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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Phenotypic, Phylogenetic Characterization and Antimicrobial Susceptibility Determination of *Chryseobacterium piscicola* Isolates Recovered from Diseased Rainbow Trout

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Abstract: Twelve *Chryseobacterium piscicola* isolates recovered from rainbow trout weighing 1-4 grams showing signs of anorexia, exophthalmos, darkening, and dorsal fin erosion were used in our study. In addition to conventional microbiological tests, comprehensive phenotypic characterization has been performed using the Biolog GENIII microplate. Molecular identification and characterization were performed using the 16S rRNA region. Antimicrobial susceptibilities of the isolates were determined using the Kirby-Bauer disk diffusion method. Our isolates were identified as *C. piscicola* in molecular identification performed by sequence analysis based on the 16S rRNA region. In a phylogenetic analysis of our isolates, obtained from three different hosts in America, Chile, and Finland, five genogroups were determined with high similarity rate. Isolates from Finland, Chile, the United States, and Turkey (only C-316) were found in the same genogroup. It was determined that the phylogenetic analysis created with the 16S rRNA region could not distinguish the host from which the bacteria was isolated. The phenotypic characterization of six representative isolates selected according to phylogenetic analysis was determined with the Biolog GENIII microplate. Based on the Biolog GENIII results of the representative isolates, the results of 40 out of 94 tests were found to be variable. With this result, it was found that *C. piscicola* isolates were not phenotypically homogeneous. Besides, it was found that the zone diameters of our isolates against florfenicol, enrofloxacin, and sulfamethoxazole/trimethoprim were higher than the other isolates, in addition to that C-41 was the most resistant isolate.

Keywords: Antibiogram, *Chryseobacterium piscicola*, *Flavobacteriaceae*, Rainbow trout.

Hastalık Semptomları Gösteren Gökkuşağı Alabalıklarından İzole Edilen *Chryseobacterium piscicola* İzolatlarının Fenotipik, Filogenetik Karakterizasyonu ve Antimikrobiyal Duyarlılıklarının Belirlenmesi

Öz: Çalışmamızda iştahsızlık, ekzoftalmus, renkte kararma ve sırt yüzgeci erozyonu bulgularını gösteren 1-4 gram ağırlıkları arasında gökkuşağı alabalıklarından izole edilen oniki adet *Chryseobacterium piscicola* izolat kullanılmıştır. Konvansiyonel mikrobiyolojik testlerin yanısıra Biolog GENIII mikroplate kullanılarak geniş kapsamlı fenotipik karakterizasyon yapılmıştır. 16S rRNA bölgesi kullanılarak moleküler identifikasyon ve karakterizasyon yapılmıştır. Çalışmada kullanılan izolatların antimikrobiyal duyarlılıkları Kirby-Bauer disk difüzyon yöntemi kullanılarak belirlenmiştir. 16S rRNA bölgesine dayalı dizi analizi ile yapılan moleküler identifikasyonda izolatlarımız %99 oranında *C. piscicola* olarak tanımlanmıştır. İzolatlarımız ile Amerika, Şili ve Finlandiya'daki üç farklı konakçıdan elde edilen izolatlarla yapılan filogenetik analizde, beş farklı genogrup yüksek benzerlik oranı ile belirlenmiştir..

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Finlandiya, Şili ve ABD'den izole edilen izolatlar ve C-316 (Türkiye) izolatı aynı genogrupta bulunmuştur. 16S rRNA bölgesi ile oluşturulan filogenetik analizin bakterinin izole edildiği konakçı ayrımının yapamadığı görülmüştür. Filogenetik analize göre seçilen altı temsili izolatın fenotipik özellikleri Biolog GENIII mikroplakası ile belirlenmiştir. Temsili izolatların Biolog GENIII sonuçlarına göre, 94 testin 40'ının sonuçlarının değişken olduğu bulunmuştur. Bu sonuçla, *C. piscicola* izolatlarının fenotipik olarak homojen bir yapıda olmadığı görülmüştür. Ayrıca çalışmamızda izolatlarımızın florfenikol, enrofloksasin ve sülfametoksazol/trimetoprime karşı oluşan zon çaplarının diğerlerine göre daha yüksek olduğu ve C-41'in en dirençli izolat olduğu bulunmuştur.

Anahtar kelimeler: Antibiyogram, *Chryseobacterium piscicola*, *Flavobacteriaceae*, Gökkuşağı alabalığı.

INTRODUCTION

Economic loss due to fish diseases in aquaculture varies according to the farming conditions but varies between 10-40% on average. The *Flavobacteriaceae* family has an extensive ecological habitat. The species in this family can also cause diseases in invertebrates, amphibians, reptiles, birds, and even mammals. Among the species that cause infection in fish in the *Flavobacteriaceae* family, there are important species such as *Flavobacterium* spp., *Tenacibaculum* spp., and *Chryseobacterium* spp. (Hugo et al., 2020; Loch & Faisal, 2015). Until now, 120 different *Chryseobacterium* species have been reported (Parte, 2018). *Chryseobacterium* species are gram-negative, oxidase and catalase-positive, non-motile, colonies range from pale to a bright yellow color due to the presence of non-diffusible flexirubin type pigment. Colonies have not to display gliding or swarming motility. Members of the genus *Chryseobacterium* grow well on commercial media (such as Tryptic Soy Agar, Brain Heart Infusion agar, or Blood agar) ranged between 4–42°C incubation temperature (Hugo et al., 2020). It has been reported that *Chryseobacterium* species have been isolated from different clinical cases (pneumonia, peritonitis, surgical wound infections, burn wounds, eye infections, pneumonia in newborns) in human medicine (Holmes et al., 1984; Hugo et al., 2020; Kämpfer et al., 2009; Loch & Faisal, 2015; Vaneechoutte et al., 2007).

While *Chryseobacterium* spp. have not been reported as a pathogen for domestic animals, there are many reports from the aquatic ecosystem and aquatic animals. Infections caused by *Chryseobacterium* species such as *C. piscicola*, *C. chaponense*, *C. aahli*, *C. oncorhynchi*, and *C. joostei* in fish have increased in the last decade (Didinen et al., 2016; Hugo et al., 2020; Loch & Faisal, 2014; Loch & Faisal, 2015). There are not many studies on whether the detected *Chryseobacterium* species are the main cause of the disease. A limited number of studies, *C. balustinum*, *C. piscicola,* and *C. aahli,* have been reported to fulfill the Koch postulates, but there are no studies on other *Chryseobacterium* species. *C. piscicola* has been reported to cause mortalities in farmed Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) in Chile and Finland (Hugo et al., 2020; Ilardi et al., 2009; Ilardi et al., 2010; Loch et al., 2013).

Our study aimed to determine the detailed phenotypic, molecular, and antimicrobial susceptibilities of twelve *C. piscicola* isolates recovered from rainbow trout showing disease symptoms.

MATERIAL AND METHOD

Bacterial Isolate and Phenotypic Characterization: In our study, the bacteria were isolated from rainbow trout weighing 1-4 grams in the trout farm located in Aegean, Eastern Anatolia, and Central Anatolia region between 2013 and 2017, showing signs of loss of appetite, exophthalmos, darkening in color, and dorsal fin erosion. This research was approved by Bursa Uludag University, the Local Ethics Commission (report 2012-02/05)

In bacteriological isolation, Tryptone Yeast Extract Salts (TYES) agar was used, and the isolates were incubated at 15°C for 72 hours. Conventional microbiological tests such as colony morphology, gram staining, motility, oxidase, presence, or absence of flexirubin pigment, catalase activity were used in phenotypic identification of isolates (Ilardi et al., 2009). The morphological and biochemical profiles of isolates were determined using the Biolog GENIII microplate with 94 different tests. Unlike the manufacturer's protocol, the incubation temperature and time were modified to optimum growth values of *C. piscicola* (72 hours incubation at 15°C).

Antimicrobial Susceptibility Testing (AST): Antimicrobial susceptibility level was determined by the Kirby-Bauer Disk Diffusion test against amoxicillin (CT0061B,25µg), oxytetracycline (CT0041B, 30µg), oxolinic acid (CT0181B, 2µg), sulfamethoxazole/trimethoprim (CT0052B, 25µg), enrofloxacin (CT0639B, 5µg), erythromycin (CT0020B, 15µg), and florfenicol (CT1754B, 30µg), Oxoid discs were

used for the test. *E. coli* ATCC 25922 was used as a quality control (QC) strain (CLSI, 2014). After incubation at 15 °C for 48-72h, the inhibition zone diameter of antibiotic discs was measured (Ilardi et al., 2009; Michel et al., 2005).

Molecular Identification and Characterization of *C. piscicola:* According to the manufacturer's instructions, the isolates' DNA was extracted with a spin colon filtration method (QIAamp DNA mini kit). Identification and sequence analysis were performed using universal primers 27F (5′-AGA GTT TGA TCM TGG CTC 118 AG-3′) and 1387R (5′-GGG CGG WGT GTA CAA GGC-3′), which amplified the 16S rRNA gene region of bacteria (Loch et al., 2013). The chromatograms obtained were aligned and compared with other isolates registered in the database using GenBank and BLAST (Basic Local Alignment Search Tool) server in the National Center for Biotechnology Information (NCBI).

The sequences of our isolates and retrieved sequences from the NCBI database that was recovered from different fish species and countries, such as rainbow trout (Chile), Atlantic salmon (Chile and Finland), and brown trout (USA), were compared by a phylogenetic tree. Phylogenetic trees of all isolates were constructed based upon partial 16S rRNA sequences. The DNA sequences were aligned using the multiple alignment program of Bionumerics software (Applied Maths, Belgium). The phylogenetic tree was constructed by the unweighted pair group method with arithmetic mean (UPGMA) method with 1000 bootstraps replicates using Bionumerics software (Saticioglu et al., 2018; Schloss & Westcott, 2011).

RESULTS AND DISCUSSION

The significance of *Chryseobacterium* infections in veterinary medicine is mostly limited to fish, though cases of hemorrhagic septicemia caused by *C. indologenes* were reported from both wild and captive leopard frogs and bullfrogs (Hugo et al., 2020; Loch & Faisal, 2015). Hence, *Chryseobacterium* species represent emerging fish pathogens with a significant impact on fresh- and sea-water fisheries worldwide. However, only the pathogenicity of *C. aahli*, *C. piscicola*, and *C. balustinum* in fish was confirmed by experimental infection. *C. piscicola* was isolated from Atlantic Salmon with ulcerative skin and muscle lesions in Chile in the first (Ilardi et al., 2009). It was later found to occur in the diseased Atlantic salmon and hatchery-reared brown trout in Finland and the USA, respectively (Ilardi et al., 2010; Loch et al., 2013). In our study, *C. piscicola* isolates were recovered from rainbow trout, weighing between 1 and 4 g, showing disease signs such as exophthalmos, darkening in color, and dorsal fin erosion. Thus, the clinical signs are similar to the previous reports, and no mass mortality was observed in fish. With the earlier reports, our study supports the pathogenic characteristic of *C. piscicola* for rainbow trout.

Ilardi et al.(2009, 2010) were described the type strain of *C. piscicola* as a gram-negative, non-motile, catalase, oxidase, production of flexirubin pigments, and hydrolysis of gelatin and esculin positive, but tyrosine, agar, starch, and casein are negative. Based on the conventional microbiological test results in the present study, all isolates were found as gram-negative, non-motile, oxidase catalase, and production of flexirubin pigment positive. Phenotypic characteristics of six representative isolates selected according to phylogenetic analysis were determined by the Biolog GENIII microplate. This microplate analyzes a microorganism in 94 phenotypic tests: 71 carbon source utilization assays and 23 chemical sensitivity assays. We described the first time phenotypic fingerprint of *C. piscicola*. Based on Biolog GENIII results of representative isolates in 40 of 94 tests (including utilization of D-Cellobiose, D-Turanose, D-Salicin, D-Galactose, etc.) were found as a variable (Table 1). It was concluded that *C. piscicola* isolates seem to be phenotypically heterogeneous.

Table 1. Biolog GENIII results of representetive six *C. piscicola* isolates.

	Dextrin	D-Maltose	D-Trehalose	D-Cellobiose	Gentiobiose	Sucrose	D-Turanose	D-Turanose	Positive Control	pH 6	pH 5
	+	+	+	4/6	+	+	2/6	4/6	+	+	-
D-Raffinose	α-D-Lactose	D-Melibiose	β-Methyl-DGlucoside	D-Salicin	N-Acetyl-D-Glucosamine	N-Acetyl-β-DMannosamine	N-Acetyl-DGalactosamine	N-Acetyl Neuraminic Acid	1% NaCl	4% NaCl	8% NaCl
5/6	1/6	3/6	4/6	3/6	4/6	+	3/6	4/6	+	-	-
α-D-Glucose	D-Mannose	D-Fructose	D-Galactose	3-Methyl Glucose	D-Fucose	L-Fucose	L-Rhamnose	Inosine	1% Sodium Lactate	Fusidic Acid	D-Serine
+	+	+	3/6	4/6	2/6	4/6	+	5/6	+	-	-
D-Sorbitol	D-Mannitol	D-Arabitol	Myo-inositol	Glycerol	D-Glucose 6-PO4	D-Fructose6-PO4	D-Aspartic Acid	D-Serine	Troleandomycin	Rifamycin SV	Minocycline
3/6	4/6	4/6	4/6	4/6	+	+	-	-	5/6	+	-
Gelatin	Glycyl-L-Proline	L-Alanine	L-Arginine	L-Aspartic Acid	L-Glutamic Acid	L-Histidine	L-Pyroglutamic Acid	L-Serine	Lincomycin	Guanidine HCl	Niaproof 4
+	+	-	+	+	+	-	3/6	1/6	-	1/6	-
Pectin	D-Galacturonic Acid	L-Galactonic Acid Lactone	D-Gluconic Acid	D-Glucuronic Acid	Glucuronamide	Mucic Acid	Quinic Acid	D-Saccharic Acid	Vancomycin	Tetrazolium Violet	Tetrazolium Blue
+	+	+	1/6	+	1/6	+	4/6	4/6	-	+	+
p-Hydroxy Phenylacetic Acid	Methyl Pyruvate	D-Lactic Acid Methyl Ester	L-Lactic Acid	Citric Acid	α-Keto-Glutaric Acid	D-Malic Acid	L-Malic Acid	Bromo-Succinic Acid	Nalidixic Acid	Lithium Chloride	Potassium Tellurite
-	4/6	+	5/6	4/6	1/6	2/6	1/6	-	3/6	-	-
Tween 40	γ-Amino-Butryric Acid	α-Hydroxy Butyric Acid	β-Hydroxy-D, LButyric Acid	α-Keto-Butyric acid	Acetoacetic Acid	Propionic Acid	Acetic Acid	Formic Acid	Aztreonam	Sodium Butyrate	Sodium Bromate
+	3/6	1/6	2/6	-	1/6	-	+	-	+	-	-

+: All isolates are positive, **-:** All isolates are negative, **x/y:** Positive isolates/all isolates ratio.

Five different antimicrobial drugs have been licensed by the Ministry of Agriculture and Forestry to treat fish diseases in Turkey. Among these products, the most commonly used antimicrobials in aquaculture; florfenicol, oxytetracycline and sulfadiazine/trimethoprim. Numerous reports have been reported on the development of antimicrobial resistance in bacteria isolated from aquaculture (Balta et al., 2010; Balta et al., 2016; Duman et al., 2017; Durmaz et al., 2012; Onuk et al., 2017; Saticioglu et al., 2019). Aquatic and other environmental organisms play a significant role in developing, acquiring, and spreading antimicrobial resistance (Fletcher, 2015; Taylor et al., 2011). It has been reported that many species in the *Flavobacteriaceae* family isolated from fish and clinical cases in humans can grow even at high concentrations of antimicrobials (Verner-Jeffreys et al., 2017). However, there is no antimicrobial susceptibility determination and interpretation model for *Chryseobacterium* species in EUCAST and CLSI standards. In this context, we adapted the antimicrobial susceptibility test protocol to previous studies on *Chryseobacterium* species (Loch et al., 2013; Michel et al., 2005). Different species in the *Chryseobacterium* genus have been reported resistant to tetracyclines, erythromycin, and linezolid, but susceptibility to vancomycin, clindamycin, or sulfamethoxazole-trimethoprim is found variable. It is assumed that *Chryseobacterium* species is intrinsically resistant to many antibiotics class, including polymyxins, aminoglycosides (streptomycin, gentamicin, and kanamycin), β-lactams (penicillins, cephalosporins, and carbapenems) and chloramphenicol (Hugo et al., 2020; Michel et al., 2005; Verner-Jeffreys et al., 2017). In previous studies using *C. piscicola* isolates, no antibiotic susceptibility characterization was determined. When the zone diameters of our isolates were examined, it was seen that the zone diameters of florfenicol, enrofloxacin, and sulfamethoxazole/trimethoprim were higher than the others (Table 2). C-41 was found to be the most resistant isolate. While the isolates have not assumed a major pathogen or primary pathogen in aquaculture, they have acquired resistance for many antimicrobials. This situation shows that antimicrobial resistance is not a major problem for only primary or well-known pathogens.

Table 2. Inhibition zones (mm) of isolates against selected antibiotics.

Isolate No	E 15µg	OA 2µg	SXT 25µg	AML 25µg	OT 30µg	FFC 30 µg	ENR 5µg
C-28	12	10	15	6	15	16	20
C-41	0	0	15	3	0	0	10
C-46	20	0	15	18	5	10	10
C-48	12	15	17	10	13	18	21
C-49	8	12	13	10	10	15	15
C-50	13	12	17	7	10	18	17
C-54	20	20	10	20	5	20	20
C-55	10	13	12	10	16	20	19
C-56	15	0	12	22	7	20	10
C-316	13	13	15	10	15	20	20
C-356	13	10	13	10	10	0	0
C-357	10	5	15	0	12	0	17

E: erythromycin, **OA:** Oxolinic acid, **SXT:** Sulfamethoxazole/trimethoprim, **AML:** Amoxicillin, **OT:** Oxytetracycline, **FFC:** Florfenicol, **ENR:** Enrofloxacin

Our isolates were identified as *C. piscicola*, based on high index similarities with the 16S rRNA reference sequence registered to the GenBank database (similarity of each isolate was 99% and above). Each isolates sequence was registered in the GenBank database with accession numbers ranging from MW072838 to MW072849. The phylogenetic trees were constructed using the 16S rRNA gene sequences of other *C. piscicola* isolates from three different countries registered in the GenBank database. In this study, phylogenetic analysis *of C. piscicola* isolates recovered from different countries and hosts were determined for the first time. It was found that *Elizabethkingia meningoseptica*, which is in the *Flavobacteriaceae* family selected as an outgroup, showed a 93.4% similarity from *C. piscicola* isolates. Our isolates were assigned to five genogroups with a very high similarity ratio (Figure 1). The majority of the isolates were in genogroup 2. Isolates from Finland, Chile, and the USA and C-316 (Turkey) were found the same in genogroup. In the phylogenetic analysis created with the 16S rRNA region, it was observed that the host where the bacteria was isolated could not be distinguished.

CONCLUSION

This study is the first in which comprehensive phenotypic tests of *C. piscicola* were performed, and its antimicrobial susceptibilities were determined. *C. piscicola* isolates are phenotypically heterogeneous, as seen from detailed phenotypic tests on representative isolates that differ phylogenetically. Besides, this study showed that the 16S rRNA region of *C. piscicola* is insufficient to distinguish between host and geographic regions.

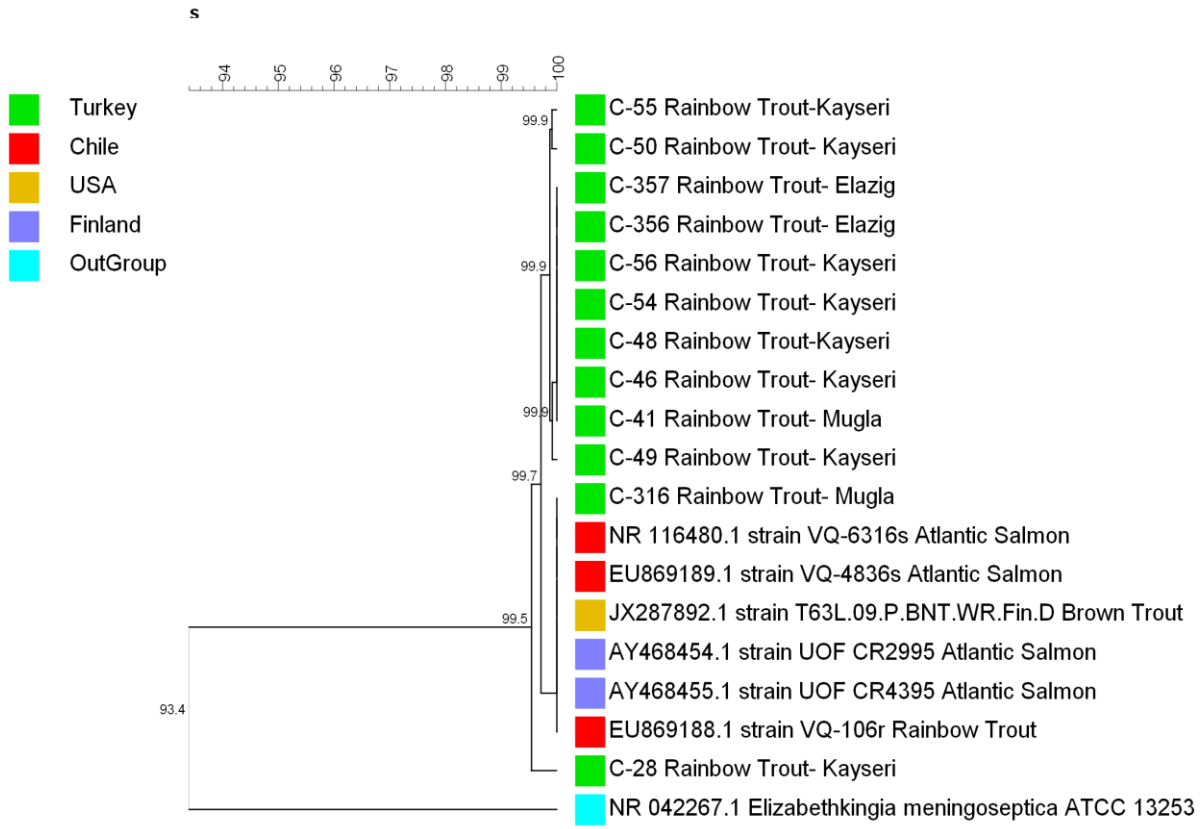

Figure 1. The phylogenetic tree of *C. piscicola* isolates based on the 16S rRNA gene sequence.

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REFERENCES

Balta, F., Sandalli, C., Kayis, S. & Ozgumus, O.B. (2010). Molecular analysis of antimicrobial resistance in *Yersinia ruckeri* strains isolated from rainbow trout (*Oncorhynchus mykiss*) grown in commercial fish farms in Turkey. *Bulletin of the European Association of Fish Pathologists*, ***30***(6), 211-219.

Balta, F., Dengiz Balta, Z., Özgümüş, O.B. & Çağirgan, H. (2016). The Antimicrobial resistance and investigation of *Yersinia ruckeri* from rainbow trout (*Oncorhynchus mykiss*) farms in the Eastern Black Sea Region. *Journal of Anatolian Environmental and Animal Sciences*, 1(3), 72-76.

CLSI. (2014). Performance standards for antimicrobial susceptibility testing of bacteria isolated from aquatic animals; second informational supplement. CLSI document VET03/04-S2. *Clinical and Laboratory Standards Institute*, PA.

Didinen, B.I., Onuk, E.E., Ozturk, T., Metin, S., Meryem, Ö.Z., Cayli, Ö. & Kubilay, A. (2016). First report of *Chryseobacterium* sp. from koi (*Cyprinus carpio*) in Turkey. *The Israeli Journal of Aquaculture-Bamidgeh*, ***68***, 1-8.

Duman, M., Saticioglu, I.B., Buyukekiz, A.G., Balta, F. & Altun, S. (2017). Molecular characterization and antimicrobial resistance profile of atypical *Citrobacter gillenii* and *Citrobacter* sp. isolated from diseased rainbow trout (*Oncorhynchus mykiss*). *Journal of Global Antimicrobial Resistance*, ***10***, 136-142.

Durmaz, Y., Onuk, E.E. & Ciftci, A. (2012). Investigation of the presence and antibiotic susceptibilities of *Flavobacterium psychrophilum* in rainbow trout farms (*Oncorhynchus mykiss* Walbaum, 1792) in The Middle and Eastern Black Sea Regions of Turkey. *Ankara Üniv. Vet. Fak. Derg*, ***59***, 141-146.

Fletcher, S. (2015). Understanding the contribution of environmental factors in the spread of antimicrobial resistance. *Environmental Health and Preventive Medicine*, ***20***(4), 243-252.

Holmes, B., Owen, R. J., Steigerwalt, A.G. & Brenner, D.J. (1984). *Flavobacterium gleum*, a new species found in human clinical specimens. *International Journal of Systematic and Evolutionary Microbiology*, ***34***(1), 21-25.

Hugo, C., Bernardet, J.F., Nicholson, A. & Kämpfer, P. (2020). *Chryseobacterium*. In B. H. and S. D.

W.B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos (Ed.), *Bergey's Manual of Systematics of Archaea and Bacteria* (1–107pp). Wiley & Sons.

Ilardi, P., Fernandez, J. & Avendaño-Herrera, R. (2009). *Chryseobacterium piscicola* sp. nov., isolated from diseased salmonid fish. *International Journal of Systematic and Evolutionary Microbiology*, ***59***(12), 3001-3005.

Ilardi, P., Abad, J., Rintamäki, P., Bernardet, J. & Avendaño-Herrera, R. (2010). Phenotypic, serological and molecular evidence of *Chryseobacterium piscicola* in farmed Atlantic salmon, *Salmo salar* L., in Finland. *Journal of Fish Diseases*, ***33***(2), 179-181.

Kämpfer, P., Vaneechoutte, M., Lodders, N., De Baere, T., Avesani, V., Janssens, M., Busse, H.J. & Wauters, G. (2009). Description of *Chryseobacterium anthropi* sp. nov. to accommodate clinical isolates biochemically similar to *Kaistella koreensis* and *Chryseobacterium haifense*, proposal to reclassify *Kaistella koreensis* as *Chryseobacterium koreense* comb. nov. and emended d. *International Journal of Systematic and Evolutionary Microbiology*, ***59***(10), 2421-2428.

Loch, T.P., Fujimoto, M., Woodiga, S.A., Walker, E.D., Marsh, T.L. & Faisal, M. (2013). Diversity of Fish-Associated Flavobacteria of Michigan. *Journal of Aquatic Animal Health*, ***25***(3), 149-164.

Loch, T.P. & Faisal, M. (2014). *Chryseobacterium aahli* sp. nov., isolated from lake trout (*Salvelinus namaycush*) and brown trout (*Salmo trutta*), and emended descriptions of *chryseobacterium ginsenosidimutans* and *chryseobacterium gregarium*. *International Journal of Systematic and Evolutionary Microbiology*, ***64***(5), 1573-1579.

Loch, T.P. & Faisal, M. (2015). Emerging flavobacterial infections in fish: a review. *Journal of Advanced Research*, ***6***(3), 283-300.

Michel, C., Matte-Tailliez, O., Kerouault, B. & Bernardet, J.F. (2005). Resistance pattern and assessment of phenicol agents' minimum inhibitory concentration in multiple drug resistant *Chryseobacterium* isolates from fish and aquatic habitats. *Journal of Applied Microbiology*, ***99***(2), 323-332.

Onuk, E.E., Tanriverdi, Ç.Y., Çoban, A.Y., Ciftci, A., Balta, F., Didinen, B.I. & Altun, S. (2017). Determination of antimicrobial susceptibility patterns of fish and rearing water originated *Aeromonas* isolates. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, ***64***(1), 69-73.

Parte, A.C. (2018). LPSN–List of Prokaryotic names with Standing in Nomenclature (bacterio. net), 20 years on. *International Journal of Systematic and Evolutionary Microbiology*, ***68***(6), 1825-1829.

Saticioglu, I.B., Duman, M. & Altun, S. (2018). Antimicrobial resistance and molecular characterization of *Pantoea agglomerans* isolated from rainbow trout (*Oncorhynchus mykiss*) fry. *Microbial Pathogenesis*, ***119***, 131-136.

Saticioglu, I.B., Duman, M., Smith, P., Wiklund, T. & Altun, S. (2019). Antimicrobial resistance and resistance genes in *Flavobacterium psychrophilum* isolates from Turkey. *Aquaculture*, ***512***, 734293.

Schloss, P.D. & Westcott, S.L. (2011). Assessing and improving methods used in operational taxonomic unit-based approaches for 16S rRNA gene sequence analysis. *Applied and Environmental Microbiology*, ***77***(10), 3219-3226.

Taylor, N.G.H., Verner-Jeffreys, D.W. & Baker-Austin, C. (2011). Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance? *Trends in Ecology & Evolution*, ***26***(6), 278-284.

Vaneechoutte, M., Kämpfer, P., De Baere, T., Avesani, V., Janssens, M. & Wauters, G. (2007). *Chryseobacterium hominis* sp. nov., to accommodate clinical isolates biochemically similar to CDC groups II-h and II-c. *International Journal of Systematic and Evolutionary Microbiology*, ***57***(11), 2623-2628.

Verner-Jeffreys, D.W., Brazier, T., Perez, R.Y., Ryder, D., Card, R.M., Welch, T. J. & Bartie, K.L. (2017). Detection of the florfenicol resistance gene *flo*R in *Chryseobacterium* isolates from rainbow trout. Exception to the general rule?. FEMS Microbiology Ecology, ***93***(4), fix015.