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Kars'ta Tüketime Sunulan Gökkuşağı Alabalığından (*Oncorhynchus Mykıss*) İzole Edilen Hareketli Aeromonas Türlerinin Varlığı ve Yaygınlığı

Presence and Prevalence of Motile Aeromonas Species Isolated from Rainbow Trout (*Oncorhynchus Mykıss*) Offered for Consumption in Kars

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Öz

Bu çalışma, Kars ilinde tüketime sunulmuş Gökkuşağı alabalıklarında hareketli Aeromonas türlerinin varlığını ve yaygınlığını belirlemek amacıyla yapılmıştır. Araştırmada, 50 çiğ alabalıktan kas ve deri dokuları incelenmiş ve toplam 100 numuneden 42'sinin *Aeromonas spp*. yönünden pozitif olduğu belirlenmiştir. Kas örneklerinin 20 (% 40.0)'si hareketli *Aeromonas spp.* yönünden pozitif bulunurken, deri örneklerinin ise 22 (% 44.0)'sinin hareketli *Aeromonas spp*. bakımından pozitif olduğu belirlenmiştir. Bu 42 örneğin % 24'ünün *A. hydrophila*, % 38'inin *A. caviae*, % 16'sının *A. sobria*, % 14'ünün ise hem *A. hydrophila* hem de *A. caviae* içerdiği tespit edilmiştir.

Sonuç olarak Kars'ta satışa sunulmuş çiğ gökkuşağı alabalıklarının hareketli *Aeromonas spp.* yönünden risk taşıdıkları belirlenmiştir. Halk sağlını tehdit eden bu durum için gerekli hijyen ve pişirme önlemlerin alınması olası tehlikenin en az düzeye indirilebilmesi için önemlidir.

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Abstract

This study aimed to determine the presence and prevalence of motile Aeromonas species in rainbow trout offered for consumption in Kars province. Muscle and skin tissues were examined from 50 raw trout, and out of 100 total samples, 42 were found positive for *Aeromonas spp.* While 20 (40.0 %) of the muscle samples were positive for motile *Aeromonas spp*., 22 (44.0 %) of the skin samples were also positive. Among these 42 positive samples, 24 % were identified as *A. hydrophila*, 38 % as *A. caviae*, 16 % as *A. sobria*, and 14 % contained both *A. hydrophila* and *A. caviae*.

Consequently, it was determined that raw rainbow trout offered for sale in Kars is at risk with regard to motile Aeromonas spp. It is important to take the necessary hygiene and cooking precautions to minimize the possible danger for this situation that threatens public health.

1. INTRODUCTION

Fish is a frequently consumed food product due to its high nutritional value, flavor, and ease of preparation. In addition to being a good source of protein, it is also notable for its low

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fat and cholesterol content. It is a rich source of dietary fibers, antioxidants, omega-3 fatty acids, and many vitamins and minerals essential for human health, making it an important component of a healthy diet (Deveci et al., 2021; Nur and Deveci 2018). However, certain considerations should be taken into account when consuming fish. Fish may be exposed to microbial contamination, particularly in the case of motile Aeromonas species, which are naturally found in stagnant waters and aquatic environments. These bacteria can cause diseases in various fish species. Although there are many species in this bacterial group, *A. hydrophila*, *A. caviae*, and *A. sobria* species are of particular importance for food hygiene (Holt et al., 1994; Palumbo et al., 1992).

Motile Aeromonas bacteria can cause infections, particularly in freshwater fish. When fish meat is processed or stored under unhygienic conditions, or when fish is in poor health, these bacteria can pose a risk of infection to humans. In particular, consumption of raw or undercooked fish increases the risk of Aeromonas infection. Aeromonas infections in humans usually cause gastrointestinal symptoms, including diarrhea, nausea, vomiting, and abdominal pain. Two distinct forms of gastroenteritis may be caused by Aeromonas bacteria: cholera and dysentery. Cholera-type gastroenteritis is typified by a mild fever and diarrhea, whereas dysentery-type gastroenteritis is characterised by bloody and mucous diarrhea (Falcao et al., 2002; Popoff 1984). *A. hydrophila*, *A. caviae*, and *A. sobria* species have been associated with a range of clinical manifestations, including various skin and soft tissue infections, persistent diarrhea, bacteremia, and septicemia (Zhiyong et al., 2002). Additionally, they have been reported to cause eye infections, urinary tract infections, gynecological infections, osteomyelitis, pneumonia, peritonitis, endocarditis, and meningitis (Chan et al., 2004; Ellison and Mostow 1984; Mellersh et al., 1984). Individuals with compromised immune systems, including pregnant women, young children, and the elderly, are at greater risk of developing foodborne illnesses such as Aeromonas infection. Therefore, it is crucial to exercise caution when consuming fish meat (Baddour and Baselski 1988). To ensure the safety of fish, it is essential to obtain it from fresh and reliable sources and to prepare it under hygienic conditions during consumption.

The objective of this study is to determine the presence and prevalence of motile Aeromonas spp., a significant foodborne pathogen, in the muscle and skin tissues of rainbow trout offered for consumption in Kars province. Furthermore, the data obtained aims to evaluate whether this pathogen poses a potential risk to public health.

2. MATERIAL VE METOD

2.1. Material

In this study, a total of 50 whole Rainbow trouts were obtained from fishmongers and supermarkets in the city center of Kars province, during December, January, and February. The fish samples were transported to the laboratory under cold chain conditions and immediately examined for the presence of motile Aeromonas spp. in muscle and skin tissues without delay (Zeaur and Aziz 1994).

2.2. Method

2.2.1. Isolation and Identification of Motile Aeromonas Species

Enrichment

The skin and muscle tissues of fish samples were separated under aseptic conditions using sterile scalpels. A total of 25 grams of each tissue was placed in stomacher bags, and 225 milliliters of 0.1 % alkaline peptone water (APW) with a pH of 8.4 - 8.6 was added. Following homogenization of the samples in a stomacher for a period of two minutes, the skin and muscle homogenates were incubated at a temperature of 28 °C for a period of 18 to 24 hours.

Isolation

The enrichment homogenates were inoculated on GSP Agar (Pseudomonas Aeromonas Selective Agar Base (Oxoid CM833)+100.000 IU/L penicillin G (Oxoid SR136E)) by drawing method and the petri plates were incubated at 28^{o}C for 3 days. Following incubation, colonies with a diameter of 2-3 mm and surrounded by a yellow halo were considered suspect *Aeromonas* colonies (Figure 1).

Five suspected colonies were selected and inoculated onto Tryptone Soy Agar (TSA; Oxoid CM131) and incubated at 30°C for 24 hours. The isolates were identified as motile Aeromonas species based on their resistance to the vibriostatic agent 2,4-diamino-6,7 - diisopropyl-pteridine (O/129; Sigma D-0656), growth in Nutrient Broth without NaCl, no growth in Nutrient Broth containing 6 % NaCl, positivity on DNase Agar (Oxoid CM321), and gram-negative characteristics, oxidase and catalase positivity after 18 - 24 hours of incubation at 35°C in SIM Medium (Oxoid CM435)

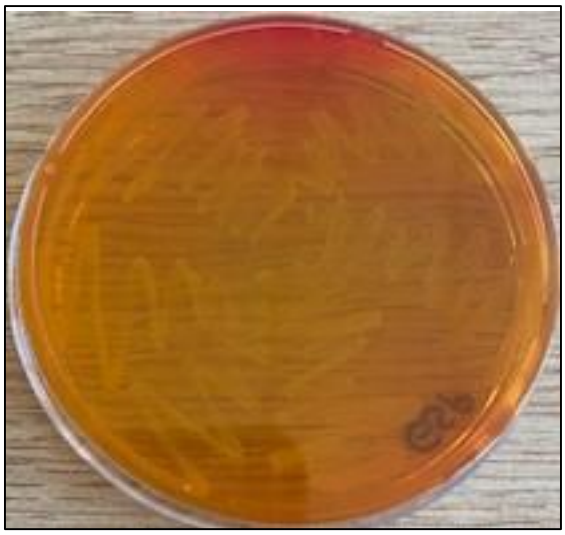

Figure 1: Typical appearance of *Aeromonas* spp. colonies on GSP Agar

Identification

The tests employed for the identification of motile *Aeromonas* species are presented in Table 1. The reference strain of *A. hydrophila* (95080) used in this study was obtained from the culture collection of the Food Processing Department, Kars Vocational School, Kafkas University.

Table 1: Identification Tests of Motile *Aeromonas* Species (2).

Biochemical Tests	***A. hydrophila***	***A. caviae***	***A. sobria***
Methyl Red Test	+	+	-
Voges-Proskauer Test	+	-	V
H_2S formation from cysteine	+	-	+
Esculin hydrolysis	+	+	-
Indole production	+	+	+
Gas formation from glucose	+	-	+
Mannitol fermentation	+	+	+
Salicin fermentation	+	+	-
L-arabinose use	+	+	-
Growth in KCN Broth	+	+	-

(+) Pozitive, (–) Negative, (V) Variable

Statistical analysis

Correlation analyses were utilized to ascertain the significance of the difference between the groups (Hayran 2012)

3. RESULTS

In the raw rainbow trout meat samples, 20 (40.0 %) tested positive for motile *Aeromonas spp*.. Of these, five (10.0 %) samples were positive for *A. hydrophila*, ten (20.0 %) for *A. caviae*, three (6.0 %) for *A. sobria*, and two (4.0 %) for both *A. hydrophila* and *A. caviae*. Additionally, 22 (44.0 %) of the analyzed fish skin samples tested positive for motile *Aeromonas spp*.. *A. hydrophila* was isolated from 7 (14.0 %), *A. caviae* from 9 (18.0 %), *A. sobria* from 5 (10.0 %) and both *A. hydrophila* and *A. caviae* from 5 (10.0 %) samples.

A total of 100 samples were analyzed, with 42 exhibiting positive results for the presence of *Aeromonas spp*.. Of the samples, 24 % were identified as *A. hydrophila*, 38 % as *A. caviae*, 16 % as *A. sobria*, and 14 % contained both *A. hydrophila* and *A. caviae*.

Statistical analysis revealed no significant difference between fish skin and muscle tissue in terms of Aeromonas species and isolation rates. Motile Aeromonas species isolated from fish are presented in Table 2 and Figures 2 and 3.

Table 2: Distribution of Motile *Aeromonas* Species in Muscle and Skin Tissues

Sample type	**Number of samples**	***Aeromonas spp.***		***A. hydrophila***		***A. caviae***		***A. sobria***		***A. hydrophila A. caviae***		***A. hydrophila A. sobria***	
	n	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Muscle	50	20	(40.0)	5	(10.0)	10	(20.0)	3	(6.0)	2	(4.0)	-	-
Skin	50	22	(44.0)	7	(14.0)	9	(18.0)	5	(10.0)	5	(10.0)	-	-
Total	100	42	(42.0)	12	(24.0)	19	(38.0)	8	(16.0)	7	(14.0)	-	-

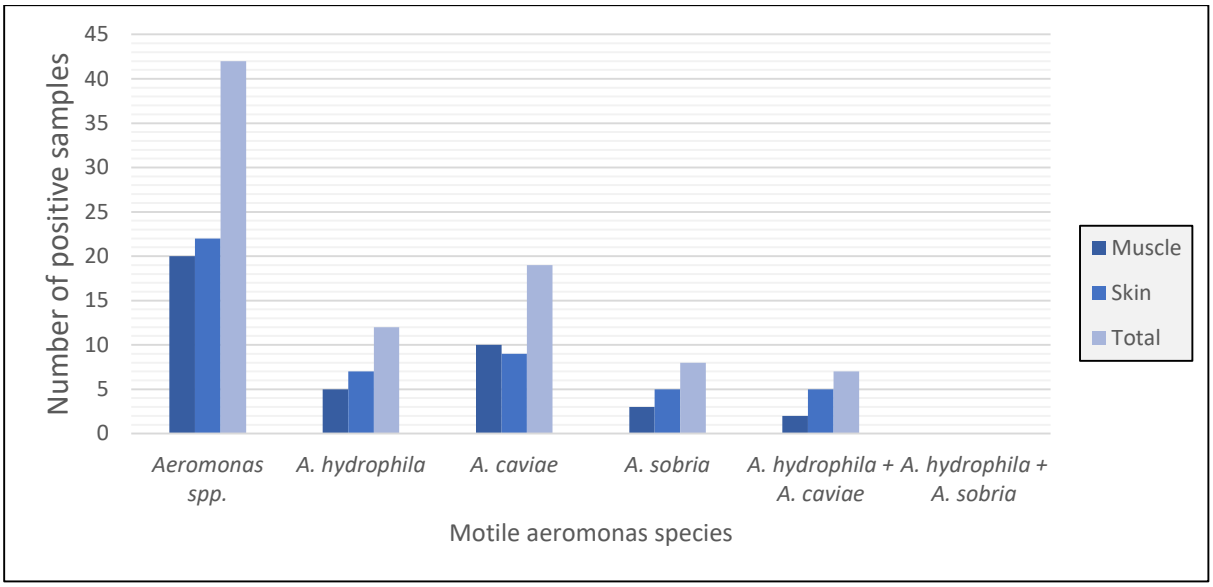

Figure 2. Distribution of Motile Aeromonas Species in Muscle and Skin Tissues

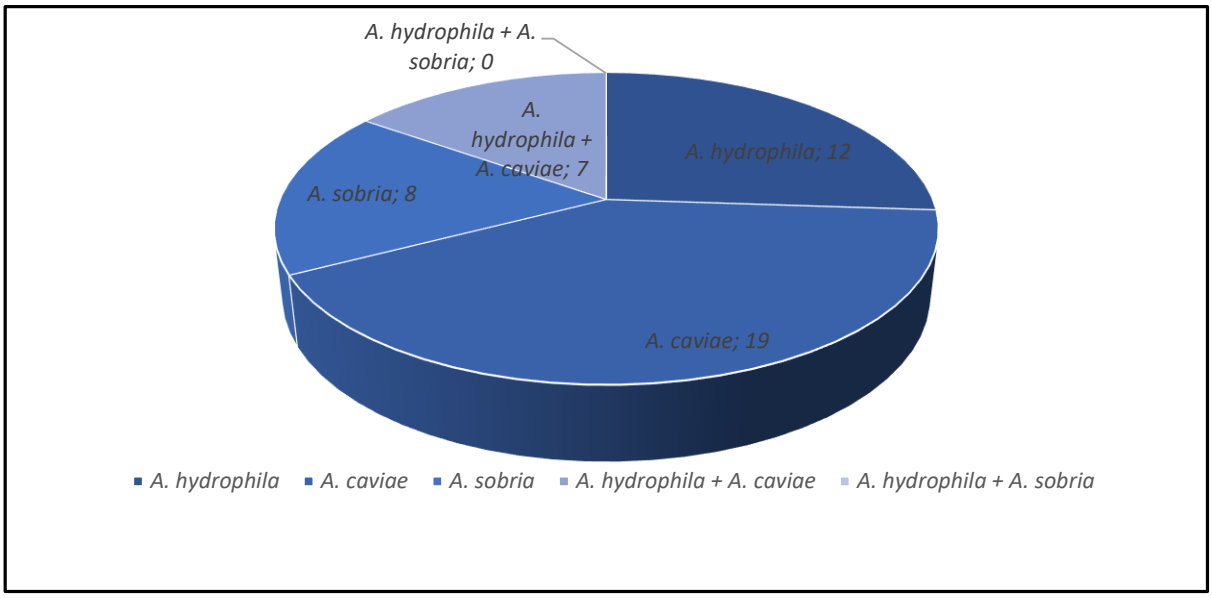

Figure 3. Distribution of total motile *Aeromonas* isolates identified from fish

4. DISCUSSION AND CONCLUSION

Aeromonas species are a group of microorganisms commonly found, particularly in stagnant water environments such as lake waters. Therefore, fish and other aquatic products living in these environments play a significant role in the transmission of the pathogen to humans. The detection of motile Aeromonas presence in fish and aquatic products is of great importance in preventing potential public health risks and ensuring food safety (Vivekanandhan and Hatha 2005; Castro-Escarpulli et al.,2003). In this study, the presence of motile *Aeromonas spp*. was investigated in the muscle and skin tissues of rainbow trout obtained from local markets and sales points in Kars province. It was found that 42 % of the

samples were positive for Aeromonas spp. While this finding falls below the high isolation rates of 120% and 93.9 % reported by İşleyici et al. (2003) and Gobat and Jemmi (1993), respectively, it is higher than the 30.07 % *Aeromonas spp*. isolation rate detected by Koç (2011) in shrimp and squid samples in Antalya.

The isolation rates and species diversity of motile Aeromonas species can vary significantly depending on the sampling site, sample type, and the sampling method used. Studies have shown that seasonal variations also play a prominent role in the number of motile Aeromonas species found in fish and marine samples (Boynukara et al., 1988-b; Wang and Silva 1999). In our study, no significant difference was observed in the prevalence and species distribution of motile Aeromonas species between the skin and muscle tissues of fish. The isolation rates obtained from both skin and muscle samples were similar, with *A. caviae* identified as the dominant species with a rate of 38 %. This finding is consistent with the study conducted by Nishikawa and Kishi (Nishikawa and Kishi 1988). On the other hand, in a research conducted in Switzerland in 1999, it was reported that the dominant species in fish samples was *A. hydrophila* with a rate of 89.9 %, followed by *A. sobria* (10.20 %) and *A. caviae* (20.41 %) (15).

In the literature, studies suggest that *A. hydrophila* is dominant among the motile Aeromonas species isolated from fish, followed by *A. sobria* and *A. caviae* (Sharma and Kumar 2011; Yadav and Kumar 2000). *A. hydrophila*, a facultative pathogen, causes infections when the host's immune system is compromised (Popovic, et. al., 2000). Because *A. hydrophila* is a pathogen commonly found in marine and lake waters and can multiply at low temperatures, seafood contaminated with *A. hydrophila* poses a potential health risk to consumers. Consumption of raw or undercooked fish and cross-contamination can increase this risk (Alcestis and Rogelio 1987; Popovic et al., 2000). *A. hydrophila*, which is frequently isolated from fish skin tissues, was detected in 24 % of the fish samples in this study, and it was observed that fish skin contained a higher proportion of *A. hydrophila* than muscle tissue.

A number of studies have demonstrated that motile Aeromonas species are also a significant pathogen in freshwater fish. In a study by Ruzica et al. (2002), it was determined that out of 8 motile *Aeromonas spp*. strains isolated from freshwater fish, 6 were identified as *A. hydrophila* and 2 as *A. sobria*. Similarly, Wang and Silva (18) detected motile Aeromonas in 82.7 % of 238 channel catfish samples from 3 different fish processing plants, with species distribution being *A. hydrophila* (36.1 %), *A. sobria* (35.7 %), and *A. caviae* (10.9 %). In a study

conducted by Boynukara et al. (1998-a) on rainbow trout in Van, 39 motile Aeromonas strains were isolated, of which 89.7 % were identified as *A. sobria*, 7.7 % as *A. caviae*, and 2.6% as *A. hydrophila*. In another study by Leitao and Silveir (1991), motile Aeromonas was detected in 22 (22.22 %) of the examined fish, and the species were identified as *A. hydrophila* (66.6 %), *A. sobria* (27.27 %), and *A. caviae* (9.09 %).

The primary source of motile Aeromonas contamination of fish meat is the widespread presence of these bacteria in aquatic environments. This prevalence facilitates the transmission of the agent to fish and other seafood and the rapid spread of contamination. Furthermore, fish carrying the agent can contaminate other fish during cleaning and preservation (Abeyta et al., 1986). Raw fish meat represents a significant source of contamination for motile Aeromonas species and has the potential to cause foodborne infections (Boulanger et al., 1977). The ability of motile Aeromonas species to reproduce at low temperatures allows them to remain viable for extended periods under refrigerated conditions. Moreover, in instances where the cold chain is disrupted, these bacteria can proliferate rapidly, dominating the ambient flora and intensifying contamination (Escarpulli et al., 2003). In this study, motile Aeromonas species were identified in 20 (40 %) of the 50 fish muscle samples analyzed. The predominant species was *A. caviae* (20 %), and *A. caviae* and *A. hydrophila* were isolated together in two samples.

In light of these findings, it is crucial to implement necessary hygienic measures during production and sales stages to prevent potential health risks associated with motile Aeromonas species. Additionally, procuring fish from reliable sources, preventing cross-contamination, implementing adequate heat treatment practices, and conducting regular health inspections of products are other crucial measures that should be taken to prevent Aeromonas infections.

Availability of Data and Materials

Datasets analyzed during the current study are available in the author on reasonable request.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

S.H. has designed the study and has collected the data. S.H. and A.H. wrote the article. S.H. has conducted the experiment together with A.H. All authors have read, revised, and approved the manuscript.

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