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Assessment of Antibiotic Resistance Genes in Bacteria Obtained from Various Fish Species: A Study in Beymelek Lagoon

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Abstract

In this study, we investigated the presence of common antibiotic resistance genes in bacteria isolated from the internal organs of various fish collected from Beymelek Lagoon. A total of 50 fish, representing 11 different species, were collected from the point where the lagoon connects to the sea using the barricade and net method on two occasions, with an interval of one week, during May 2022. Bacteria isolated from various internal organs of the fish were identified using the VITEK 2 Compact automated identification system. Among the ten bacterial isolates obtained, five were classified as Gram-negative and five as Gram-positive. Sixteen antibiotic resistance genes (*aadA1*, *ampC*, *blaTEM*, *cfr*, *dfrA1*, *ermB*, *floR*, *intI1*, *mphA*, *qnrA1*, *sul1*, *sul2*, *sul3*, *tetA*, *tetB*, and *tetW*) were screened in these isolates. Resistance genes were detected in five distinct bacterial strains isolated from four different fish species. The *intI1* gene was identified in five isolates, while the *sul1* gene was detected in one isolate. None of the other 14 resistance genes were present in the isolated bacteria. These findings provide insight into the distribution of antibiotic resistance genes in bacterial strains associated with marine fish and highlight the potential risks of antimicrobial resistance in aquatic environments.

Introduction

The composition of bacterial flora in aquatic organisms such as fish can differ depending on the levels of bacterial presence in their surrounding environment. In fish inhabiting marine and freshwater environments, the bacterial community predominantly consists of aerobic or facultative anaerobic bacteria, as well as psychrophilic Gram-negative bacteria such as *Vibrio* spp., *Pseudoalteromonas* spp., *Shewanella* spp., *Pseudomonas* spp., *Moraxella* spp., *Alteromonas* spp., *Haemophilus* spp., *Acinetobacter* spp., *Flavobacterium* spp., and *Cytophaga* spp. (Arda, 1974; Brian-Jaisson et al., 2014; Cahill, 1990; Pond et al., 2006). It has been reported that the aquatic environment in which fish reside significantly influences the bacterial communities recovered from them, with salinity and bacterial load being key determining factors (Austin & Austin, 2016).

Given the close relationship between fish-associated and environmental bacteria, the potential impact of antibiotic resistance in these ecosystems warrants careful consideration. The emergence and dissemination of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in aquatic environments pose increasing threats to both biotic integrity and public health (Bhat & Altinok, 2023; Gillings, 2013; RAH et al., 2022). Water serves as a primary vector for pollutants and plays a crucial role in the spread of ARB and ARGs within ecosystems (Lupo et al., 2012; Stalder et al., 2012). Bacterial resistance can

develop through two primary mechanisms: intrinsic (natural) resistance and acquired resistance. Intrinsic resistance refers to the inherent ability of certain bacterial species to withstand specific antibiotics. In contrast, acquired resistance arises from non-species-specific genetic mutations or horizontal gene transfer (HGT) (Amábile-Cuevas, 2021).

HGT is recognized as a critical mechanism in the acquisition of ARGs, as it facilitates the transfer of resistance determinants between different bacterial species (Von Wintersdorff et al., 2016). This resistance often emerges through mechanisms such as transformation, transduction, and conjugation, resistance genes can be effectively disseminated among bacterial populations (Amábile-Cuevas, 2015; Guo et al., 2015; Qiu et al., 2012; Zhang et al., 2018).

Over time, ARB have the potential to evolve into human pathogens and act as reservoirs for resistance genes (Rasul & Majumdar, 2017). Certain species, such as *Vibrio* spp. and *Lactococcus* spp., which are well-known fish pathogens, have been shown to transfer tetracycline resistance genes to human-associated bacteria like *E.coli* and *Enterococcus faecalis* (Neela et al., 2009). The potential transmission of ARB from aquatic environments to humans is a significant public health concern, primarily occurring through the consumption of contaminated aquatic organisms (Petersen et al., 2002). The prevalence of resistant bacteria in the microbiota of commercially valuable aquatic species poses risks not only for human consumption but also for the effective management of diseases in aquaculture systems (Jiang et al., 2018; Sucato et al., 2021).

Coastal lagoons play a vital ecological role as breeding grounds for fish and invertebrates, contributing significantly to biodiversity. Additionally, they serve as essential migratory routes for waterfowl and fish (Breber et al., 2008). The accumulation and spread of antibiotic-resistant bacteria in coastal lagoons, which are exposed to intense human activities, pose a significant threat. These lagoons can become sources of contamination, affecting the environment as well as animals and humans (Dolejska & Papagiannitsis, 2018; H. Wang et al., 2021)

This study aims to investigate the presence of antibiotic resistance genes in cultivable bacteria isolated from the internal organs of fish collected from Beymelek Lagoon in the Demre district of Antalya, Türkiye. Beymelek Lagoon, a protected natural ecosystem, was selected due to its ecological significance, its role as a breeding ground for various fish species given the absence of similar studies in terms of scope, this research provides the basis for future studies. Additionally, the lagoon is safeguarded from human activities such as aquaculture, fishing, and wastewater discharge, making it an ideal site for investigating the relationship between antibiotic-resistant bacteria and resistance genes in fish from protected aquatic environments.

Materials and Methods

Sample Collection

A total of 50 fish samples were collected from the point at which Beymelek Lagoon connects to the sea on two occasions, with an interval of one week, in May 2022, as indicated by the blue dot and red arrow in Figure 1. Of these, 27 were obtained during the initial sampling, and 23 during the subsequent sampling, using the barricade-net method. The collection of fish samples was conducted through direct participation in the routine survey studies of the Fisheries Department of the Mediterranean Fisheries Research Production and Training Institute (MEDFRI). The fish were placed in ice-filled transport boxes following their catch and promptly transported into the Fish Disease Laboratory of MEDFRI. The necropsy and bacteriological cultivation processes commenced promptly, and the samples were maintained in refrigerators set at +4°C during this period to ensure their integrity.

Isolation and Identification of Bacteria

Before conducting necropsies, the skin of the fish was thoroughly cleaned with 70% ethanol to maintain aseptic conditions. Subsequently, liver, spleen, and anterior kidney samples were collected from each fish and homogenized using a sterile disposable tube tissue homogenizer (Capkin et al., 2015). One gram of homogenized tissue was suspended in 9 mL of sterile saline to create a 1:10 dilution and vortexed for two minutes to ensure thorough mixing. Then, 1 mL of this tissue-saline mixture was transferred into the first of five serial dilution tubes, each containing 9 mL of sterile saline. From each dilution, 0.1 mL was plated onto Marine Agar in triplicate and incubated at 22°C for five days (Canak & Akayli, 2019). The total average bacterial count was calculated after incubation, and morphologically distinct colonies were selected for subculturing. All bacterial isolates were assessed for catalase and oxidase activity as well as Gram staining (Arda, 2006). The selected bacteria were then identified using the VITEK 2 Compact identification system (Biomerieux) at the Konya Veterinary Control Institute Microbiology Laboratory.

Detection of the ARGs

A single colony measuring approximately 2 mm in diameter was selected using a disposable sterile loop and transferred into a 1.5 mL Eppendorf tube containing 500 µL of sterile distilled water. The mixture was vortexed for two minutes until fully suspended, followed by heat treatment at 100°C for 10 minutes in a heat block to lyse the bacterial cells and release their genetic material. After heat treatment, the tubes were centrifuged at 2500 rpm for 10 seconds, and the supernatant was collected for polymerase chain

reaction (PCR) analysis (Bergkessel & Guthrie, 2013; Midilli, 2022). The PCR process began with an initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50–60 °C for 30 seconds, and extension at 72°C for 1 minute. A final extension was carried out at 72°C for 5 minutes (Bergkessel & Guthrie, 2013; Woodman et al., 2016).

A total of 16 ARGs were examined in this study, selected based on their prevalence in aquatic environments. The primers used for the detection of resistance genes included *aadA1* (Aminoglycoside Adenyltransferase A1), *ampC* (Ampicillin Cephalosporinase), *blaTEM* (Beta-Lactamase TEM), *cfr* (Chloramphenicol-Florfenicol Resistance), *dfrA1* (Dihydrofolate Reductase A1), *ermB* (Erythromycin Ribosome Methylation), *floR* (Florfenicol Resistance), *intI1* (Class 1 Integron Integrase), *mphA* (Macrolide Phosphotransferase A), *qnrA1* (Quinolone Resistance Gene A1), *sul1, sul2, sul3* (Sulfonamide Resistance Genes 1, 2, and 3), and *tetA, tetB, tetW* (Tetracycline Resistance Genes A, B, and W). The complete list of primers is provided in Table 1.

A 2% agarose gel supplemented with 1% ethidium bromide was prepared for the electrophoresis of PCR products. Following the completion of the PCR process,

Figure 1. Lagoon-Sea connection point.

10 µL of the products were combined with 3 µL of a 5x loading dye solution. A total of 6 µL of DNA marker was loaded into the first well of the prepared gel, and 13 µL of the mixture containing DNA product and dye was added to wells numbered one through ten (Lee et al., 2012). The electrophoresis experiments were conducted at 80V and 500A for 15 minutes, followed by 40V and 500A for 40 minutes (Midilli, 2022). The gels were subsequently visualized using the OmniDOC™ Gel Documentation device (Cleaver Scientific).

Results

In this study a total of 50 fish categorized into 11 distinct species (*Lithognathus mormyrus, Diplodus sargus, Sardinella maderensis, Sardinella aurita, Mugil cephalus, Chelon ramada, Chelon labrosus, Chelon auratus, Chelon saliens, Dicentrarchus labrax, Sparus aurata)* were examined. Of these, 27 were belong to the initial sampling (*Lithognathus mormyrus, Sardinella maderensis, Sardinella aurita, Chelon ramada, Chelon labrosus, Chelon auratus, Chelon saliens, Dicentrarchus labrax, Sparus aurata*), and 23 were belong to the subsequent sampling (*Diplodus sargus, Sardinella maderensis, Sardinella aurita, Mugil cephalus, Chelon labrosus, Chelon auratus, Chelon saliens, Dicentrarchus labrax, Sparus aurata*). Table 2 presents a comprehensive overview of the fish species included in the study, detailing their individual weights as well as the average weights for each fish species.

Bacterial growth was observed in the internal organs, including the liver, spleen, and kidneys, of ten out of 50 fish examined during this study. The fish exhibiting bacterial growth in their internal organs are presented in Figure 2.

The highest total bacterial count was observed in the liver of fish sample number 46, which was a Gilthead seabream (*Sparus aurata*), with a count of 1.33×10^2. In contrast, the lowest average total bacterial count was found in fish sample number 12, a Sardine (*Sardinella aurita*), with a count of 1×10^2. Table 3 details the organs where bacterial growth was observed, along with the average total bacterial counts obtained after three consecutive bacterial cultivations from serial dilution tubes.

After conducting the classical biochemical tests such as Gram stain, catalase, oxidase, it was determined that five out of the ten examined bacteria were Gram-

Table 1. Primers used in the study

ARGs	Primers	Sequence (5' – 3')	T_a (°C)	PCR Product Size (bp)	References
aadA1	aadA1-FW	TATCCAGCTAAGCGCGAACT	58	447	(San Martín et al., 2008)
	aadA1-RV	ATTTGCCGACTACCTTGGTC			
ampC	ampC-FW	CCTCTTGCTCCACATTTGCT	58	189	(Yang et al., 2012)
	ampC-RV	ACAACGTTTGCTGTGTGACG			
blaTEM	blaTEM-FW	CATTTTCGTGTCGCCCTTAT	58	167	
	blaTEM-RV	GGGCGAAAACTCTCAAGGAT			
cfr	cfr-FW	TGTGCTACAGGCAACATTGGAT	55	148	(He et al., 2016)
	cfr-RV	CAAATACTTGACGGTTGGCTAGAG			
dfrA1	dfrA1-FW	GGAATGGCCCTGATATTCCA	55	95	(Johnson et al., 2016)
	dfrA1-RV	AGTCTTGCGTCCAACCAACAG			
ermB	ermB-FW	CATGCGTCTGACATCTATCTGA	56.8	190	(Xu et al., 2017)
	ermB-RV	CTGTGGTATGGCGGGTAAGTT			
floR	floR-FW	CGGTCGGTATTGTCTTCACG	56	171	(Li et al., 2013)
	floR-RV	TCACGGGCCACGCTGTAT			
intI1	intI1-FW	GGCTTCGTGATGCCTGCTT	57	146	(Luo et al., 2010)
	intI1-RV	CATTCCTGGCCGTGGTTCT			
mphA	mphA-FW	GCAGGCGATTCTTGAGCATT	57	214	(Dang et al., 2017)
	mphA-RV	GCCGATACCTCCCAACTGTA			
qnrA1	qnrA-FW	ATTTCTCACGCCAGGATTTG	53	516	(Robicsek et al., 2006)
	qnrA-RW	GATCGGCAAAGGTTAGGTCA			
sul1	sul1-FW	CGCACCGGAAACATCGCTGCAC	55.9	163	(Pei et al., 2006)
	sul1-RV	TGAAGTTCCGCCGCAAGGCTCG			
sul2	sul2-FW	TCCGGTGGAGGCCGGTATCTGG	60.8	191	
	sul2-RV	CGGGAATGCCATCTGCCTTGAG			
sul3	sul3-FW	CCCATACCCGGATCAAGAATAA	57	143	(Luo et al., 2010)
	sul3-RV	CAGCGAATTGGTGCAGCTACTA			
tetA	tetA-FW	GCTACATCCTGCTTGCCTTC	60	210	(Tamminen et al., 2011)
	tetA-RV	CATAGATCGCCGTGAAGAGG			
tetB	tetB-FW	CGAAGTAGGGGTTGAGACGC	56	192	(Luo et al., 2010)
	tetB-RV	AGACCAAGACCCGCTAATGAA			
tetW	tetW-FW	GAGAGCCTGCTATATGCCAGC	60	168	(Xu et al., 2017)
	tetW-RV	GGGCGTATCCACAATGTTAAC			

negative, while the remaining were Gram-positive. Subsequently, the identification process for these bacteria commenced at the Konya Veterinary Control Institute Microbiology Laboratory. The results of the biochemical tests carried out for identification using the VITEK 2 system are presented in Table 4 and Table 5, while the identified bacterial species can be found in Table 6.

Following the PCR and electrophoresis procedures conducted in this study, we determined that 40% of the previously identified bacterial isolates contained only the *intI1* gene. Additionally, 10% of the isolates exhibited the presence of both the *intI1* and *sul1* genes. It is noteworthy that no other ARGs, including *aadA1*, *ampC*, *blaTEM*, *cfr*, *dfrA1*, *ermB*, *floR*, *mphA*, *qnrA1*, *sul2*, *sul3*, *tetA*, *tetB*, and *tetW*, were detected among the ten isolates analyzed. The results of the 16 antibiotic resistance genes examined in the isolated bacteria are shown in Table 7.

Figures 3 and 4 present electrophoresis images that demonstrate positive results for the *intI1* and *sul1* genes in isolated bacterial samples respectively. In Figure 3, isolates 4, 5, 6, 8, and 9 yielded positive results for the *intI1* gene. Meanwhile, Figure 4 shows that only isolate number 6 exhibited positive result for the *sul1* gene.

Table 2. Total number of fish species and weights

Sample No.	Fish Species	Weight (g)	Number of fish	Mean Weight (g)
1	Sand steenbras (*Lithognathus mormyrus*)	144.10	3	174.27
2		208.10		
3		170.60		
4	White seabream (*Diplodus sargus*)	234.00	2	212.75
5		191.50		
6	Sardine (*Sardinella maderensis*)	42.00	3	46.77
7		58.00		
8		40.30		
9	Sardine (*Sardinella aurita*)	42.80	4	47.83
10		47.60		
11		50.60		
12		50.30		
13	Flathead grey mullet (*Mugil cephalus*)	237.50	3	290.83
14		320.00		
15		315.00		
16	Thinlip grey mullet (*Chelon ramada*)	122.30	2	121.23
17		120.15		
18	Thicklip grey mullet (*Chelon labrosus*)	120.60	3	124.35
19		126.48		
20		125.96		
21	Leaping mullet (*Chelon auratus*)	120.00	7	119.93
22		117.65		
23		119.80		
24		115.55		
25		122.75		
26		122.60		
27		118.90		
28	Golden grey mullet (*Chelon saliens*)	109.84	7	123.55
29		118.55		
30		125.45		
31		123.50		
32		142.20		
33		122.00		
34		123.30		
35	European seabass (*Dicentrarchus labrax*)	275.40	6	293.74
36		322.28		
37		280.18		
38		273.34		
39		324.10		
40		287.15		
41	Gilthead seabream (*Sparus aurata*)	317.40	10	312.17
42		318.38		
43		302.63		
44		305.50		
45		299.80		
46		311.72		
47		323.22		
48		302.46		
49		324.54		
50		316.00		

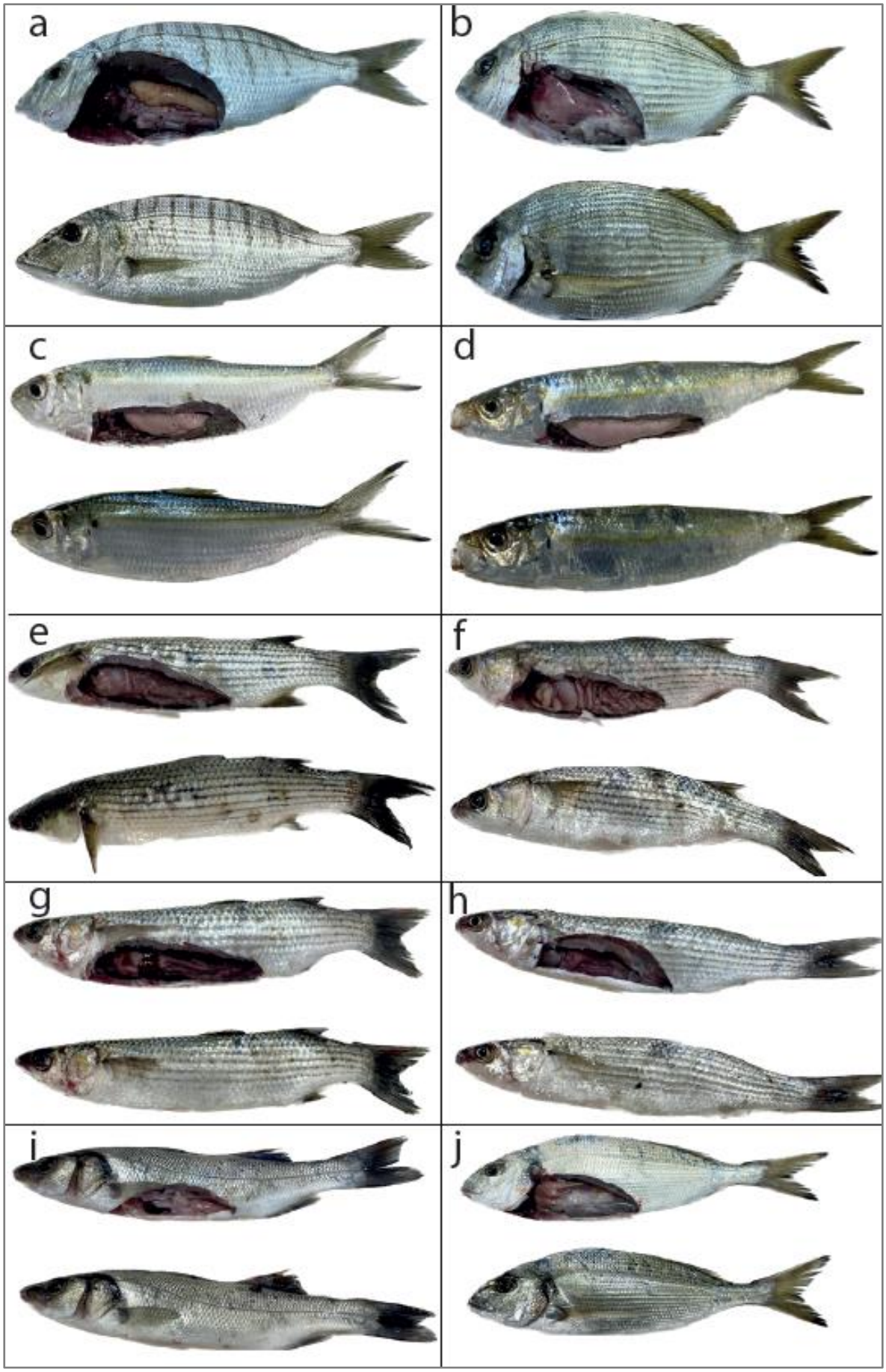

Figure 2. **a)** Sample 1: Sand steenbras (*Lithognathus mormyrus*), **b)** Sample 4: White seabream (*Diplodus sargus*), **c)** Sample 7: Sardine (*Sardinella maderensis*), **d)** Sample 12: Sardine (*Sardinella aurita*), **e)** Sample 18: Thicklip grey mullet (*Chelon labrosus*), **f)** Sample 19: Thicklip grey mullet (*Chelon labrosus*), **g)** Sample 25: Golden grey mullet (*Chelon auratus*), **h)** Sample 34: Leaping mullet (*Chelon saliens*), **i)** Sample 40: Sea bass (*Dicentrarchus labrax*), **j)** Sample 46: Sea bream (*Sparus aurata*).

Table 3. Total Bacterial Count in organs

Sample No:	Fish Species	Organs	Total Bacterial Count (cfu/g)
1	Sand steenbras (*Lithognathus mormyrus*)	Liver	1.2×10^2
4	White seabream (*Diplodus sargus*)	Kidney	1.02×10^2
7	Sardine (*Sardinella maderensis*)	Spleen	1.13×10^2
12	Sardine (*Sardinella aurita*)	Liver	1×10^2
18	Thicklip grey mullet (*Chelon labrosus*)	Spleen	1.08×10^2
19	Thicklip grey mullet (*Chelon labrosus*)	Liver	1.02×10^2
25	Leaping mullet (*Chelon auratus*)	Liver	1.15×10^2
34	Golden grey mullet (*Chelon saliens*)	Kidney	1.04×10^2
40	European seabass (*Dicentrarchus labrax*)	Spleen	1.26×10^2
46	Gilthead seabream (*Sparus aurata*)	Liver	1.33×10^2

Table 4. VITEK 2 biochemical test results (Gram-negative bacterial species)

	1	2	4	6	10
APPA	-	-	-	-	-
H_2S	-	+	-	-	-
BGLU	-	+	-	-	+
ProA	-	+	-	-	+
SAC	-	-	-	-	-
ILATk	-	-	-	-	-
GlyA	-	-	-	-	-
O129R	-	-	-	-	-
ADO	-	-	-	-	-
BNAG	-	-	-	-	-
dMAL	-	+	-	-	+
LIP	-	-	-	-	-
dTAG	-	-	-	-	-
AGLU	-	+	+	-	-
ODC	-	-	-	-	-
GGAA	-	-	-	-	-
PyrA	-	+	-	-	+
AGLTp	-	-	-	-	-
dMAN	-	-	-	-	-
PLE	-	-	-	-	-
dTRE	-	+	-	-	+
SUCT	-	+	-	-	-
LDC	-	-	-	-	-
IMLTa	-	+	-	-	-
IARL	-	-	-	-	-
dGLU	-	+	-	-	+
dMNE	-	+	-	-	+
TyrA	+	+	+	+	+
CIT	-	+	-	-	-
NAGA	-	-	-	-	-
IHISa	-	-	-	-	-
ELLM	-	-	-	-	-
dCEL	-	-	-	-	-
GGT	-	+	-	-	-
BXYL	-	-	+	-	-
URE	+	-	-	+	+
MNT	-	+	+	-	-
AGAL	-	-	-	-	-
CMT	-	+	-	-	+
ILATa	-	-	-	-	-
BGAL	-	-	-	-	-
OFF	-	-	-	-	-
BAlap	-	-	-	-	-
dSOR	-	-	-	-	-
5KG	-	-	-	-	-
PHOS	-	-	-	-	-
BGUR	-	-	-	-	-

Discussion

Approximately 45% of the global population resides within 100 km of coastlines (Zhu et al., 2017) Due to their geomorphological characteristics, lagoons represent highly sensitive aquatic ecosystems that are particularly vulnerable to anthropogenic influences. Environmental alterations within these ecosystems can serve as critical indicators for assessing ecological integrity, as well as potential impacts on animal and public health.

Bacteria are the primary causative agents of diseases in living organisms, and their resistance to antibiotics used in treatment is a growing concern. Interaction with aquatic environments is one of the potential pathways for ARGs transfer from environmental bacteria to human pathogens, leading to significant public health challenges such as the emergence of resistant bacterial strains and decreased treatment efficacy (Altuğ et al., 2013; Chen et al., 2013). For instance, there is epidemiological and molecular evidence suggesting that fish pathogens such as *Aeromonas* spp. have the potential to transfer antibiotic resistance genes to human-associated pathogens such as *Escherichia coli* (Rasul & Majumdar, 2017). Organisms inhabiting aquatic ecosystems are inevitably exposed to bacterial contamination. Additionally, it is known that marine fish species ingest water to maintain osmoregulation (Evans, 2008).

In this case, the interaction between fish and the bacteria presents in their aquatic environments, along with the resistance genes carried by these bacteria, becomes unavoidable. Numerous studies have documented the common presence of resistance genes, such as *blaTEM*, *tetA*, *tetB*, *sul1*, *sul2*, *sul3*, *floR*, *qnrA*, and *dfrA*, in various aquaculture environments. (Watts et al., 2017).

In a study on sea bream conducted by Canak & Akayli (2019), the bacterial species *Pseudomonas stutzeri*, commonly found in aquatic environments, and

Table 5. VITEK 2 biochemical test results (Gram-positive bacterial species)

	3	5	7	8	9
AMY	-	-	-	-	-
APPA	-	-	-	+	-
LeuA	+	-	+	+	+
AlaA	+	-	+	+	+
dRIB	-	+	-	+	-
NOVO	-	-	-	+	-
dRAF	-	-	-	-	-
OPTO	-	+	-	+	-
PIPLC	-	-	-	-	-
CDEX	-	-	-	+	-
ProA	+	-	-	+	-
TyrA	+	-	+	+	+
ILATk	+	+	+	-	-
NC6.5	-	+	-	+	-
O129R	-	-	-	+	-
dXYL	-	+	-	-	-
AspA	-	-	-	+	-
BGURr	-	-	-	-	-
dSOR	-	+	-	-	-
LAC	-	-	-	-	-
dMAN	-	+	-	-	-
SAL	-	+	-	+	-
ADH1	+	+	+	-	+
BGAR	-	-	-	-	-
AGAL	-	+	-	-	-
URE	-	-	-	-	-
NAG	-	+	-	+	-
dMNE	-	+	-	+	-
SAC	-	+	-	+	-
BGAL	-	-	-	-	-
AMAN	-	-	-	-	-
PyrA	-	-	-	+	-
POLYB	-	-	-	+	-
dMAL	-	+	-	+	-
MBdG	-	+	-	+	-
dTRE	-	+	-	+	-
AGLU	-	-	-	-	+
PHOS	+	+	-	-	-
BGUR	-	-	-	-	-
dGAL	-	-	-	-	-
BACI	-	-	-	+	-
PUL	-	-	-	-	-
ADH2s	-	-	-	-	-

Micrococcus luteus were identified. Antibiogram tests revealed that *M. luteus* was fully susceptible to ampicillin, erythromycin, florfenicol, and oxytetracycline, while *P. stutzeri* exhibited resistance to erythromycin but remained susceptible to enrofloxacin. In another study on sea bream (*Sparus aurata*), *Pseudomonas putida*, *P. stutzeri*, and *Kocuria rhizophila* were isolated, with antibiogram tests indicating florfenicol resistance in *P. stutzeri* and *P. putida* (Salgueiro et al., 2020). Additionally, Pękala et al. (2018) isolated *K. rhizophila* and *M. luteus* from the liver and spleen of two different trout species exhibiting disease symptoms in Poland. In our study, all resistance genes were negative in *K. rhizophila* strains isolated from leaping mullet (*Chelon auratus*), whereas only the *intI1* gene was detected in the *P. stutzeri* strain isolated from sardine (*Sardinella aurita*).

Ferri et al. (2023) isolated *Pseudomonas luteola*, *Kocuria kristinae*, and *Staphylococcus sciuri* in samples taken from processed seafood and their processing environments. Their molecular analysis detected *ermB*, *blaTEM*, and *cfr* in *S. sciuri*, and *blaTEM* in *P. luteola*, but no resistance genes in *K. Kristinae*. In our study, the *intI1* gene was detected in *S. sciuri* isolated from thicklip grey mullet (*Chelon laborus*). On the other hand, *K. kristinae*, previously unreported in marine animals, was first isolated from large yellow croaker (*Larimichthys crocea*) by Meng et al. (2023), suggesting its potential presence in aquatic organisms.

In our study, *Kocuria kristinae* was isolated and identified for the first time in Türkiye from a fish species. It was isolated from fish sample number 7, which is one of the three sardine samples (*Sardinella maderensis*) and this isolated bacterial did not show any antibiotic resistance genes.

Capkin et al. (2015) conducted a study in which they isolated three strains of *Pseudomonas luteola* from the spleens, kidneys, and livers of trouts. Molecular analysis revealed that these strains were positive only for *tetB*, *sul2*, *ampC*, and *blaTEM*, among the *tetA*, *tetB*, *sul1*, *sul2*, *sul3*, *ampC*, *blaTEM*, *aadA*, and *intI1* genes investigated. Türe & Alp (2016) isolated *P. luteola* from the liver and spleen of turbot, but the strain tested negative for the resistance genes *tetB*, *ampC*, *blaTEM*, *floR*, *sul2*, *sul3*, and *dfrA1*. No resistance genes were detected in *P. luteola*, which was isolated from Gilthead seabream (*Sparus aurata*).

It is known that *Kocuria rosea* is primarily isolated from human infections, though it has also been reported in extreme environments such as high-mineral groundwater and petroleum-contaminated sites (Akbari et al., 2021; Gholami et al., 2015; Timkina et al., 2022). In this study, both *K. rosea* strains isolated from European seabass (*Dicentrarchus labrax*) tested positive for the *intI1* gene, but negative for other resistance genes.

Lactococcus lactis is an important bacterial species traditionally associated with dairy products. However, *L. lactis* strains have also been isolated from various sources, including surface waters (Švec & Sedláček, 2008), the intestines of freshwater fish (Sugita et al., 2007), and marine fish (Itoi et al., 2008). However, Wang

Table 6. Identified bacterial species from organs

Sample No:	Fish Species	Organ	Isolate No:	Gram Stain	Bacteria
1	Sand steenbras (*Lithognathus mormyrus*)	Liver	1	-	*Acinetobacter lwoffii*
4	White seabream (*Diplodus sargus*)	Kidney	2	-	*Pseudomonas luteola*
7	Sardine (*Sardinella maderensis*)	Spleen	3	+	*Kocuria kristinae*
12	Sardine (*Sardinella aurita*)	Liver	4	-	*Pseudomonas stutzeri*
18	Thicklip grey mullet (*Chelon labrosus*)	Spleen	5	+	*Staphylococcus sciuri*
19	Thicklip grey mullet (*Chelon labrosus*)	Liver	6	-	*Acinetobacter lwoffii*
25	Leaping mullet (*Chelon auratus*)	Liver	7	+	*Kocuria rhizophila*
34	Golden grey mullet (*Chelon saliens*)	Kidney	8	+	*Lactococcus lactis* subsp. *lactis*
40	European seabass (*Dicentrarchus labrax*)	Spleen	9	+	*Kocuria rosea*
46	Gilthead seabream (*Sparus aurata*)	Liver	10	-	*Pseudomonas luteola*

Table 7. Results of ARGs profile

Isolate No:	Bacteria / ARGs	*aadA1 ampC blaTEM cfr dfrA1 ermB floR intI1 mphA qnrA sul 1 sul 2 sul 3 tetA tetB tetW*
1	*Acinetobacter lwoffii*	
2	*Pseudomonas luteola*	
3	*Kocuria kristinae*	
4	*Pseudomonas stutzeri*	
5	*Staphylococcus sciuri*	
6	*Acinetobacter lwoffii*	
7	*Kocuria rhizophila*	
8	*Lactococcus lactis subsp. lactis*	
9	*Kocuria rosea*	
10	*Pseudomonas luteola*	

*(Pink: Gram-negative bacteria, Blue: Gram-positive bacteria, Green: ARG positive for relevant bacteria)

et al. (2008) were the first to demonstrate that it can infect aquatic animals, beginning with shrimps, and followed by Khoo et al. (2014) in silver carp. In a study conducted by Chen et al. (2012), *Lactococcus lactis subsp*. *lactis* was isolated from the internal organs of sick fish after an outbreak among cultured sturgeon. According to antibiotic susceptibility tests, they found the bacteria were resistant to oxytetracycline, flumequine, and erythromycin, but were sensitive to ampicillin and florfenicol. Wünnemann et al. (2018) found that all *Lactococcus lactis* subsp. *lactis* strains, isolated from the internal organs of allis shad (*Alosa alosa*) exhibiting disease symptoms, were susceptible to gentamicin, erythromycin, tetracycline, and ampicillin. We detected only the *intI1* gene in *Lactococcus lactis* subsp. *lactis* strain isolated from golden grey mullet (*Chelon saliens*) fish samples collected from the lagoon-sea connection point and no other resistance genes were identified.

Kozińska et al. (2014) isolated two strains of *Acinetobacter lwoffii*, a bacterium known for its potential to transfer ARGs, from carp obtained from various fish farms. One of these strains exhibited resistance to sulfamethoxazole/trimethoprim and amoxicillin, while the other displayed resistance to amoxicillin and ampicillin. In this study we isolated two *A. lwoffii* strains from both sand steenbras (*Lithognathus mormyrus*) and thicklip grey mullet; however, only the strain from thicklip grey mullet harbored the *intI1* and *sul1* genes.

In our study, the bacteria isolated from fish samples collected from Beymelek Lagoon showed similarities to those isolated in the aforementioned studies. The presence of integrons in bacterial communities within aquatic environments is considered an indicator of the degree of water body pollution (Lupo et al., 2012). Moreover, various scientific studies conducted globally have revealed a strong correlation between the *sul1* and *intI1* genes (De los Santos et al., 2021). The *sul1* gene, which serves as a reliable marker for both horizontal gene transfer and multidrug resistance, is frequently detected in environmental and clinical bacterial populations and is typically associated with class 1 integrons (Blanco-Picazo et al., 2020; Sköld 2000).

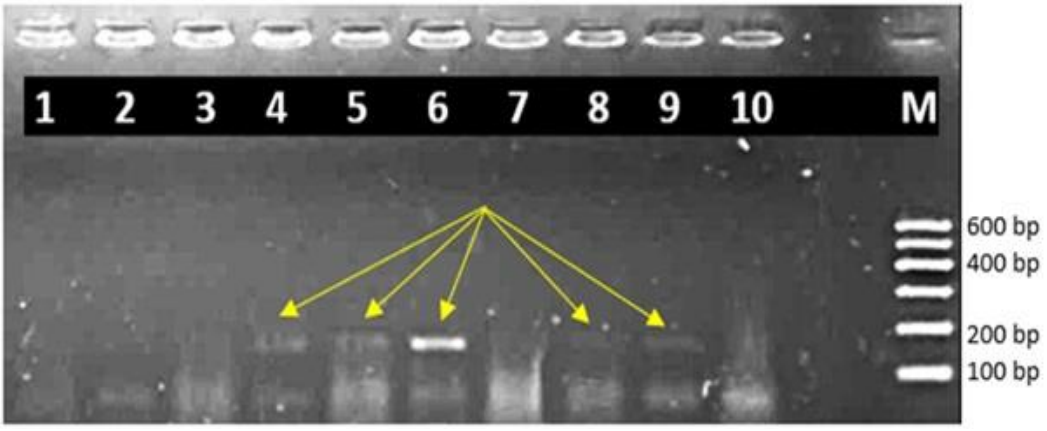

Figure 3. Electrophoresis of *intI1* for bacteria numbered 1-10 (Marker 100 bp, *intI1*: 146 bp).

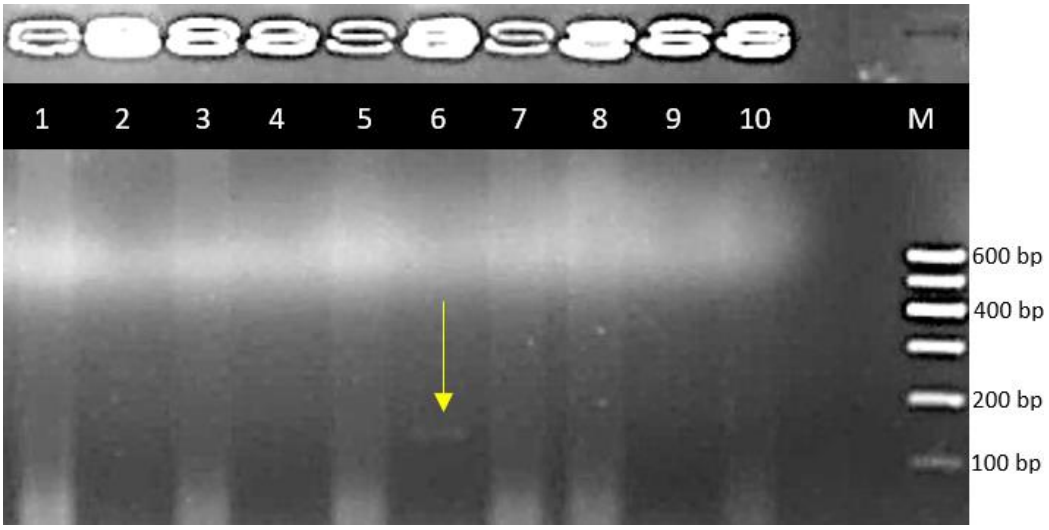

Figure 4. Electrophoresis of *sul1* for bacteria numbered 1-10 (Marker 100 bp, *sul1*: 163 bp).

In the context of our study, the coexistence of the *intI1* gene and the *sul1* gene aligns with the findings reported in the literature. Our previous findings further suggest that the presence of antibiotic resistance genes, including *blaTEM*, *ermB*, *intI1*, *sul1*, *sul2*, and *tetW*, in bacterial species isolated from the water samples of Beymelek Lagoon underscores their significance as potential biomarkers of environmental pollution (Boyraz et al., 2024).

Conclusion

Coastal lagoon regions are of significant ecological importance and provide valuable opportunities for fishery and aquaculture practices. However, it is essential to acknowledge that the interactions between these environments and human communities can have both positive and negative impacts. The increasing effects of global warming, urbanization, and growing human populations, along with their associated activities, are contributing to the degradation of environmental ecosystems.

In this study, various fish species were collected from Beymelek Lagoon, one of the most well-preserved natural lagoons in Türkiye. Bacterial species were isolated from the internal organs of these fish. Notably, one of these species was identified for the first time in fish from Türkiye. Additionally, several ARGs were detected in these bacterial isolates. The study also revealed that the isolated bacteria may potentially serve as disease agents for fish. In this context, this study represents the first investigation conducted in the Beymelek Lagoon, offering novel insights into the significance of monitoring antibiotic resistance in aquatic organisms within natural ecosystems. The findings contribute to a deeper understanding of the potential risks associated with antimicrobial resistance in aquatic environments and highlight the importance of continuous surveillance for environmental and public health impacts.

The presence of ARGs has recently emerged as a significant indicator of environmental pollution. Previous studies on ARB and the resistance genes they carry highlight the seriousness of this issue. Furthermore, it is crucial to recognize that food products, whether it is wild-caught or produced by aquaculture, can serve as reservoirs for bacteria and potential carriers for ARGs transmission to humans. Within the framework of the One Health concept, we advocate for the integrated monitoring of environmental, animal, and human health as a comprehensive strategy to mitigate the spread of antimicrobial resistance.

Ethical Statement

No live animals were used in the study thus there is no need for Ethical Approval.

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Author Contribution

All authors attended the laboratory studies and data analyses. Additionally, all of them were involved in writing the manuscript and approved the final version.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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