Phenotypic characteristics of *Listonella anguillarum* strains isolated from rainbow trout (*Oncorhynchus mykiss*, Walbaum) farms in Turkey

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Summary: In the present study, nine isolates of *Listonella anguillarum* were characterized isolated from epizootics at five rainbow trout fresh water farms in Middle and Mediterranean region of Turkey. Infected rainbow trout exhibited lethargy, dark skin, bilateral exophthalmos, spilling of scales, furuncles on the sides of the body, haemorrhage in the skin, mouth, eyes, fins and anus. Necropsy findings included haemorrhage in the liver, adipose tissue and muscles, pale kidney and liver, enlarged spleen, ascites in the body cavity, yellowish-bloody fluid in the stomach and intestine. Samples for bacteriological examinations were collected from the kidney and liver by using sterile swabs; these samples were streaked onto trypticase soy agar supplemented with 1% NaCl plates, and incubated at 25°C for 48 h. Nine bacterial isolates were obtained from sick fish. *L.anguillarum* isolates was identified by conventional methods and API 20 E. Phenotypic characteristics of the isolates were found as homogenous. All of *L.anguillarum* isolates were sensitive to trimethoprim, tetracycline, oxytetracycline, enoxacin and resistance to flucloxacillin, sulfamethoxazole, amoxicillin and ampicillin.

Key words: *Listonella anguillarum*, *Oncorhynchus mykiss*, phenotypic characteristics, rainbow trout, vibriosis.

Türkiye'de gökkuşağı alabalığı çiftliklerinden izole edilen *Listonella anguillarum* suşlarının fenotipik özellikleri

Özet: Bu çalışmada Türkiye'de İç Anadolu ve Akdeniz bölgesinde bulunan 5 gökkuşağı alabalığı çiftliklerinde meydana gelen salgınlardan izole edilen 9 *Listonella anguillarum* izolatının fenotipik özellikleri belirlenmiştir. Enfekte gökkuşağı alabalıklarında letarji, deri renginde koyulaşma, bilateral ekzoftalmus, pullarda dökülme, vücut yüzeyinde furunkuller, deri, ağız, göz, yüzgeç ve anüste hemorajiler görülmüştür. İç bakıda asites, karaciğer, yağ doku ve kaslarda hemoraji, karaciğer ve böbrekte solgunluk, dalakta genişleme, mide ve bağırsakta sarı renkli kanlı sıvı birikimi tespit edilmiştir. Bakteriyolojik incelemeler için karaciğer ve böbrekten alınan örnekler, %1 NaCl ilave edilmiş Triptik Soy Agara ekilmiş ve 25°C de 48 saat süre ile inkübe edilmiştir. *L.anguillarum* izolatları (n=9) geleneksel bakteriyolojik yöntemler ve API 20 E kullanılarak identifiye edilmiştir. İzolatların fenotipik özelliklerinin homojen olduğutespit edilmiştir. Yapılan antibiyogram testinde tüm izolatların trimethoprim, tetrasiklin, oksitetrasiklin, enoksasin'e duyarlı ve flukloksasillin, sülfamethakzol, amoksisillin ve ampisilline karşı dirençli oldukları belirlenmiştir.

Anahtar kelimeler: Fenotipik özellikler, gökkuşağı alabalığı, *Listonella anguillarum*, *Oncorhynchus mykiss*, vibriosis.

Introduction

Vibriosis is one of the most prevalent fish diseases caused by several bacterial species belonging to the genus *Vibrio.*

Bacteria species belong to *Vibrio* genus exist normally in the microflora of intestine and skin of healthy marine and freshwater fishes. However, epizootics of vibriosis appear to the stressing conditions such as grading, transporting, malnutrition, negative environmental status and unsuitable water quality. *Listonella anguillarum* is the causative agent of vibriosis isolated from marine fishes, mainly sea bream, sea bass (1, 3, 8, 12, 20). However, vibriosis infections have been reported in salmonid fish especially, rainbow trout in the past years (9, 11, 13, 14, 19). Recently, has reported that it also caused important losses in rainbow trout (*Oncorhynchus mykiss*) cultured in freshwater in Turkey (16, 17, 18).

The aim of present study was to determine the phenotypic characteristics and antimicrobial sensi-

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tivity caused by *L.anguillarum* isolated from rainbow trout in different farms located in the Middle and Mediterranean regions of Turkey.

Materials and Methods

Sampling: The outbreaks were occurred rainbow trout (body weight 50-300 g) in five different fresh water farms in the Middle and Mediterranean regions of Turkey. Samples came from 5 fish farms during 2004-2005. The postmortem examinations of external and internal disease signs were performed.

Isolation and identification of bacteria: For bacterial isolation, samples were obtained from the kidney and liver of 10 fish, streaked on trypticase-soy agar (TSA, Merck) supplemented with 1% NaCl plates, and incubated at 25°C for 48 h. Single colonies were restreaked on the same media to obtain pure isolates. Isolated colonies were identified by physiological and biochemical characterization. Routine tests were carried out for determination of biochemical characteristics of the bacteria, as described previously (6, 7, 10).

A presumptive identification of the strain was performed using the following tests: Gram staining, motility, fermentative degradation of glucose with O/F basal medium (Merck) supplemented with 1% glucose, oxidase, catalase, production of indole, citrate utilization, hydrolysis of starch and gelatine, Methyl red-Voges proskouer tests, production of H_2S, growth at peptone water with 0, 2, 7, 8 and 10% NaCl, growth in thiosulphate citrate bile salts sucrose agar (TCBS), growth at Tryptic Soy Broth at 4°C, 25°C, 37°C, inhibition by the vibriostatic agent O/129 (2,4-diamino-6,7-di isopropylpteridine; Oxoid) and fermentation of carbohydrates (glucose, arabinose, inositol, saccharose, maltose, fructose, lactose, galactose, rhamnose, mannitol, sorbitol, amygdalin, mellibiose). Additional test were performed using API 20 E (bioMérieux) (2). In all the tests, *L.anguillarum* ATCC 14181 was used as a reference.

Antimicrobial sensitivity: Antimicrobial tests were performed on the isolate at 25°C by using a disc diffusion assay on Mueller-Hinton agar (Oxoid). CLSI standards were used for the evaluation of the results (5).

Results

Necropsy findings of moribund rainbow trout included haemorrhage in the liver, adipose tissue and muscles, pale kidney and liver, ascites in the body cavity, yellowish-bloody fluid in the stomach and the intestine, enlarged spleen. The external signs were lethargy, dark skin, exophthalmos, spilling of scales, haemorrhage and furuncle in skin, haemorrhage in the eyes, the fins, the mouth and the anus (Figure 1). The mortality changed from 25 to 30% in the farms.

For isolation, samples taken from kidneys and livers of sick fishes streaked onto tryptic soy agar (TSA, Merck) supplemented with 1% NaCl. Colonies on the TSA appear white, entire, smooth, convex and 2-3 mm in diameter after 48 h growth at 25°C. Isolates were found to be Gram negative, straight or slightly curved rods, fermentative, motile, producing yellow colonies on TCBS Agar, positive for oxidase, catalase and hydrolysis of gelatine and sensitive to 0/129 Vibriostat test (10 and 150 µg), positive for indol production, gas production from saccharose and mannitol and negative for decarboxylation of lysine and ornithine (Table 1).

All *L.anguillarum* isolates were sensitive to oxytetracycline, tetracycline, enoxacin and trimethoprim and resistant to sulfamethoxazole, amoxicillin, ampicillin and flucloxacillin. However, the isolates showed different antimicrobial sensitivities for trimethoprim+ sulfamethoxazole, doxycycline and furazolidone (Table 2).

Table 1. The phenotypic characteristics of *L.anguillarum* strains

	L.anguillarum strains									
Charecteristics	A1	B1	K1	C1	C2	C3	A4	A6	M1	ATCC 14181
Growth in TCBS	+, Y	+,Y	+,Y	+, Y	+, Y	+, Y	+, Y	+, Y	+, Y	+, Y
Gr staining	–	–	–	–	–	–	–	–	–	–
Motility	+	+	+	+	+	+	+	+	+	+
S.O	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
O/F	F	F	F	F	F	F	F	F	F	F
O/129 (10µg)	S	S	S	S	S	S	S	S	S	S
O/129 (150µg)	S	S	S	S	S	S	S	S	S	S
Arginine dihydrolase	+	+	+	+	+	+	+	+	+	+
β galactosidase	+	+	+	+	+	+	+	+	+	+
Lysine decarboxylase	–	–	–	–	–	–	–	–	–	–
Ornithine decarboxylase	–	–	–	–	–	–	–	–	–	–
H_2S	–	–	–	–	–	–	–	–	–	–
Urea	–	–	–	–	–	–	–	–	–	–
Hydroliyis of gelatine	+	+	+	+	+	+	+	+	+	+
Hydrolysis of starch	+	+	+	+	+	+	+	+	+	+
Production of indole	+	+	+	+	+	+	+	+	+	+
MR	+	+	–	–	+	+	–	–	–	–
VP	–	–	+	+	+	+	+	+	+	+
NO_3 reduction	+	+	+	+	+	+	+	+	+	+
Growth:										
in 0% NaCl	+	+	+	+	+	+	+	+	+	+
in 2% NaCl	+	+	+	+	+	+	+	+	+	+
in 7% NaCl	+	+	+	+	+	+	+	+	+	–
in 8% NaCl	–	–	–	–	–	–	–	–	–	–
in 10% NaCl	–	–	–	–	–	–	–	–	–	–
at 4°C	+	+	+	+	+	+	+	+	+	+
at 25°C	+	+	+	+	+	+	+	+	+	+
at 37°C	+	+	+	+	+	+	+	+	+	+
at 42°C	+	+	+	+	+	+	+	+	+	+
Utilization of citrate	+	+	+	+	+	+	+	+	+	+
Fermentation of carbohydrates										
Glucose	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	–	–	+	–
Inositol	–	–	–	–	–	–	–	–	–	–
Saccharose	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+
Lactose	–	–	–	–	–	–	–	–	–	–
Galactose	+	+	+	+	+	+	+	+	+	+
Rhamnose	–	–	–	–	–	–	–	–	–	–
Mannitol	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	+
Amygdalin	–	–	–	–	–	–	–	–	–	–
Mellibiose	–	–	–	–	–	–	–	–	–	–

(+): positive, (-): negative, (Y): Yellow; (F): fermantative; A, B, C, K, M codes refer to different farms: A, B, C, M in Mediterranean region, K in Middle region

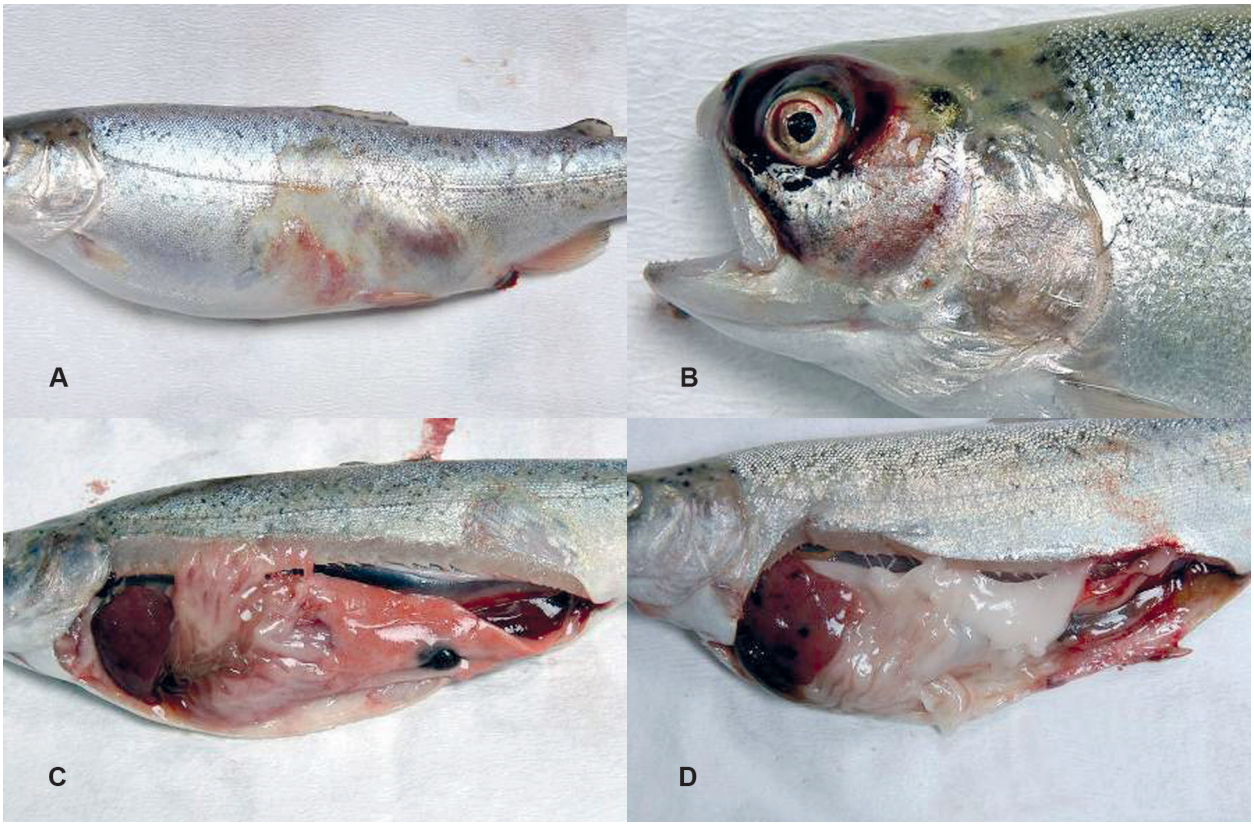

Figure 1. Rainbow trout naturally infected with *L.angullarum*. a) Furuncle, spilling of scales and hemorrhage on the side of the body. b) Exophthalmos and hemorrhage in eye. c) Hyperemia and hemorrhage in adipose tissue. d) Hemorrhage in the liver

Table 2. Antibiotics sensitivities of *L.anguillarum* strains

	L.anguillarum strains								
Antibiotics (µg/disc)	A1	B1	K1	C1	C2	C3	A4	A6	M1
Oxolinic Acid (2)	S	S	S	S	S	S	R	R	R
Amoxicillin (25)	R	R	R	R	R	R	R	R	R
Trımethoprim+Sulfamethoxazole (25)	S	S	S	S	S	S	R	R	R
Doxycycline (30)	S	S	S	S	S	S	R	S	R
Tetracycline (30)	S	S	S	S	S	S	S	S	S
Oxytetracycline (30)	S	S	S	S	S	S	S	S	S
Enoxacin (10)	S	S	S	S	S	S	S	S	S
Furazolidone (100)	S	I	S	I	S	S	S	S	S
Flucloxacillin (5)	R	R	R	R	R	R	R	R	R
Sulfamethoxazole (100)	R	R	R	R	R	R	R	R	R
Trimethoprim (5)	S	S	S	S	S	S	S	S	S
Ampicillin (10)	R	R	R	R	R	R	R	R	R

Discussion and Conclusion

Clinical findings during epizootics at five rainbow trout fresh water farms in Middle and Mediterranean region of Turkey were similar to those previously described by Bullock (4), Timur and Korun (18), Balebona et al. (3) and Tanrıkul (17).

The biochemical and culture characteristics of *L.anguillarum* strains were found substantially similar in the present study. Tanrıkul (17) also was reported same biochemical characteristics *L.anguillarum* strains from different localities and different times in rainbow trouts in Turkish farms. However, different results were found among strains in terms of MR-VP and fermentation of arabinose. Myhr et al.(15) noted variable results for VP test in *L.anguillarum* strains from salmonid fish (15).

All *L.anguillarum* isolates were sensitive to oxytetracycline in the present study, while Tanrıkul (17) was found variable results for oxytetracycline sensitivitiy (17). All *L.anguillarum* isolates were sensitive to oxytetracycline, tetracycline, enoxacin and trimethoprim. However, Tanrıkul (17) reported that all *L.anguillarum* isolates (n=12) were sensitive to amoxicillin, ampicillin, furazolidone, oxolinic acid (17).

In conclusion, the biochemical and culture characteristics of *L.anguillarum* strains isolated from rainbow trout in different farms located in the Middle and Mediterranean regions of Turkey were found substantially similar. In addition all *L.anguillarum* isolates were sensitive to oxytetracycline, tetracycline, enoxacin and trimethoprim in this study. Thus, this antibiotics have been used in controlling vibriosis in rainbow trout.

References

1. **Akaylı T,** (2001). *Diagnosis of Vibriosis by ELISA and methods in cultured sea bream (Sparus auratus, L 1758).* Ph.D Thesis, İstanbul Univ Fisheries Faculty, İstanbul.
2. **Austin B, Austin DA,** (2007). *Bacterial Fish Pathogens: Disease in Farmed and Wild Fish*. 4 th ed. Praxis Publishing, Chichester, p.594.
3. **Balebona CM, Zorilla I, Morinigo MA, Borrege JJ,** (1998). *Survey of bacterial pathologies affecting farmed gilt head sea bream (Sparus aurata, L) in south western Spain from 1990 to 1996.* Aquacult. 166, 19-35.
4. **Bullock GL, Sniesko SF,** (1971). *Bacterial Diseases of Fish. Diseases of Fishes, book 2A*, T.F.H., p. 56-80.
5. **CLSI (Clinical Laboratory Standards Institute),** (2006). *Performance standards for antimicrobial susceptibility testing.*16th Informational Supplement.
6. **Collins CH, Lyne PM,** (1976). *Microbiological Methods.* 4 th ed. Butterworths, p.521.
7. **Cowan ST, Steel KJ,** (1970). *Manual for the Identification of Medical Bacteria*. Cambrige at University Press, p.16.
8. **Çağırgan H,** (1993). *An investigation on the diagnosis and the treatment of cultured sea bass and sea bream.* Ph. D Thesis, Ege University Fisheries Faculty, İzmir.
9. **Giorgetti G, Ceschia G,** (1982). *Vibriosis in rainbow trout, Salmo gairdneri Richardson, in fresh water in north-eastern Italy*. J Fish Dis. 5, 125-130.
10. **Holt J, Krieg NR, Sneath PHA, Staley JI, Willians ST,**(1994). *Bergey's Manual of Determinative Bacteriology*. 9 th ed. Williams & Wilkins, p. 787.
11. **Holt G,** (1970). *Vibriosis (Vibrio anguillarum) as an epizootic disease in rainbow trout (Salmo gairdneri).* Acta Vet Scand. 11, 600-603.
12. **Korun J,** (2006). *A study on Listonella anguillarum infection occurred in cultured gilt-head sea bream (Sparus aurata L.).* EU J Fish Aquat Sci. 23, 259-263.
13. **Malnar L, Čož-Rakovac R, Hacmanjek M, Teskeredžić Z, Teskeredžić E, Strunjak-Perović I, McLean E, Naglić T,** (1996). *Vibriosis in rainbow trout cultured in the Krka estuary, Croatia: occurrence and comments*. Vet Med. 41, 77-81.
14. **McCarthy DH, Stevenson, JP, Roberts MS,** (1974). *Vibriosis in rainbow trout.* J Wildlife Dis. 10, 10-17.
15. **Myhr E, Larsen JL, Lillehaug A. Gudding R, Heum M, Hastein T,** (1991). *Characterization of Vibrio anguillarum and closely related species isolated from farmed fish in Norway.* Appl Environ Microbiol. 57, 2750-2757.
16. **Tanrıkul T, Gültepe N,** (2011). *Mix Infections in rainbow trout (Oncorhynchus mykiss Walbaum): Lactococcus garvieae and Vibrio anguillarum O1.* J Anim Vet Adv. 10, 1019-1023.
17. **Tanrıkul T,** (2007). *Vibriosis as an epizootic disease of rainbow trout (Oncorhynchus mykiss) in Turkey.* Pakistan J Biolog Sci. 10, 1733-1737.
18. **Timur G, Korun J,** (2004). *First outbreak of vibriosis in farmed rainbow trout (Oncorhynchus mykiss) in Turkey.* Istanbul Univ J Aquat Sci. 18, 1-9.
19. **Ross AJ, Martin JE, Bressler V,** (1968). *Vibrio anguillarum from an epizootic in rainbow trout (Salmo gairdneri) in the U.S.A.* Bull Off Int Epizoot. 69. 1139-1148.
20. **Zorrilla I, Arijo S, Chabrillon M, Diaz P, Martinez-Manzanares E, Balebona M, Moriñigo MA,** (2003). *Vibrio species isolated from diseased farmed sole, Solea senegalensis (Kaup) and evaluation of the potential virulence role of their extracellular products.* J Fish Dis. 26, 103-108.