 × Master Mix PCR mixture (Qiagen, Master PCR kit), and 9.5 μL of distilled water. $MgCl_2$ concentration was optimised for each primer set. As a negative control, all primer sets were tested with sterile water. DNA amplification was performed in a thermocycler (SensQuest, Germany) under the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 38-60°C for 30 s, extension at 72°C for 45 s, and final extension at 72°C for 10 min. Ten microliters of PCR products were subjected to electrophoresis in 1.2% (w/v) agarose gel prepared with 1 × TAE (Tris-Acetate-EDTA) buffer and run at 100 V for 45 min. Then, the DNA bands were stained with ethidium bromide and viewed by UV transillumination. The sizes of the PCR products were determined by comparing them with the migration of 100-bp DNA ladder (Bio Basic, Canada). The plasmids were extracted using QIAprep (Qiagen) Mini Kit according to the procedure described by the manufacturer. This procedure was modified, and the lysozyme was added. Extracted DNA plasmid was subjected to electrophoresis in 0.7% (w/v) agarose gel (1×TBE) and run at 100 V for 1 h. The products were stained in ethidium bromide, and viewed by UV transillumination.

Results

A total of 20 *Lactococcus* isolates showed a multiple-antibiotic-resistant phenotype. The following antibiotic resistance of the isolates was observed: florfenicol - 0%, penicilin, ampicillin, tetracycline, and oxytetracycline - 10%, amoxicilline and erytromycine - 16.6%, gentamycin - 46.6%, enrofloxacine - 36.6%, and sulphamethaxazole + trimethoprim - 86.6%. The isolates had the highest resistance rates to sulphamethaxazole + trimethoprim. The results also demonstrated available individual and multiple resistance to antibiotics in *L. garvieae*. A total of 30 isolates of *Lactococcus* showed resistance to all antibiotics tested (except florfenicol) (Table 2). In total, 93% of the isolates were resistant to 2 or more antibiotics, and multi-resistance was detected to at least 2 different classes of antibiotics in all isolates.

Table 1. Primer sequences and annealing temperatures used in the PCR reactions

Primers	Sequences (5′–3′)	Target gene	PCR product size (bp)	Annealing temperature (°C)	References
ereA-F	AACACCCTGAACCCAAGGGACG				
ereA-R	CTTCACATCCGGATTCGCTCGA	*ereA*	420	59	(22)
ereB-F	AGAAATGGAGGTTCATACTTACCA				
ereB-R	CATATAATCATCACCAATGGCA	*ereB*	546	52	(3)
tetS-F	ATCAAGATATTAAGGAC				
tetS-R	TTCTCTATGTGGTAATC	*tetS*	590	38	(6)
TetB-F	TTGGTTAGGGGCAAGTTTTG				
TetB-R	GTAATGGGCCAATAACACCG	*tetB*	659	53	(20)
SulI-F	CGGCGTGGGCTACCTGAACG				
SulI-R	GCCGATCGCGTGAAGTTCCG	*sulI*	433	61	(13)
SulII-F	GCGCTCAAGGCAGATGGCATT				
SulII-R	GCGTTTGATACCGGCACCCGT	*sulII*	293	60	(13)
dhfr1-F	CTGATATTCCATGGAGTGCCA				
dhfr1-R	CGTTGCTGCCACTTGTTAACC	*dhfr1*	433	57	(23)
Tem_{OT}.F	ATGAGTATTCAACATTTCCG				
Tem_{OT}.R	CAATGCTTAATCAGTGAGG	*blaTEM*	859	45	(21)
PSE1-F	CGCTTCCCGTTAACAAGTAC				
PSE1-R	CTGGTTCATTTCAGATAGCG	*blaPSE*	465	50	(30)
AmpC-F	TTCTATCAACACTGGCARCC				
AmpC-R	CCYTTTTATGTACCCATGA	*ampC*	550	49	(24)
aadA-F	TGATTTGCTGGTTACGGTGAC				
aadA-R	CGCTATGTTCTCTTGCTTTTG	*aadA*	284	52	(28)
floR-F	TATCTCCCTGTCGTTCCAG				
floR-R	AGAACTCGCCGATCAATG	*floR*	399	50.5	(28)

Table 2. Antibiotic resistance profiles of *Lactococcus* isolates

Antibiotics	Resistant (%)	Intermediate (%)	Susceptible (%)
Erythromycin	16.6	33.3	50.1
Tetracycline	10	16.6	73.4
Oxytetracycline	10	30	60
Sulpha+trimet	86.6	3.3	10.1
Penicillin G	10	0	90
Amoxicilline	16.6	3.3	80.1
Ampicillin	10	3.3	86.7
Gentamycin	46.6	26.6	26.8
Enrofloxacin	36.6	20	43.4
Florfenicol	0	16.6	83.3

The presence of all 12 different ARGs was determined in 30 *Lactococcus* isolates individually. The results of the PCR assays are shown in Table 3. PCR amplification was performed for 9 of these gene regions and these ARGs resulted in the amplification of DNA fragments with various band intensities, and their sizes ranging between 284 (*aadA*) and 859 (*blaTEM*) bp (Fig. 1). All bacterial isolates had two or more ARGs (multi-resistant bacteria). The most prevalent ARGs were found to be tetracycline (*tetB*), erythromycin (*ereB*), and β-lactam (bla_{TEM}). Although 1 of the 2 tetracycline ARGs (*tetS*) was only found in 2 strains, the other (*tetB*) were detected in most of the strains.

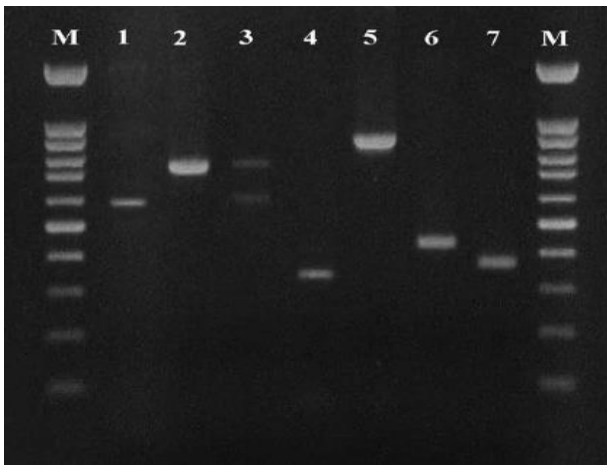

Fig. 1. Gel electrophoresis image of different resistance determinants in *L. garvieae* isolates. M - 100-bp DNA marker; 1 – *ereB*; 2 – *tetB*; 3 – *tetS*; 4 - *dhfr*; 5 - bla_{TEM};6 – *sulI*; 7 - *aadA*

Plasmids extracted were seperated on 0.7% agarose gels for 1 h. In total, 2 strains (164 A/03 and 8053) belonging to *L. garvieae*, were found to harbour one of the plasmids, with sizes ranging from 3 to 9 kb (Fig. 2). The ARGs patterns of these strains harbouring one plasmid were similar, and all of them had 3 ARGs (Table 3).

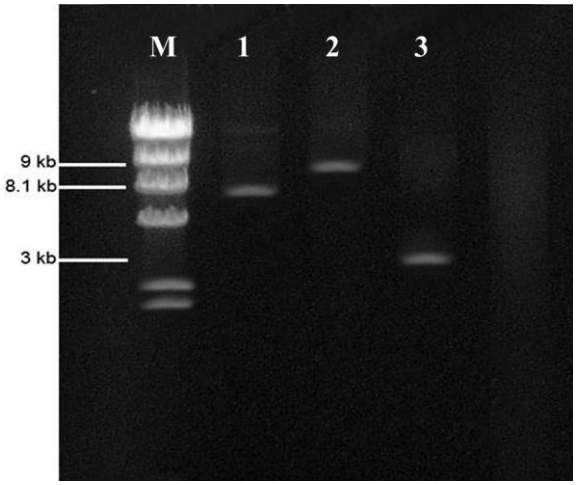

Fig. 2. Gel electrophoresis of DNA plasmid from *L. garvieae* strains. M - Lambda *HindIII* marker; 1 - *E. coli dh5*; 2 - *L. garvieae* 164 A/03; 3 - *L. garvieae* 8053

Discussion

In aquaculture, the use of antimicrobial compounds when they are necessary is the most appropriate method for control of bacterial diseases, as well as environmental conditions and protective measures (14). Historically, antimicrobial agents have been used to control infections caused by the *Streptococcus* genus in fish. However, the unrestrained use of these agents has led to an increase in antibiotic resistance (29). In the present study, we observed the antibiotic resistance of *Lactococcus* strains using phenotypic methods, and then multi-resistant isolates were selected for further analyses of ARGs and plasmid.

All isolates investigated in this study had two or more ARGs. The *tet(B)* and *ereB* RGs were detected more frequently than others, reflecting the higher use of encoding antibiotics in intensive fish farming. The rapid spread of tetracycline resistant determinants among the bacterial populations is due to the location of tetracycline genes on mobile elements such as plasmids, transposons, and gene cassettes (25). In a previous study, most of the tested *L. garvieae* strains showed high levels of resistance to erythromycin and tetracycline, and some of these strains were shown to carry transferable R-plasmids (17). In the present study, it was found that tetracycline ARGs were common in *Lactococcus* strains. The *tet(B)* gene was one of the most prevalent ARGs detected in this study. A possible transfer of *tet(M)* from the *L. garvieae* chromosome to *Enterococcus faecalis* chromosome was described (19). On the other hand, bacterial resistance is usually associated with the presence of high molecular weight plasmids (16). Two *L. garvieae* strains have been found to harbour small plasmids, with sizes ranging from 3 to 9 kb. It can be assumed that the spread of resistant determinants among the *Lactococcus* isolates is due to the location of these genes on chromosomes.

Erythromycin can be used to treat infections caused by Gram-positive bacteria including *Lactococcus*, *Streptococcus*, *Staphylococcus*, and *Haemophilus* genera (27). However, we found that oxytetracycline and ampicillin were phenotypically more effective than erythromycin. Erythromycin resistance determinants include *Erm* methylases, efflux pumps, and drug inactivating enzymes. We used primer sets of *ereA* and *ereB* genes for detection of the drug inactivation. The *ereB* gene was commonly detected (63.3% of the isolates), while *ereA* was not detected in *Lactococcus* strains. In a previous study, the drug resistance of *L. garvieae* isolated from cultured yellowtail was investigated, and it was found that 44% of the isolates were resistant to erythromycin and oxytetracycline. All resistant isolates possessed *ermB* and *tetS* genes (11). In our study, 10% and 16.6% of *L. garvieae* isolates were resistant to oxytetracycline and erythromycin respectively. Moreover, most of the strains had related ARGs (*tetB*, *ereB*).

It was demonstrated that β-lactam antibiotics were the most effective compounds in growth inhibition of *L. garvieae* (10). In our study, florfenicol and β-lactam antibiotics, including penicillin, amoxicillin, and ampicillin, were found to be the most effective agents against *L. garvieae* strains. However, bla_{TEM} ARG were found in 12 of all investigated strains, while bla_{PSE} and *ampC* genes were not detected in any of the strains. Florfenicol is a new antibiotic introduced to aquaculture a few years ago, and it has been successfully used for the treatment of bacterial fish diseases in Turkey (12). In the present study, most of the isolates (83.3%) were sensitive to this antibiotic. The *floR* ARGs were also detected in 14 (46.6%) isolates. It was demonstrated that the use of florfenicol has extremely increased in recent years.

Therapeutic options of antibiotics, including aminoglycoside and sulphonamides, may be limited in the case of infections caused by Gram-positive bacteria (18). For this reason, although *Lactococcus* isolates were resistant to sulphamethoxazole and gentamycin, most strains had no related ARGs. The investigation of the presence of ARGs in bacteria isolated from fish farms is important with regard to the potential transportation of ARGs from fish pathogens to human bacterial pathogens (16).

The results of this study revealed phenotypic and genotypic antibiotic resistance in *Lactococcus* spp. isolates, and provided insights into understanding resistance mechanisms of *Lactococcus* isolates in fish. There is a poor relationship between the phenotypic and genotypic resistance in *Lactococcus* strains. The study opens the opportunity to perform further studies that could determine the possible role of ARGs in fish.

Table 3. Presence of antimicrobial resistance genes in *Lactococcus* isolates

	ereA	*ereB*	*tetS*	*tetB*	*SulI*	*SulII*	*dhfrI*	*blaTEM*	*blaPSE*	*ampC*	*aadA*	*floR*
637-5	-	-	-	+	+	-	-	-	-	-	-	-
K9	-	+	-	+	-	-	-	-	-	-	-	-
Ard 30	-	+	-	+	-	-	+	-	-	-	-	-
Sider-17	-	+	-	+	-	-	-	-	-	-	-	-
İyi. Şfk.	-	+	-	+	-	+	-	-	-	-	-	-
225-1	-	+	-	+	+	+	-	-	-	-	-	-
Şer114	-	-	-	+	-	+	-	-	-	-	-	+
Trb	-	+	-	+	-	-	-	-	-	-	-	-
235-16	-	+	-	+	-	-	-	-	-	-	-	-
Muğ 1	-	+	-	+	-	+	-	-	-	-	-	-
Muğ 2	-	+	-	+	-	+	-	-	-	-	-	+
Lgper	-	+	-	+	-	-	-	+	-	-	-	+
M 300	-	+	-	+	-	-	-	-	-	-	-	-
G-27	-	+	-	+	-	+	-	-	-	-	-	-
PP6O	-	+	-	+	+	+	-	+	-	-	-	+
2398	-	-	-	+	-	-	+	-	-	-	-	-
1684	-	-	-	-	-	+	-	-	-	-	-	+
164 A/03	-	-	+	-	-	+	-	-	-	-	-	+
532	-	-	+	-	-	+	-	-	-	-	-	+
ATCC	-	-	-	-	-	+	-	-	-	-	-	+
Akoluk	-	-	-	-	-	-	-	+	-	-	-	-
399-18	-	+	-	+	-	-	-	+	-	-	-	+
Ö. Eskit	-	+	-	+	-	-	-	+	-	-	-	+
671-14	-	-	-	-	-	-	-	+	-	-	-	+
Muğ 3	-	-	-	+	-	-	-	+	-	-	-	-
A-58	-	+	-	+	-	-	-	+	-	-	-	-
FTPI	-	+	-	-	-	+	-	+	-	-	+	+
498	-	+	-	-	-	+	-	+	-	-	+	+
8053	-	+	-	-	-	-	-	+	-	-	-	+
L. lactis	-	-	-	-	-	-	+	+	-	-	+	-

The frequency of resistance to sulphamethoxazole + trimethoprim and gentamycin remained high in *L. garvieae*. Moreover, most of the strains possessed *tetB* and *ereB* ARGs. This study is important in terms of reporting antibiotic resistance genotype and phenotype of *Lactococcus* isolates, which may be a significant source of possible diseases occurring in Turkish aquaculture.

Conflict of Interest Statement: The authors declare that there is no conflict of interest.

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Animal Rights Statement: Experiments on animals were conducted in accordance with National and Institutional Guidelines for the protection of animal welfare.

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References

1. Acar J., Rostel B.: Antimicrobial resistance: an overview. Sci Tech Rev 2001, 20, 797–810.
2. Altinok I.: Multiplex PCR assay for detection of four major bacterial pathogens causing rainbow trout disease. Dis Aquat Organ 2011, 93, 199–206.
3. Arthur M., Autissier D., Courvalin P.: Analysis of the nucleotide sequence of the *ereB* gene encoding the erythromycin esterase type II. Nucleic Acids Res 1986, 14, 4987–4999.
4. Cabello F.C., Godfrey H.P., Tomova A., Ivanova L., Dölz H.: Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. Environ Microbiol 2013, 15, 1917–1942.
5. Chan J.F.W., Woo P.C.Y., Teng J.L.L., Lau S.K.P., Leung S.S.M., Tam F.C.C., Yuen K.Y.: Primary infective spondylodiscitis caused by *Lactococcus garvieae* and a review of human *L. garvieae* infections. Infection 2011, 39, 259–264.
6. Charpentier E., Gerbaud G., Courvalin P.: Characterization of a new class of tetracycline-resistance gene *tet*(S) in *Listeria monocytogenes* BM4210. Gene 1993, 131, 27–34.
7. CLSI.: Performance standarts for antimicrobial susceptibility testing; Twenty-first Informational Supplement, 2011.
8. Devriese L.A., Hommez J., Laevens H., Banadme P., Haesebrouck F.: Identification of aesculin hydrolyzing streptococci and enterococci from subclinical intramammary infections in dairy cows. Vet Microbiol 1999, 70, 87–94.
9. Diler O., Altun S., Adiloglu A.K., Kubilay A., Isikli B.: First occurrence of *Streptococcosis* affecting farmed rainbow trout (*Oncorhynchus mykiss*) in Turkey. Bull Eur Assn Fish P 2002, 22, 21–25.
10. Kav K., Erganis O.: Isolation, identification and determination of phenotypic characteristics of *L. garvieae* from rainbow trout (*Oncorhynchus mykiss*) farms in Konya, Turkey. Vet Bil Derg 2008, 23, 7–17.
11. Kawanishi A., Kojima A., Ishihara K., Esaki H., Kijima M., Takahashi T.: Drug resistance and pulsed-field gel electrophoresis patterns of *Lactococcus garvieae* isolates from cultured seriola (Yellowtail, Amberjack and Kingfish) in Japan. Lett Appl Microbiol 2005, 40, 322–328.
12. Kayis S., Capkin E., Balta F., Altinok I.: Bacteria in rainbow trout (*Oncorhynchus mykiss*) in the southern Black Sea Region of Turkey - A survey. Isr J Aquacult-Bamid 2009, 61, 339–344.
13. Kerrn M.B., Klemmensen T.F., Moller N., Espersen F.: Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. J Antimicrob Chemother 2002, 50, 513–516.
14. Kubilay A., Altun S., Ulukoy G., Diler O.: Determination of antimicrobial susceptibility of *Lactococcus garvieae* strains. S.D.Ü. Eğirdir Su Ür Fak Derg 2005, 1, 39–48.
15. Kusuda K., Kawai K., Salati F., Banner C.R., Fryer J.L.: *Enterococcus seriolicida* sp. nov., a fish pathogen. Int J Syst Bacteriol 1991, 41, 406–409.
16. Li J., Yie J., Foo R.W.T., Ling J.M.L., Xu H., Woo N.Y.S.: Antibiotic resistance and plasmid profiles of *Vibrio* isolates from cultured silver sea bream, *Sparus sarpa*. Mar Pollut Bull 1999, 39, 245–249.
17. Maki T., Hirono I., Kondo H., Aoki T.: Drug resistance mechanism of the fish-pathogenic bacterium *Lactococcus garvieae*. J Fish Dis 2008, 31, 461–468.
18. Muziasari W.I., Managaki S., Pärnänen K., Karkman A., Lyra C.: Sulphonamide and trimethoprim resistance genes persist in sediments at Baltic Sea aquaculture farms but are not detected in the surrounding environment. PLoS ONE 2014, 9, e92702. doi:10.1371/journal.pone.0092702.
19. Neela F.A., Nonaka L., Rahman M.H., Suzuki S.: Transfer of the chromosomally encoded tetracycline resistance genes *tet*(M) from marine bacteria to *Escherichia coli* and *Enterococcus faecalis*. World J Microbiol Biotechnol 2009, 25, 1095–1101.
20. Ng L.K., Martin I., Alfa M., Mulvey M.: Multiplex PCR for the detection of tetracycline resistant genes. Mol Cell Probe 2001, 15, 209–215.
21. Olesen I., Hasman H., Aarestrup F.M.: Prevalence of β-lactamases among ampicillin-resistant *Escherichia coli* and *Salmonella* isolated from food animals in Denmark. Microb Drug Resist 2004, 10, 334–340.
22. Ounissi H., Courvalin P.: Nucleotide sequence of the gene *ereA* encoding the erythromycin esterase in *Escherichia coli*. Gene 1985, 35, 271–278.
23. Schmidt A.S., Bruun M.S., Larsen J.L., Dalsgaard I.: Characterization of class 1 integrons associated with R-plasmids in clinical *Aeromonas salmonicida* isolates from various geographical areas. J Antimicrob Chemoth 2001, 47, 735–743.
24. Schwartz T., Kohnen W., Jansen B., Obst U.: Detection of antibiotic–resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiol Ecol 2003, 43, 325–335.
25. Sunde M., Nordstrom M.: The prevalence of associations between and conjugal transfer of antibiotic resistance genes in *Escherichia coli* isolated from Norwegian meat and meat products. J Antimicrob Chemoth 2006, 58, 741–747.
26. Tejedor J.L., Vela A.I., Gibello A., Casamayor A., Dominguez L., Garayzábal J.F.: A genetic comparison of pig, cow and trout isolates of *Lactococcus garvieae* by PFGE analysis. Lett Appl Microbiol 2011, 53, 614–619.
27. Turker H., Yildirim A.B., Karakas F.P.: Sensitivity of bacteria isolated from fish to some medicinal plants. Turk J Fish Aquat Sc 2009, 9, 181–186.
28. Van T.T.H., Chin J., Chapman T., Tran L.T., Coloe P.J.: Safety of raw meat and shellfish in Vietnam: an analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. Int J Food Microbiol 2008, 124, 217–223.
29. Vendrell D., Balcázar J.L., Ruiz-Zarzuela I., Gironés O., Múzquiz J.L.: *Lactococcus garvieae* in fish: A review. Comp Immunol Microb 2006, 29, 177–198.
30. Zühlsdorf M.T., Wiedemann B.: Tn21-specific structures in Gram–negative bacteria from clinical isolates. Antimicrob Agents Chemother 1992, 36, 1915–1921.