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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Isolation of *Aeromonas sobria* from Fish, Determination of Antibiotic Resistances and Genotyping

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Abstract: This study aimed to isolate *Aeromonas sobria* strains from sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*), determine their antibiotic resistance profiles, and assess their phylogenetic similarities. In 2023, 100 fish samples were collected from the Izmir region. Isolates were identified using the VITEK-2 automated system, while antibiotic resistance was evaluated through the Kirby-Bauer disk diffusion technique. Genotyping was performed using the RAPD-PCR method. Among the samples, 11% tested positive for *A. sobria*. Antibiograms revealed the highest resistance to ampicillin and amoxicillin (90.9%), while enrofloxacin demonstrated the highest sensitivity (72.7%). Genotyping identified 11 distinct genotypes, with phylogenetic similarities ranging from 17% to 92%. The findings highlight the zoonotic potential and diverse resistance patterns of *A. sobria*, emphasizing the importance of selecting appropriate antimicrobials for treatment.

Keywords: *Aeromonas sobria*, antibiotic resistance, fish, genotyping.

Balıklardan *Aeromonas sobria* İzolasyonu, Antibiyotik Dirençliliklerinin Belirlenmesi ve Genotiplendirilmesi

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Öz: Bu çalışma, levrek (*Dicentrarchus labrax*) ve çipura (*Sparus aurata*) balıklarından *Aeromonas sobria* suşlarını izole etmeyi, bu izolatların antibiyotik direnç profillerini belirlemeyi ve filogenetik benzerliklerini değerlendirmeyi amaçlamıştır.2023 yılında İzmir bölgesinden 100 balık örneği toplanmıştır. İzolatların tanımlanmasında VITEK-2 otomatik sistemi kullanılmış, antibiyotik dirençleri Kirby-Bauer disk difüzyon yöntemiyle değerlendirilmiştir. Genotiplendirme işlemi RAPD-PCR yöntemiyle gerçekleştirilmiştir. Örneklerin %11'inde *A. sobria* pozitif olarak saptanmıştır. Antibiyogram sonuçlarına göre, en yüksek direnç ampisilin ve amoksisilin (%90,9) karşısında belirlenmiş, en yüksek duyarlılık ise enrofloksasin (%72,7) için tespit edilmiştir. Genotiplendirme sonucunda 11 farklı genotip tanımlanmış olup, filogenetik benzerlik oranları %17 ile %92 arasında değişmiştir.Elde edilen bulgular, *A. sobria*'nın zoonotik potansiyelini ve farklı direnç desenlerini ortaya koymakta; tedavide uygun antimikrobiyal seçiminin önemini vurgulamaktadır.

Anahtar kelimeler: *Aeromonas sobria*, antibiyotik dirençliliği, balık, genotiplendirme.

INTRODUCTION

Aquatic animals, particularly farmed fish species, are frequently exposed to various bacterial pathogens that significantly affect the sustainability and profitability of aquaculture operations. Among these, *Aeromonas sobria* is a mesophilic, motile, Gram-negative bacterium commonly isolated from freshwater and marine environments, and is recognized as both an opportunistic fish pathogen and a zoonotic agent capable of causing disease in humans (Gauthier et al., 2017; Song et al., 2019). In fish, *A. sobria* is known to cause motile *Aeromonas* septicemia (MAS), a disease characterized by hemorrhagic ulcers, ascites, exophthalmia, and internal organ necrosis, leading to considerable economic losses in aquaculture (Soliman et al., 2022).

The increasing use of antibiotics in aquaculture to control bacterial infections has contributed to the emergence of multidrug-resistant *Aeromonas* strains (Zhang, 2023). Resistance to β-lactam antibiotics such as ampicillin and amoxicillin has been widely reported in *A. sobria*, often mediated by plasmid-encoded β-lactamases (Zhang et al., 2021). Moreover, this species possesses numerous virulence factors, including hemolysins, aerolysins, and the ability to

form biofilms, which further complicate treatment efforts and increase public health risks (Majeed et al., 2023; Ye et al., 2023).

In Türkiye, several studies have reported the presence of *Aeromonas* species in cultured marine and freshwater fish. Onuk et al. (2017) determined varying antibiotic resistance in strains isolated from trout and aquaculture waters. They detected *A. sobria* isolates carrying the plasmid-mediated quinolone resistance gene qnrS2 in fish and water samples collected from the Aegean, Mediterranean, and Black Sea regions. Additionally, Şahin et al. (2019) identified *A. sobria* from ornamental fish and reported diverse antimicrobial resistance profiles. These findings highlight the occurrence and resistance potential of *Aeromonas* species in aquatic environments in Türkiye (Balta, 2020).

Despite its clinical and economic relevance, data regarding the genotypic diversity and antimicrobial resistance profiles of *A. sobria* isolated from aquaculture species in Türkiye remain limited. Therefore, this study aims to isolate *A. sobria* strains from sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) in the İzmir region, determine their antibiotic resistance patterns using disk diffusion method, and assess their genetic diversity through RAPD-PCR analysis.

MATERIAL AND METHOD

In this study, a total of 100 fish samples, including gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*), were collected in 2023 from aquaculture farms operating in the İzmir province. The samples were transported to the laboratory under cold chain conditions. Swab samples were aseptically taken from internal organs (liver, kidney, spleen) and inoculated onto Tryptic Soy Agar (TSA; Merck) supplemented with 1% NaCl. Plates were incubated at 28 ± 1 °C for 24 to 48 hours. Colonies exhibiting morphological characteristics consistent with *Aeromonas* spp. (creamy, round, convex) were selected. After Gram staining, Gram-negative rod-shaped bacteria were subcultured for purification. The examined sea bass showed hemorrhagic lesions on the body surface and fins (Figure 1).

lesions on the body surface, fins, and head region.

Preliminary phenotypic identification was conducted using catalase and oxidase tests. Biochemical characterization was performed using the VITEK® 2 Compact System (bioMérieux, France) with the GN (Gram-Negative) identification card. Isolates confirmed as *Aeromonas sobria* were subjected to molecular typing and antimicrobial susceptibility testing.

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to CLSI guidelines (CLSI, 2013). Isolates were grown overnight in nutrient broth, and suspensions equivalent to 0.5 McFarland standard were prepared and spread on Mueller-Hinton Agar (MHA; Merck). The following antibiotic disks were used: ampicillin (10 µg), amoxicillin (10 µg), tetracycline (30 µg), oxytetracycline (30 µg), trimethoprim/sulfamethoxazole (25 µg), enrofloxacin (5 µg), and gentamicin (10 µg). Plates were incubated at 28 ± 1 °C for 24 hours. Inhibition zones were measured in millimeters and interpreted according to CLSI breakpoints (Bauer et al., 1966; CLSI, 2014).

Figure 1. Photographs of diseased sea bass (*Dicentrarchus labrax*) showing hemorrhagic lesions on the body surface, fins, and head region.

Genomic DNA was extracted from each *A. sobria* isolate using the High Pure PCR Template Preparation Kit (Roche, Germany), following the manufacturer's instructions. To determine the genotypic profiles of the isolates, Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) was performed using the ERIC-2 (Enterobacterial Repetitive Intergenic Consensus) primer (5'-AAG TAA GTG ACT GGG GTG AGC G-3'), as described by Versalovic et al. (1991). The PCR reaction mixture (25 µL total volume) consisted of 1× PCR buffer, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 2.5 U Taq DNA polymerase, 25 pmol of ERIC-2 primer, and 5 µL of template DNA. Amplification was carried out in a Techne TC-412 thermal cycler using the following protocol: initial denaturation at 94 °C for 5 minutes, followed by 40 cycles of denaturation at 94 °C for 1 minute, annealing at 40 °C for 1 minute, and extension at 72 °C for 3 minutes, with a final extension at 72 °C for 7 minutes.

The RAPD-PCR products were electrophoresed on a 1.5% agarose gel containing ethidium bromide (2 µg/mL) and visualized under a UV transilluminator. Banding patterns were analyzed using the Quantity One image analysis software (Bio-Rad, USA). Dendrograms were generated using the Unweighted Pair Group Method with

Arithmetic Averages (UPGMA). Genetic similarity among isolates was evaluated using the Dice coefficient, and clusters were defined based on a 70% similarity threshold (Savasan & Goksoy, 2018). No reference strains were used in the RAPD-PCR study. According to the provisions of the Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees (Official Gazette No. 28914, dated February 15, 2014), ethical committee approval was not required for this study, as only clinical samples were used and no experimental procedures were performed on live animals.

RESULTS

Out of 100 fish samples examined, a total of 11 (11%) isolates were identified as *Aeromonas sobria* based on biochemical analysis using the VITEK® 2 Compact GN card. These isolates originated from both *Sparus aurata* and *Dicentrarchus labrax* specimens. Additionally, the VITEK-2 results showing the biochemical characteristics of the isolates are presented in Figure 2.

2	APPA	-	3	ADO	-	4	PyrA	+	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	(+)	61	IMLTa	-	62	ELLM	(+)	64	ILATa	-			

Figure 2. VITEK-2 results showing the biochemical characteristics of *Aeromonas sobria* isolates obtained from gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*).

In addition to the *A. sobria* isolates, a total of 13 (13%) *A. hydrophila*, 8 (8%) *A. veronii*, and 6 (6%) *A. salmonicida* strains were isolated from the 100 fish samples examined. Antimicrobial susceptibility testing of the 11 *A. sobria* isolates was performed using the Kirby-Bauer disk diffusion method, and the results were interpreted according to CLSI 2014 criteria. The isolates demonstrated the highest susceptibility to ciprofloxacin and enrofloxacin, with 72.7% of strains categorized as susceptible and no resistant isolates detected. Gentamicin also showed relatively good effectiveness, with 18.2% of isolates fully susceptible and 54.5% demonstrating intermediate susceptibility. In contrast, high resistance rates were observed for ampicillin (90.9%) and amoxicillin (90.9%), followed by oxytetracycline (63.6%) and erythromycin (36.4%). Trimethoprim/sulfamethoxazole exhibited the lowest susceptibility, with 85.7% of isolates classified as resistant. The complete distribution of susceptibility patterns is presented in Table 1.

As a result of RAPD-PCR performed using the ERIC-2 primer for the genotyping of 11 isolates identified as *A. sobria*, 11 distinct genotypes were detected (Figure 2). Phylogenetic analysis revealed that the genotypes exhibited similarity levels ranging from 17% to 92% (Figure 3).

Table 1. Antimicrobial resistance profiles of isolates

Antibiotic Name	Susceptible	Intermediate	Resistant	Referans
Amoxicillin (25 µg)	1 (9.1%)	0 (0.0%)	10 (90.9%)	CLSI, 2014
Ampicillin (10 µg)	1 (9.1%)	0 (0.0%)	10 (90.9%)	CLSI, 2014
Ciprofloxacin (5 µg)	8 (72.7%)	3 (27.3%)	0 (0.0%)	CLSI, 2014
Erythromycin (15 µg)	1 (9.1%)	6 (54.5%)	4 (36.4%)	CLSI, 2014
Enrofloxacin (5 µg)	8 (72.7%)	3 (27.3%)	0 (0.0%)	CLSI, 2014
Gentamicin (10 µg)	2 (18.2%)	6 (54.5%)	3 (27.3%)	CLSI, 2014
Oxytetracycline (30 µg)	3 (27.3%)	1 (9.1%)	7 (63.6%)	CLSI, 2014

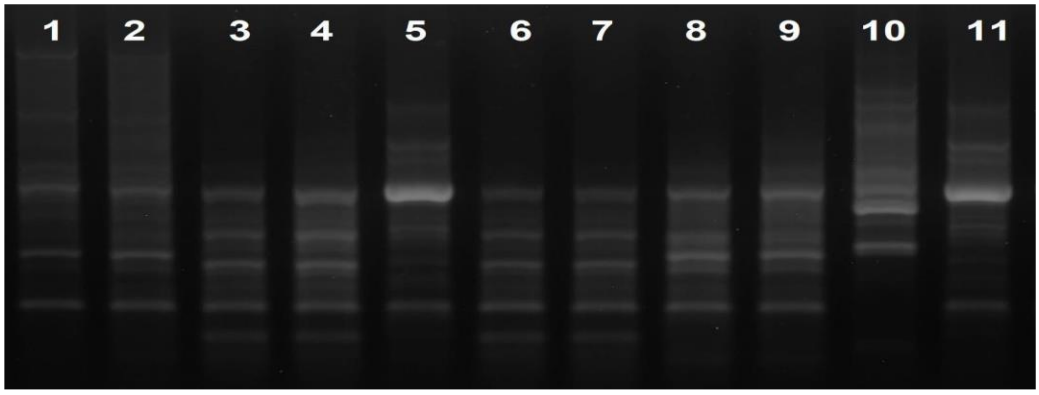

Figure 3. RAPD profiles of the *A. sobria* strains analyzed

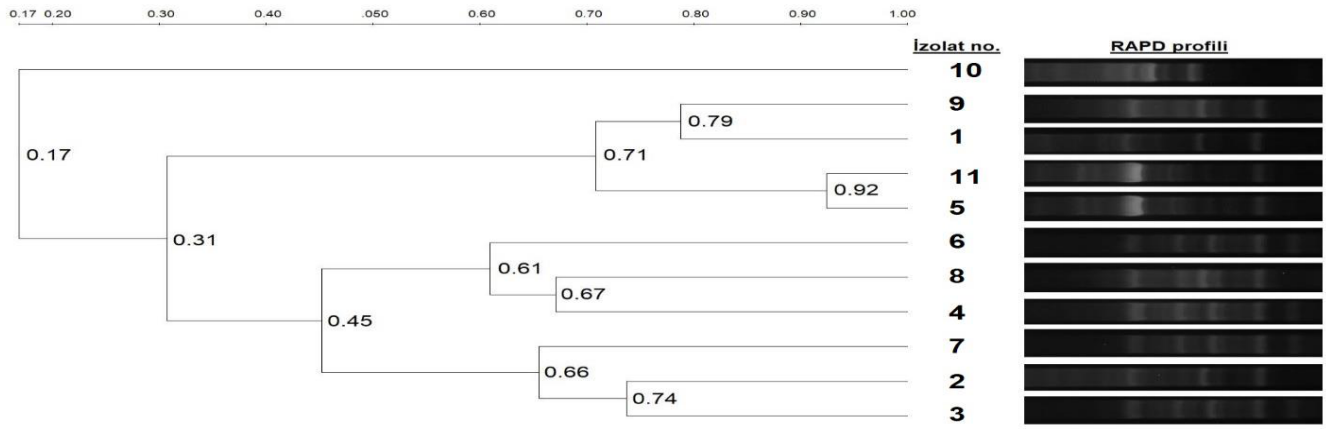

Figure 4. Phylogenetic similarity analysis of the examined *A. sobria* isolates

DISCUSSION AND CONCLUSION

Aeromonas species are globally distributed, Gram-negative, oxidase-positive, facultatively anaerobic rods commonly found in aquatic environments, food products, and the natural microbiota of fish (Aravena-Román et al., 2012). Motile *Aeromonas* species are frequently isolated from a wide range of freshwater habitats, including fish ponds, and are known to cause significant infections in freshwater fish (Guz & Kozińska, 2004). Among these, *A. sobria* is a clinically important species that causes ulcerative dermatitis, tail and fin rot, and septicemia in fish. Moreover, it is recognized as a zoonotic pathogen capable of causing serious infections in immunocompromised individuals (Ahmed et al., 2020).

In the present study, 11 *A. sobria* strains were isolated and identified from 100 fish samples collected from different regions of İzmir. Antibiotic susceptibility testing revealed the highest resistance to ampicillin and amoxicillin (90.9%), while the greatest sensitivity was observed against enrofloxacin (72.7%). Consistent with our findings, Onuk et al. (2017) reported that motile *Aeromonas* isolates, including *A. sobria*, showed high susceptibility to gentamicin, ciprofloxacin, and florfenicol, whereas they exhibited the highest resistance to amoxicillin and ampicillin.

Similarly, Guz and Kozińska (2004) tested the susceptibility of 18 *A. hydrophila* and 3 *A. sobria* isolates obtained from carp with motile *Aeromonas* infections in Poland. They reported 100% resistance to ampicillin and penicillin, and moderate resistance to cephalothin (57%) and erythromycin (52%). In another study from Slovakia, Majtán et al. (2012) examined mass mortalities in Garra rufa fish and found that A. sobria isolates displayed resistance only to ampicillin when tested against eight antibiotics using the disk diffusion method.

The findings of the present study are partially consistent with those reported by Onuk et al. (2017), who identified *Aeromonas* species from various fish samples in Türkiye and observed high resistance rates to ampicillin and tetracycline. Similarly, in our study, *A. sobria* isolates obtained from sea bass (Dicentrarchus labrax) and gilthead sea bream (*Sparus aurata*) showed the highest resistance to ampicillin and amoxicillin (90.9%). However, unlike the results of Onuk et al. (2017), enrofloxacin was found to be the most effective antibiotic against our isolates (72.7% sensitivity). This difference may be attributed to variations in fish species, environmental conditions, and antibiotic usage practices in aquaculture systems.

The results of the present study are in partial agreement with those of Çiftçi et al. (2015), who compared the phenotypic and genotypic properties of *A.sobria* strains isolated from rainbow trout. In both studies, high resistance to β-lactam antibiotics such as ampicillin and amoxicillin was detected. However, while Çiftçi et al. (2015) used ERIC-PCR for genotypic comparison, the present study employed RAPD-PCR and revealed greater genetic diversity among the isolates (17–92% similarity). Moreover, enrofloxacin was found to be the most effective antibiotic in this study (72.7% sensitivity), whereas Çiftçi et al. (2015) reported moderate sensitivity to quinolones. These differences may be attributed to the variation in fish species, environmental conditions, and molecular typing methods.

Durmaz and Türk (2009) isolated 52 motile *Aeromonas* strains from 95 samples (73 fish and 22 water) collected from trout farms and reported that over 80% of the isolates were resistant to oxytetracycline, streptomycin, and carbenicillin, while over 90% were sensitive to amikacin, ciprofloxacin, and enrofloxacin. All *A. sobria* strains in their study were resistant to carbenicillin.

Accurate identification of *Aeromonas* species in aquatic environments requires both phenotypic and genotypic characterization. While phenotypic methods such as biochemical testing are commonly used, the high diversity within the genus often leads to ambiguous results, making genotyping a more reliable approach (Dubey et al., 2021).

In our study, genotyping by RAPD-PCR using the ERIC-2 primer revealed 11 distinct genotypes among the 11 *A. sobria* isolates. Phylogenetic similarity analysis showed genetic similarities ranging between 17% and 92%. Similarly, Duman (2017) reported that *A. sobria* isolates showed up to 90% genetic similarity in a study involving 98 motile *Aeromonas* strains. Çiftçi et al. (2015), using RAPD-PCR on 36 *A. sobria* isolates, observed 13 different genotypes with a similarity index of 70%, indicating phenotypic and genotypic diversity among the strains. They also suggested that the use of combined typing methods could improve strain discrimination and that such results could contribute to vaccine development and diagnostic kit production for *A. sobria* infections in fish.

The originality of this study lies in its contribution to the limited data available on *A. sobria* infections in marine fish in Türkiye. Most previous studies have focused on *Aeromonas* species isolated from freshwater fish, while research on marine species such as sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) remains scarce. Moreover, the use of RAPD-PCR analysis provides additional insight into the genetic diversity of *A. sobria* strains in marine aquaculture environments. These results contribute to the limited data on *A. sobria* in marine fish in Türkiye and emphasize the need for further studies to understand the epidemiology and resistance patterns of this pathogen in aquaculture environments.

In conclusion, *A. sobria* is a widespread aquatic bacterium capable of causing infections in both fish and humans. In this study, 11 isolates were obtained from 100 fish samples collected in the İzmir region. Antibiotic susceptibility testing showed the highest resistance to ampicillin and amoxicillin (90.9%), and the highest sensitivity to enrofloxacin (72.7%). These findings are consistent with previous studies reporting β-lactam resistance in *A. sobria*. RAPD-PCR genotyping revealed 11 distinct genotypes with 17%-92% similarity, indicating considerable genetic diversity and the possibility of multiple sources of infection. These results emphasize the need for rational antibiotic use and treatment strategies based on susceptibility testing. Furthermore, the data presented here may serve as a basis for future studies on the monitoring, control, and prevention of *A. sobria* infections in aquaculture and public health contexts.

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