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Detection of Antibiotic Resistance Genes in Bacteria Isolated from Beymelek Lagoon Water Samples

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Abstract

This study investigated the prevalence of antibiotic resistance genes in bacteria isolated from water samples collected from Beymelek Lagoon, Turkey. Water samples were obtained at two different periods from five distinct locations within the lagoon. At each sampling site, environmental parameters, including temperature, pH, and salinity, were measured to provide a comprehensive understanding of the sampling conditions. A total of 16 bacterial strains were isolated from water samples collected in February, and an additional 12 strains were isolated from samples collected in August 2022. The isolates were identified using the VITEK2 Compact automated identification system. Among the 28 isolates obtained across the sampling periods, 16 were classified as Gram-negative and 12 as Gram-positive. In this study 16 antibiotic resistance genes: aadA1, ampC, blaTEM, cfr, dfrA1, ermB, floR, intI1, mphA, qnrA, sul1, sul2, sul3, tetA, tetB, and tetW investigated. In February, the blaTEM gene was detected in 25% of the isolates, ermB in 7.14%, while sul1, sul2, and tetW genes were each identified in 3.57% of the isolates. In August, blaTEM, intI1, and tetW genes were detected in 17.86%, 25%, and 3.57% of the isolates, respectively. This study highlights the presence of antibiotic resistance genes in bacteria isolated from lagoon water.

Introduction

Antibiotics have been widely used to treat bacterial infections in humans and animals since their discovery (Bhat & Altinok, 2023; Liu et al., 2018). While most antibiotics are now synthetically manufactured, they are naturally produced by bacteria and fungi, organisms that have existed for millions of years. These microorganisms synthesize antibiotics as a survival strategy, enabling them to protect themselves, compete for food resources, colonize their habitats, and eliminate other microorganisms in their environment (Martínez, 2008; Zhuang et al., 2021).

These natural antibiotic producers, especially bacteria, are naturally resistant to the antibiotics they produce. Other bacteria in the environment try to survive by developing antibiotic resistance mechanisms or by acquiring them later (Jury et al., 2010; Leiva et al., 2021). Based on this information, it is extremely important to consider that antibiotic resistance genes may also emerge in non-clinical environments and that resistance may develop against different antibiotics (Martínez, 2008). The spread of antibiotic-resistant bacteria into aquatic environments, including drinking water sources, represents one of the most significant threats to human health in the 21st century. This

growing issue poses challenges for public health, as it compromises the effectiveness of treatments and increases the risk of disease transmission through contaminated water. (Sanganyado & Gwenzi, 2019; CDC, 2019; World Health Organisaton, 2019).

Lagoons, which are a transition zone between land and seas or oceans, are very sensitive to environmental changes due to their structure and may be rich in antibiotic-resistant bacteria. The accumulation and spread of antibiotic-resistant bacteria in lagoons (H. Wang et al., 2021) causes lagoon environments to serve as a source of transmission of these bacteria to both the environment, animals and humans (Dolejska & Papagiannitsis, 2018). It is thought that a better understanding of the role of environmental and social influences in shaping antibiotic resistance in natural environments can help predict and prevent the emergence of resistance and its possible harmful effects in the future.

This study focused on Beymelek Lagoon, situated in the Demre district of Antalya province, Turkey, as the research site. The investigation aimed to detect antibiotic resistance genes in bacteria isolated from water samples collected at five distinct locations within the lagoon during two different periods. Environmental parameters, including temperature, pH, and salinity, were measured at each sampling point. Notably, the bacterial isolates and identified antibiotic resistance genes were analyzed in relation to temperature data, allowing for a comparative assessment of the findings.

Materials and Methods

Sample Collection

Water samples were collected considering the limnological characteristics of the lagoon, at a depth of 15 cm from the surface, during two distinct periods. To ensure reliable microbial profiling, three 100 mL water samples were obtained from each sampling site. Among the sampling points, Point 1 was specifically selected due to its proximity to the brackish water inflow that feeds the lagoon, Point 2 was near the highway, and Point 3 represented the connection point to the sea (Figure 1).

The sampling periods, February and August, were chosen based on data from the Mediterranean Fisheries Research, Production, and Training Institute, as these months represent the lowest and highest average lagoon water temperatures, respectively. During sampling, water temperature, salinity, and pH levels were measured and recorded. Water samples were collected in sterile, disposable 100 mL plastic containers and transported to the microbiology laboratory under cold chain conditions within a short time frame. The samples were maintained at +4°C in a cooler until bacteriological analysis was performed.

Isolation and Identification of Bacteria

The primary water sample was utilized for bacterial isolation, while two additional samples were preserved

Figure 1. Beymelek Lagoon.

at +4°C as backups. As the primary sample provided sufficient bacterial growth, the preserved samples were not processed further. Each 100 mL sample was vortexed, and 1 mL was taken from the midpoint of the water column in the container and transferred to a glass tube containing 9 mL of sterile physiological saline. After vortexing the sample in the tube, 1 mL was transferred to the second dilution tube. Serial dilutions continued until the fifth dilution tube.

From each dilution tube, 0.1 mL of the sample was spread, in triplicate, onto Marine Agar (MA) plates. MA is an effective nutrient medium for marine bacteria, supporting the isolation, cultivation, and maintenance of diverse heterotrophic marine bacterial species. In this study, MA was the primary medium used for these purposes (Nikolakopoulou et al., 2008; Rodrigues & de Carvalho, 2022). The plates were incubated for four days at 18°C for February samples and 29°C for August samples.

After incubation, morphologically distinct colonies were selected and subcultured. A loopful of colonies grown on MA was evaluated for catalase and oxidase activity, and Gram staining was performed on clean slides (Arda, 2006). Furthermore, bacteria initially cultured on MA were transferred to Trypticase Soy Agar (TSA) and Brain Heart Infusion Agar (BHIA) to determine their ability to grow on routine media.

Pure colonies were inoculated into Marine Broth (MB) medium. Subsequently, MB with 20% glycerin (Tendencia & De La Peña, 2001) was used for long-term storage in 5 mL screw-capped DNase- and RNase-free cryotubes at -20°C. Bacterial species identification was performed using the VITEK2 Compact automatic ID/AST system (Biomerieux) at the Konya Veterinary Control Institute Microbiology Laboratory.

Detection of the Antibiotic Resistance Genes

In this study, the colony PCR method was employed to obtain genetic material for detecting resistance genes (Packeiser et al., 2013; Sebastião et al., 2015). Briefly, bacterial colonies approximately 2 mm in diameter were collected using disposable sterile loops and transferred into 1.5 mL Eppendorf tubes containing 500 µL of sterile distilled water. The bacterial cells were suspended by vortexing for 2 minutes.

Following suspension, the mixture was heated at 100°C for 10 minutes to lyse the cells. The genetic material released by cell wall disruption was subsequently isolated by centrifuging the tubes at 2500 rpm for 10 seconds in a microcentrifuge. The supernatant containing the DNA was collected and used as a template for the PCR process (Woodman et al., 2016).

The primers used in this study are listed in Table 1 and were synthesized accordingly. The target genes included aadA1 (aminoglycoside resistance), ampC and blaTEM (beta-lactam resistance), cfr (amphenicol

Table 1. Primer pairs used in this study

	Primers	Sequence	T_a (°C)	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			

resistance), dfrA1 (trimethoprim resistance), ermB (macrolide resistance), floR (amphenicol resistance), Class 1 integron integrase (IntI1), mphA (macrolide resistance), qnrA1 (quinolone resistance), sul1, sul2, sul3 (sulfonamide resistance), and tetA, tetB, tetW (tetracycline resistance).

PCR was performed using Qiagen Master Mix (Qiagen 201445) following the manufacturer's instructions. Amplification was carried out with an initial denaturation 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 step was conducted at 72°C for 5 minutes (Bergkessel & Guthrie, 2013).

All PCR products were analyzed via 2% agarose gel electrophoresis at 80 V for 45 minutes. The gels were stained with 10% ethidium bromide and visualized under ultraviolet (UV) light.

Results

Temperature and salinity values for the regions where water samples were collected in February and August are presented in Table 2. In February, the region with the lowest water temperature was No: 3, measuring 16.0°C, while the highest water temperature was observed at No: 4, measuring 17.2°C. Salinity values in February were lowest at the Region No: 1 and highest at No: 2.

In August, the region with the lowest water temperature was No: 1 at 25.5°C, whereas the highest temperature was recorded at No: 4, reaching 29.5°C. Salinity values in August were lowest at the Region No: 1 and highest at No: 3.

A total of 28 bacterial strains were isolated from lagoon water samples. From the samples collected in February, seven Gram-negative and nine Gram-positive strains were isolated, while nine Gram-negative and three Gram-positive strains were isolated from the samples collected in August. The isolates were identified using the VITEK2 Compact automatic identification system. The results of the VITEK2 biochemical tests for Gram-negative and Gram-positive bacteria are presented in Tables 3 and 4, respectively. The resistance gene profiles of the identified bacteria are provided in Table 5.

The data on antibiotic resistance genes present in the bacterial strains isolated in this study are presented in Table 3. Among the isolates, blaTEM was the most commonly detected antibiotic resistance gene. The detection rates of resistance genes in the isolated bacteria were as follows: 42.86% for blaTEM, 7.14% for ermB, 25% for intI1, 3.57% for sul1, 3.57% for sul2, and 7.14% for tetW. However, some antibiotic resistance genes, including aadA1, ampC, cfr, dfrA1, floR, mphA, qnrA1, sul3, tetA, and tetB, were not detected in any of the 28 isolates.

The distribution of resistance genes among the bacterial isolates, categorized by the period of isolation, is illustrated in Figure 2. Gel electrophoresis images of bacterial strains that tested positive for antibiotic resistance genes are shown in Figures 3 through 8.

Discussion

Coastal lagoons are known to constitute approximately 13% of the world's coastlines (Barnes, 1980). The changes occurring in lagoon areas, which are more sensitive than other marine environments, are crucial for environmental, animal, and public health. Antimicrobial resistance genes detected in these ecosystems serve as indicators of pollution resulting from human activities and have been described as environmental pollutants due to their presence in these areas (Altuğ et al., 2013; Rysz & Alvarez, 2004).

The detection of resistance genes such as intI, sul1, tetW, and blaTEM in natural environments is widely accepted as an indicator of environmental antimicrobial resistance (Nappier et al., 2020). The abundance of integrons in bacterial communities within aquatic habitats is known to reflect the degree of pollution in water bodies (Lupo et al., 2012). The sul1 gene, which is a strong indicator of horizontal gene transfer and multiple resistance, is commonly found in environmental and clinical bacterial populations, often within class 1 integrons on conjugative plasmids that also harbor other resistance genes. Studies have shown that the tetW gene confers resistance to tetracycline and is frequently found in aquaculture environments (Blanco-Picazo et al., 2020; Jian et al., 2021; Roberts, 2002, Sköld, 2000).

The presence of antibiotic resistance genes such as intI, sul1, tetW, and blaTEM in the present study underscores the importance of these genes as pollution indicators. Notably, the detection of the blaTEM gene in bacteria containing the sul1 gene aligns with the expression of multiple resistance traits.

Table 2. Water parameters during sampling times

	February			August		
Region	Temperature	Salinity (%)	pH	Temperature	Salinity (%)	pH
No:1	16.2	1.80	7.82	25.5	2.15	7.70
No:2	16.8	2.41	7.94	28.5	2.58	7.85
No:3	16.0	2.25	7.99	29.4	2.78	7.89
No:4	17.2	2.35	7.91	29.5	2.76	7.86
No:5	16.5	2.33	7.94	28.3	2.74	7.89

Table 3. VITEK 2 biochemical test results of Gram-negative bacteria

	1	6	8	10	12	14	15	18	19	21	22	23	24	25	27	28
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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Most culture-based studies of aquatic bacterial communities typically focus on fecal-derived bacteria or well-known human pathogens. However, every bacterium in an ecosystem can act as both a reservoir and a vector for the spread of resistance genes (Almakki et al., 2017). In a study conducted by Gholami (2012) in Karataş Lagoon, bacteria such as *Sphingomonas paucimobilis*, *Vibrio parahaemolyticus*, *Pseudomonas stutzeri*, and *Vibrio alginolyticus* were isolated from lagoon water samples collected over two different periods. Similarly, Gürün (2014) isolated bacteria including *E. coli*, *Sphingomonas paucimobilis*, *Myroides* sp., *Micrococcus luteus*, and *Kocuria kristinae* from seawater in Güllük Bay.

Aerococcus viridans is frequently encountered as a human pathogen (Pien et al., 1984). In a study by Buu-Hoi et al. (1989), strains of *A. viridans* isolated from human and animal samples tested positive for the ermB resistance gene. In our study, only the blaTEM gene was detected in the *A. viridans* strain isolated from the Region No:1 in February.

Beach et al. (2012) conducted an antibiogram test on a *Bordetella hinzii* strain isolated from sick turkeys and found that the strain was resistant to cefotaxime and tobramycin, moderately sensitive to ampicillin and chloramphenicol, and fully sensitive to tetracycline, sulfamethoxazole/trimethoprim, and ciprofloxacin. In our study, the *B. hinzii* strain isolated from the Region No:2 in August tested negative for other resistance genes but showed a positive reaction for the blaTEM gene.

In a study conducted by Capkin et al. (2015) on trout and the environments in which they were raised, *P. luteola* was isolated from the internal organs of the

Table 4. VITEK 2 biochemical test results of Gram-positive bacteria

	2	3	4	5	7	9	11	13	16	17	20	26
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	-	-	-	-	-	-	-	-	-	-	-	-

fish, while *E. coli* and other coliforms were detected in water samples. The tetB, sul2, ampC, and blaTEM genes were found in *P. luteola* strains, while tetA, tetB, sul1, sul2, ampC, aadA, and blaTEM genes were found in varying amounts in the *E. coli* isolates. In our study, one of the three *P. luteola* strains isolated from water samples collected in August tested positive for blaTEM and tetW, another tested positive for blaTEM and intI1, and the third tested positive only for the blaTEM gene. Additionally, only the blaTEM gene was detected in the *E. coli* strain isolated from water samples taken from the Region No: 4 in February.

Enterococcus columbae is a type of enterococcus first described in pigeons (Devriese et al., 1990). It has been reported that bacteria isolated from pigeons may exhibit high resistance to antibiotics (Osman et al., 2019; Stenzel et al., 2014). Dolka et al. (2020) investigated the antibiotic resistance profiles of 50 *E. columbae* strains isolated from the cloaca of pigeons across various regions of Poland at different times. Their findings revealed that 44 of these strains were resistant to enrofloxacin, 39 to doxycycline, 33 to erythromycin, 11 to chloramphenicol, and seven to tetracycline. One strain was resistant to penicillin, while all strains were found to be susceptible to ampicillin and amoxicillin/clavulanic acid. In our study, it was determined that the *E. columbae* strain isolated from water samples collected from the Region No: 2 in February contained both blaTEM and tetW resistance genes.

Hafnia alvei is a bacterial species that has been isolated from a diverse range of organisms and environments, including mammals, birds, reptiles, fish, soil, water, and sewage. It is infrequently recognized as an opportunistic pathogen and is typically not considered directly associated with humans (Janda & Abbott, 2006; Okada & Gordon, 2003). Castello et al. (2023) reported that among the two *H. alvei* strains isolated from fresh vegetable products in their study, one exhibited a positive reaction for the blaTEM gene

Table 5. Identified bacteria and their resistance profiles (Pink: Gram-negative, Blue: Gram-positive)

	No	Region	Bacteria	aadA1	ampC	bla TEM	cfr	dfr A1	ermB	floR	intI1	mphA	qnrA1	sul 1	sul 2	sul 3	tetA	tetB	tetW
February	1	No:1	*Sphingomonas paucimobilis*			■								■					
	2	No:1	*Micrococcus luteus*																
	3	No:1	*Staphylococcus lentus*																
	4	No:1	*Kocuria kristinae*																
	5	No:1	*Aerococcus viridans*			■													
	6	No:1	*Pseudomonas stutzeri*			■													
	7	No:2	*Enterococcus columbae*			■													■
	8	No:2	*Pseudomonas stutzeri*			■			■										
	9	No:2	*Micrococcus luteus*																
	10	No:3	*Pseudomonas stutzeri*																
	11	No:3	*Micrococcus luteus*																
	12	No:4	*Hafnia alvei*			■			■						■				
	13	No:4	*Micrococcus luteus*																
	14	No:4	*E. coli*			■													
	15	No:5	*Sphingomonas paucimobilis*																
	16	No:5	*Kocuria rhizophila*																
August	17	No:1	*Kocuria rhizophila*																
	18	No:1	*Pseudomonas luteola*			■													■
	19	No:2	*Bordetella hinzii*			■													
	20	No:2	*Micrococcus luteus*								■								
	21	No:2	*Pseudomonas luteola*			■													
	22	No:3	*Vibrio parahaemolyticus*								■								
	23	No:3	*Pseudomonas putida*			■					■								
	24	No:3	*Myroides* sp.								■								
	25	No:4	*Vibrio alginolyticus*								■								
	26	No:4	*Kocuria rosea*								■								
	27	No:5	*Vibrio alginolyticus*																
	28	No:5	*Pseudomonas luteola*			■					■								

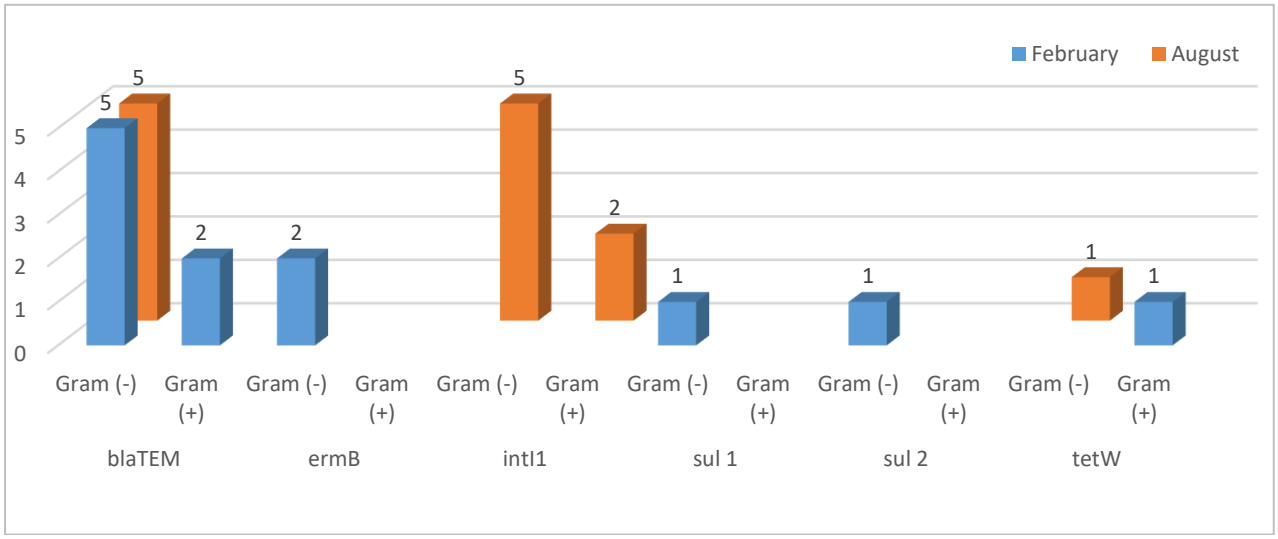

Figure 2. Distribution of bacteria according to antibiotic resistance genes.

but tested negative for tetA, tetB, tetW, sul2, and sul3, while the other strain tested negative for all these genes. In our study, the *H. alvei* strain isolated from the Region No: 4 in February demonstrated positive reactions for the blaTEM, ermB, and sul2 genes, highlighting the potential role of *H. alvei* as a significant indicator of multidrug resistance.

Pękala et al. (2018) isolated *Kocuria rhizophila* and *Micrococcus luteus* from the internal organs of two different trout species exhibiting disease symptoms in Poland. Using the disk diffusion method, they found that *Kocuria* and *Micrococcus* strains were resistant to flumequine, oxolinic acid, and sulfonamides, but sensitive to β-lactams, macrolides, amphenicols, and tetracyclines. In our study, all resistance genes tested negative in the *K. rhizophila* strains. The intI1 gene was detected in only one *M. luteus* strain isolated in August.

Marques et al. (2023) observed that *Kocuria kristinae*, isolated from a boa constrictor, was resistant to sulfamethoxazole/trimethoprim, tetracycline, and erythromycin but sensitive to gentamicin. However, no resistance genes were detected in the *K. kristinae* strains

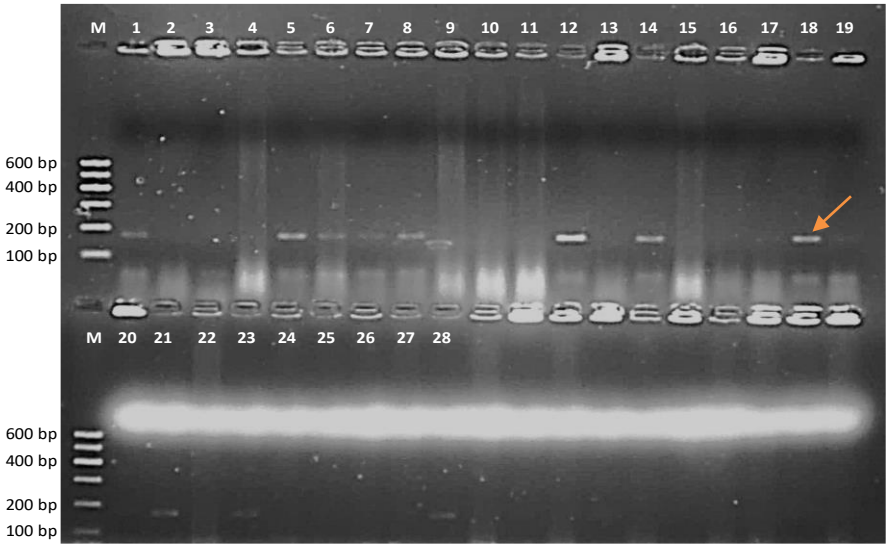

Figure 3. Electrophoresis of blaTem gene (M: Marker 100 bp, blaTem: 167 bp).

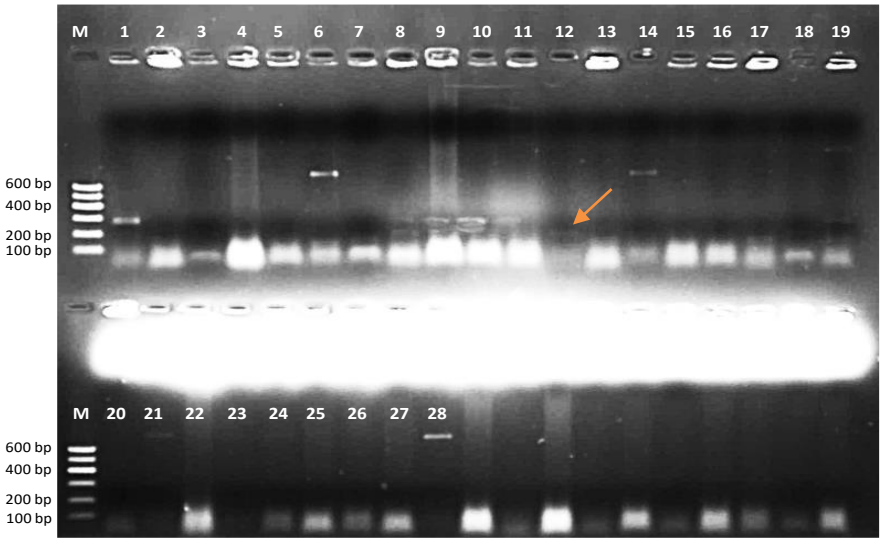

Figure 4. Electrophoresis of ermB gene (M: Marker 100 bp, ermB: 190 bp).

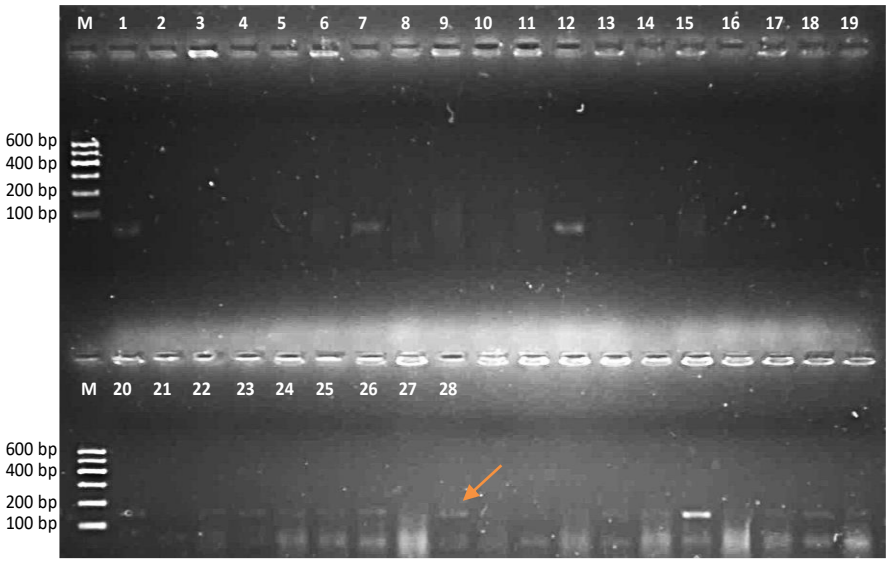

Figure 5. Electrophoresis of intI1 gene (M: Marker 100 bp, intI1: 146 bp).

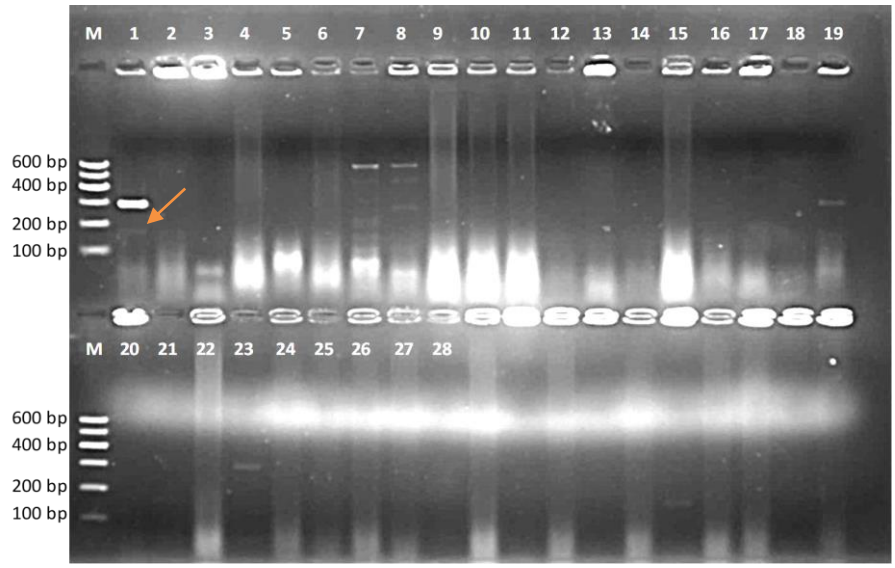

Figure 6. Electrophoresis of sul1 gene (M: Marker 100 bp, sul1: 163 bp).

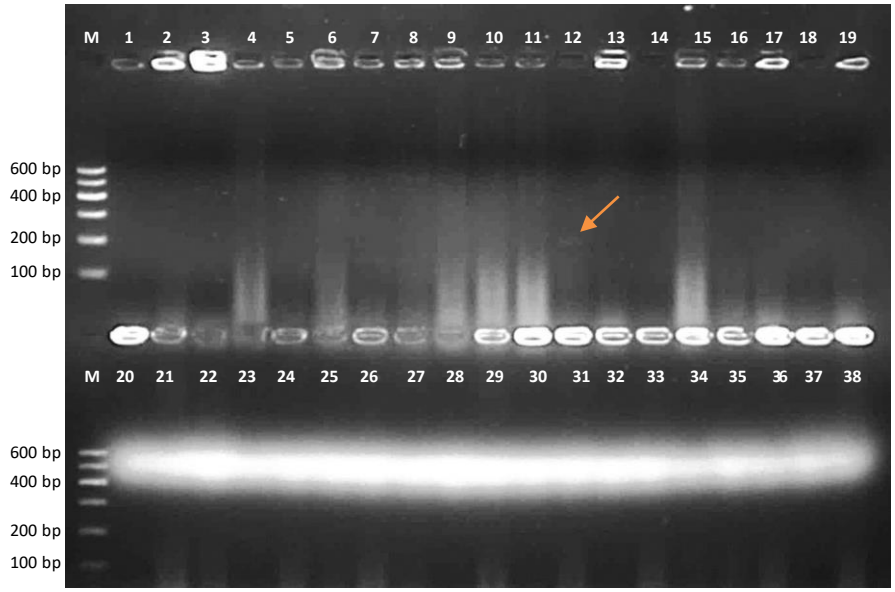

Figure 7. Electrophoresis of sul2 gene (M: Marker 100 bp, sul2: 191 bp).

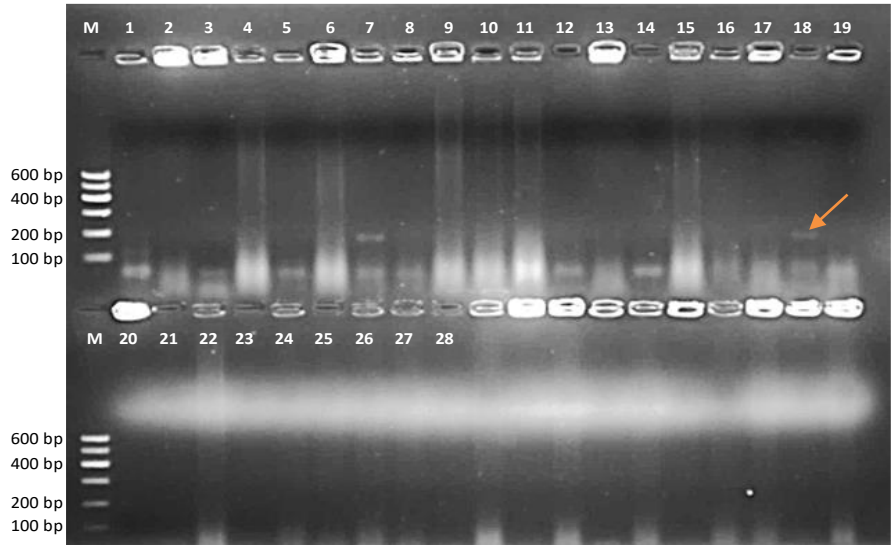

Figure 8. Electrophoresis of tetW gene (M: Marker 100 bp, tetW: 168 bp).

isolated from the samples collected from the Region No: 1 in February. Although *K. rosea* is typically associated with human disease cases, studies have shown that it can also be isolated from environmental sources (Akbari et al., 2021; Timkina et al., 2022). In our study, the *K. rosea* strain isolated from water samples tested positive for the intI1 gene; however, no resistance genes were identified.

Studies have demonstrated that species within the *Myroides* genus are commonly isolated from seafood and environmental sources such as water and soil (Licker et al., 2018; Z. D. Zhang et al., 2014). In a clinical study, *Myroides* species were reported to harbor the *sul2* and *sul3* genes (Ming et al., 2017). In our study, a *Myroides* sp. strain was isolated from samples collected from the Region No: 3 in August, and the intI1 gene was detected in this strain.

Luczkiewicz et al. (2015) reported that the dominant species among the *Pseudomonas* strains isolated from wastewater facility samples was *P. putida*. Antibiogram testing revealed that this bacterium exhibited resistance to various antibiotics, including gentamicin, ciprofloxacin, and piperacillin. It was suggested that *P. putida* could serve as a potential receptor and reservoir for antimicrobial resistance genes. In our study, the blaTEM and intI1 genes were detected in the *P. putida* strain isolated from the Region No: 3 in August. Z. Wang et al. (2023) isolated *P. stutzeri* from an industrial wastewater environment and reported that the qnrA and sul2 genes were present in the strains they examined. Among the three *P. stutzeri* strains isolated from water samples in February in our study, one strain tested positive for the blaTEM gene, while another was positive for both the blaTEM and ermB genes. The third strain tested negative for all the resistance genes analyzed.

Ferri et al. (2023) isolated *Staphylococcus lentus* and *Sphingomonas paucimobilis* from processed seafood and associated environments. They identified the ermB, cfr, sul1, and sul3 genes in *S. lentus* and the blaTEM gene in *S. paucimobilis*. In our study, no resistance genes were detected in the *S. lentus* strain isolated from samples collected from the Region No: 1 in February. Among the two *S. paucimobilis* strains isolated in February, one tested negative for all genes, while the other was positive for both the blaTEM and sul1 genes.

Vibrio alginolyticus is a marine bacterium frequently associated with diseases in various marine organisms, while *Vibrio parahaemolyticus* is a significant cause of acute gastroenteritis, wound infections, and sepsis in coastal regions worldwide (F. Zhang et al., 2024). Håkonsholm et al. (2020) detected ampC, bla, qnr, and tet genes in 53 *V. alginolyticus* strains isolated from water samples in the Norwegian Sea. Beshiru & Igbinosa (2023) investigated 67 *V. parahaemolyticus* strains from ready-to-eat foods in Nigeria, reporting resistance gene distributions as follows: 49.3% for blaTEM, 8.9% for aadA, 16.4% for tetA, 11.9% for tetB and dfrA, 20.9% for sul1, 8.9% for sul2, 13.4% for qnrA, and 16.4% for intI1. In our study, one *V. parahaemolyticus* and two *V. alginolyticus* strains were isolated from water samples collected in August. The *V. parahaemolyticus* strain and one of the *V. alginolyticus* strains tested positive for the intI1 gene, while no resistance genes were detected in the other *V. alginolyticus* strain.

Our findings reveal that most bacterial species and resistance genes identified in this study align with those reported in prior research, though some variations were observed. These discrepancies may be attributed to the unique environmental conditions and sampling locations. Furthermore, differences in resistance genes could be explained by the ability of bacteria to utilize horizontal gene transfer mechanisms, interactions within bacterial communities, and exposure to environmental factors that drive the development of resistance.

Conclusion

In our study, water samples collected from the Beymelek Lagoon during two different periods were analyzed for antibiotic-resistant bacteria and the resistance genes they carry. The lower number of bacteria isolated from water samples in August compared to February is thought to result from increased water temperature and salinity levels. However, in terms of resistance genes, bacteria isolated from August samples were found to carry more resistance genes than those from February. This finding aligns with the idea that salinity and temperature are significant factors influencing the resistance of aquatic bacterial communities to antimicrobial agents (Almakki et al., 2017).

The data obtained in this study were compared with findings from previous research conducted in various regions worldwide. Within the framework of the "One Health" concept, this study reinforces the idea that the interconnectedness of the environment, animal health, and human health should be considered a common denominator in addressing the development of antimicrobial resistance. Regular monitoring of these factors is essential to combat this global challenge effectively.

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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