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Research Article

Screening of *Aeromonas* spp. and their antimicrobial resistance profiles in wild fish and seawater from the Gulf of Antalya, Mediterranean Sea

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Abstract

Aeromonas species are widespread pathogens that cause significant diseases in humans and animals, resulting in severe economic losses in the aquaculture industry. Furthermore, these species have become a greater threat due to recent reports of multidrug-resistant *Aeromonas* species and the potential transfer of antibiotic resistance genes to important pathogenic bacteria affecting human and animal health. The aim of this study was to investigate the presence of *Aeromonas* spp., an important zoonotic pathogen, in wild fish species caught in the Gulf of Antalya and in seawater, and to identify the most common *Aeromonas* species and their antibiotic resistance profiles. Seawater samples from five different coastal points in the Gulf of Antalya and 80 fish caught by local fishermen were bacteriologically examined. Suspected *Aeromonas* spp. isolates were purified and identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry. The resistance profiles of the isolates to 12 antibiotics from 9 different classes were determined by the Kirby-Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute criteria. *Aeromonas* spp. were isolated from the liver and kidney of only one (1.25%) wild fish (*Pagellus acarne* Risso, 1826) and identified as *Aeromonas molluscorum*. The isolate was resistant to ampicillin and cefazolin. The multiple antibiotic resistance index was calculated to be 0.16. These findings highlight that wild fish and seawater in the Gulf of Antalya do not pose a risk with respect to multidrug-resistant *Aeromonas* species that could threaten human health through foodborne infections or direct contact.

Keywords: *Aeromonas* spp., antimicrobial resistance, fish, gulf of Antalya, Mediterranean Sea.

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Introduction

Türkiye is one of Europe's leading countries in producing farmed fish (Ukav 2023). Over the past last 30 years, national aquaculture production has increased significantly. However, challenges such as overfishing, the introduction of predatory/invasive species such as *Pterois volitans* and *Lagocephalus sceleratus* from the Red Sea and Indo-Pacific, rising seawater temperatures due to global warming, and the widespread occurrence of various bacterial fish pathogens have significantly affected aquaculture production (Bariche et al 2020, Çinar et al 2021, Huseyinoglu et al 2023). *Aeromonas* species are among the most common causes of infectious diseases in fish.

Aeromonas species are Gram-negative, facultatively anaerobic bacteria belonging to the family *Aeromonadaceae*. The genus *Aeromonas* currently contains 36 recognized species. Among these, *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Aeromonas caviae*, and *Aeromonas veronii* are known to cause significant diseases in fish (Fernández-Bravo and Figueras 2020). *A. hydrophila* is an important pathogen causing motile *Aeromonas* septicemia (MAS). This pathogen infects a variety of fish and shellfish species in both freshwater and marine environments, often causing mass mortalities, which represent a serious global challenge for the aquaculture industry. The infection typically manifests as hemorrhagic and ulcerative lesions on the skin and muscles, exophthalmia,

ascites, fin erosion, hemorrhages in internal organs, bloody fluid accumulation in the abdominal cavity, and enlargement of the spleen and kidneys (Janda and Abbott 2010, Noga 2010, Roberts 2012, Fernández-Bravo and Figueras 2020). Additionally, *A. salmonicida* is responsible for furunculosis, a contagious and fatal disease characterized by septicemia and the appearance of furuncles of varying sizes on the body. This disease primarily affects salmonids, but can also affect other non-salmonid fish species (Janda and Abbott 2010).

The genus *Aeromonas* is recognized as one of the most important pathogens causing disease in fish and other cold-blooded animal species. It is also an important etiologic agent responsible for various infections in both immunosuppressed and immunocompetent individuals (Janda and Abbott 2010, Fernández-Bravo and Figueras 2020). Motile *Aeromonas* species are classified among foodborne pathogenic bacteria because they can proliferate across a broad temperature range, including refrigerator temperatures, along with the presence of various virulence factors such as enterotoxins (Janda and Abbott 2010). Infections can be transmitted by ingestion of uncooked or contaminated foodstuffs, consumption of water contaminated with pathogens, and close contact with infected animals (Igbinosa et al 2012, Praveen et al 2016, Hoel et al 2019). *Aeromonas* species that cause disease in humans can lead to a variety of infections, including septicemia, skin and soft tissue infections, meningitis, peritonitis, liver dysfunction, and gastroenteritis (Janda and Abbott 2010). A recent study demonstrated that bilateral necrotizing fasciitis in an immunocompromised patient was caused by multidrug-resistant (MDR) *A. hydrophila* (Ugarte-Torres et al 2018). Similarly, it has been identified that *Aeromonas* spp. have been found to cause necrotizing fasciitis in humans after ingestion of raw or undercooked seafood or direct exposure to seawater in coastal regions (Park et al 2009).

In recent years, the emergence of MDR *Aeromonas* species has posed a significant threat due to the potential transfer of antibiotic resistance genes to important pathogenic bacteria affecting both human and animal health. This situation is of great concern to the both populations. The aquatic environment, particularly coastal waters, is recognized as a reservoir for the spread of antibiotic resistance. Consequently, seawater may serve as a potential source of antibiotic resistance for a number of organisms and species, both wild and cultivated in aquaculture. These include molluscs, sea bass, gilthead bream, and salmon, as well as humans. Acquisition of antibiotic resistance has been shown to occur through two main routes: ingestion of edible marine organisms or direct exposure to seawater (Matyar et al 2010, Onuk et al 2015, Eid et al 2022, Gambino et al 2022).

In Türkiye, numerous studies have reported the prevalence of *Aeromonas* spp. and varying rates of antibiotic resistance in farmed, retail, and frozen fish, especially in species such as trout and sea bass (Kırkan et al 2003, Karatas et al 2005, Yücel et al 2005, Tel et al 2007, Akşit and Kum 2008, Durmaz and Türk 2009, Özer et al 2009, Erdem et al 2010, Korun and Toprak 2010, Akaylı et al 2011, Boran et al 2013, Capkin et al 2015, Uzun and Ogut 2015, Türe and Alp 2016, Şık et al 2020, Filik et al 2021, Kayış et al 2021, Tanrıkul and Dinçtürk 2021, Ünver and Bakıcı 2021, Yardımcı and Turgay 2021, Telli et al 2022). However, only a limited number of studies have investigated the present of *Aeromonas* spp. and their antibiotic resistance profiles in fish and seawater from natural environments (Matyar et al 2008, Matyar et al 2010, Akkan et al 2013, Onuk et al 2015, Onuk et al 2017). Furthermore, there are no studies specifically identifying the prevalence of *Aeromonas* spp. and the dominant *Aeromonas* species in fish caught by local fishermen for human consumption or in seawater, which is considered to be a reservoir for bacterial pathogens affecting marine life and humans in the Gulf of Antalya. Similarly, there are limited data exist on the detection of resistant *Aeromonas* spp. in marine organisms and seawater from other Mediterranean countries (Alduina et al 2020, Sucato et al 2021, Eid et al 2022, Gambino et al 2022).

Onuk et al (2017) emphasized the importance of elucidating the antibiotic resistance profiles of isolates to prevent zoonoses transmitted through direct contact or food, highlighting the need for more widespread studies on this topic. The aim of this study is to investigate the presence of *Aeromonas* spp., a significant zoonotic pathogen, in wild fish species that are heavily caught by fishermen and in seawater collected from various coastal regions of the Gulf of Antalya, while also identifying the commonly found *Aeromonas* species and their antibiotic resistance profiles. Furthermore, the aim is to provide updated data on whether the aquatic environment acts as a potential reservoir for MDR *Aeromonas* spp., which could present a risk to humans through foodborne infections or direct contact.

Material and Methods

Sample Collection

Sampling was conducted between November 2023 and April 2024 in the Antalya Bay (36°42'-36°02'N-31°35'-32°10'E; 36°19,6'N 29°61,3'E-36°19,3'N 29°64,5'E; 36°17,8N 29°61'E-36°17,6'N 29°64,6'E), which is located in the eastern part of the Mediterranean Sea, within the

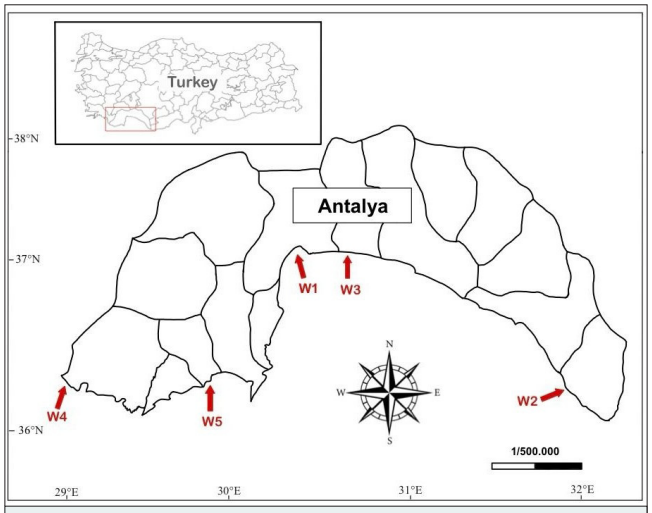

Figure 1. Map of sampling site: Antalya fishing harbor (W1); Gazipaşa (W2); Kundu (W3); Kaş (W4); Kumluca (W5).

Levantine Sea. A total of 80 fish samples were collected directly from the sea by local fishermen along the coast of Antalya, including 11 red mullet (*Mullus barbatus* Linnaeus, 1758), 9 goldband goatfish (*Upeneus moluccensis* Bleeker, 1855), 14 common pandora (*Pagellus erythrinus* Linnaeus, 1758), 6 axillary seabream (*Pagellus acarne* Risso, 1826), 20 sardines (*Sardina pilchardus* Walbaum, 1792), and 20 chub mackerel (*Scomber japonicus* Houttuyn, 1782). In addition, seawater samples (1L) were collected from five different coastal locations in Antalya Bay, including the Antalya fishing harbor, Kumluca, Kaş, Kundu, and Gazipaşa coasts (Figure 1). All water and fish samples were immediately transported to the laboratory under aseptic conditions and maintained in a cold chain at +4 °C (Nhinh et al 2021, Eid et al 2022, Gambino et al 2022).

Bacterial Isolation and Identification

To detect *Aeromonas* spp. in fish, the skin surface was disinfected with 70% ethanol prior to dissection incisions. Ventral and lateral incisions were performed to expose the internal organs (Mangus and Pessier 2021). During necropsy, fish were examined for macroscopic findings including ascites, exophthalmia, skin darkening, ulceration, hemorrhage, visceral congestion, and liver and spleen enlargement, and findings were recorded. Liver, spleen and kidney samples were collected for bacteriologic examination. These samples were cultured on 5% sheep blood agar (Oxoid, UK) and incubated at 28°C for 24 hours. To isolate *Aeromonas* spp. from water samples, 25 ml of the water sample was enriched by incubation in 225 ml of alkaline peptone water (pH 8.6) at 28°C for 24 hours, followed by inoculation onto blood agar. After the incubation period, colonies of catalase- and oxidase-positive, Gram-negative rod-shaped bacteria on blood agar were selected, subcultured onto blood agar for pure colony isolation, and incubated again at 28°C for 24 hours. After this period, the isolates were inoculated onto nutrient agar containing 6% NaCl to assess their growth characteristics. Colonies that grown on 6% NaCl nutrient agar were excluded from further analysis. For other bacterial agents, subcultures were performed on *Aeromonas* selective agar (Himedia, India) and incubated at 28°C for 24-48 hours. After this incubation period, colonies suspected to be *Aeromonas* spp. (translucent, circular, convex, 0.5-3 mm in diameter) were selected, and their hemolytic activity on blood agar was evaluated (Matyar et al 2010, Eid et al 2022, Mursalim et al 2022).

The identification of suspected *Aeromonas* spp. isolates was performed using matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS). The analyses were carried out at the Plant Health Clinic Application and Research Center of Hatay Mustafa Kemal University. Protein isolation from suspected *Aeromonas* spp. isolates was conducted according to the ethanol-formic acid protocol previously reported by Uysal et al (2019). Following data acquisition using the Biotyper 3.0 software (Microflex LT; Bruker Daltonics GmbH, Bremen, Germany), the resulting spectra were assessed for genus- and species-level identification using the Maldi Biotyper Real-Time Classification (RTC) software (version 9). A score range between 1.700 and 3.000, indicated by yellow/green, was considered a reliable threshold for identification.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of *Aeromonas* species was evaluated using Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) plates (Oxoid, UK), in

accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. The study tested 12 antibiotics from 9 different classes, including penicillins: ampicillin (10 µg); β-lactam/β-lactamase inhibitor combinations: amoxicillin-clavulanic acid (20/10 µg); cephalosporins: ceftriaxone (30 µg) and cefazolin (30 µg); aminoglycosides: streptomycin (10 µg) and amikacin (30 µg); tetracyclines: tetracycline (30 µg); fluoroquinolones: ciprofloxacin (5 µg) and ofloxacin (5 µg); quinolones: nalidixic acid (30 µg); sulfonamides: sulfamethoxazole/trimethoprim (23.75/1.25 µg); and phenicols: chloramphenicol (30 µg, Bioanalyse®, Türkiye).

The bacterial concentration was adjusted to 0.5 McFarland standard (1.5 x 108 CFU/ml) using sterile physiological saline, and 0.1 ml of the bacterial suspension was spread onto the MHA surface with a sterile swab. Antibiotic discs were placed on the MHA plates with sterile forceps, and the plates were incubated at 28°C for 24 hours. The results were interpreted by measuring the diameter of inhibition zones around the discs, which were classified as sensitive (S), intermediate (I), or resistant (R) in accordance with CLSI M100-S25 and M45 guidelines (CLSI 2015a, CLSI 2015b). Since CLSI breakpoints for *Aeromonas* spp. are available for only a limited number of antibiotic agents, the CLSI Enterobacteria breakpoints were used instead (Nhinh et al 2021, Eid et al 2022). For each isolate, the Multiple Antibiotic Resistance (MAR) index was determined according to the formula MAR = a/b, where 'a' indicates the number of antibiotics to which the isolate exhibited resistance, and 'b' is the total number of antibiotics assessed. According to Krumperman (1983), a MAR index exceeding 0.2 indicates that the isolates likely originate from high-risk environments characterized by frequent antibiotic usage. Conversely, a MAR index of 0.2 or below suggests that the isolates are associated with low-risk sources where antibiotic use is minimal or absent (Krumperman 1983, Nhinh et al 2021).

Results

Bacterial Isolation and Identification

The bacterial agents were isolated from 65 out of 80 fish samples (81.25%), while no pathogenic agents were found in the remaining 15 samples (18.75%). A single bacterial pathogen was isolated from 54 fish, while two different bacterial agents were identified in 11 samples. All isolates were determined to be Gram-negative, oxidase-positive, and catalase-positive rods. In subcultures grown on nutrient agar containing 6% NaCl, 42 of the 76 isolates (55.26%) showed growth and were excluded from further testing. Among the remaining 34 isolates, 21 showed growth on *Aeromonas* selective agar. Of the translucent, circular, convex colonies measuring 0.5–3 mm in diameter suspected to be *Aeromonas* spp., only one was confirmed as *Aeromonas molluscorum* by MALDI-TOF MS analysis. This strain showed α-hemolytic, smooth colonies on blood agar. The results of the MALDI-TOF MS analysis of 21 isolates grown on *Aeromonas* selective agar are presented in Table 1.

Aeromonas spp. were isolated from the liver and kidney of only one fish (1.25%) out of 80 samples, an axillary seabream (*Pagellus acarne* Risso, 1826). In addition to *A. molluscorum, Shewanella* spp. were also isolated from the same fish. During necropsy, hemorrhage in the internal organs and hepatomegaly were observed. No *Aeromonas* spp. was isolated from any of the five seawater samples.

Antimicrobial Susceptibility Profile

The *A. molluscorum* isolate was found to be susceptible to amoxicillin-clavulanic acid, ceftriaxone, streptomycin, amikacin, tetracycline, ciprofloxacin, ofloxacin, nalidixic acid, sulfamethoxazole/trimethoprim, and chloramphenicol, but resistant to ampicillin and cefazolin. The MAR index of the isolate was calculated to be 0.16.

Discussion

This study reports the detection of *Aeromonas* spp. and its antibiotic resistance profiles in wild fish species [*Mullus barbatus* (Linnaeus, 1758), *Upeneus moluccensis* (Bleeker, 1855), *Pagellus erythrinus* (Linnaeus, 1758), *Pagellus acarne* (Risso, 1826), *Sardina pilchardus* (Walbaum, 1792), and *Scomber japonicus* (Houttuyn, 1782)] and seawater samples from the Gulf of Antalya in the Mediterranean Sea. Additionally, it highlights the potential risks posed by *Aeromonas* spp. and its antibiotic resistance to humans, marine life, and the environment in the sampled areas.

Numerous studies have documented the presence of *Aeromonas* spp. in Türkiye, particularly in farmed, retail, and frozen fish, with a specific emphasis on trout and sea bass (Yücel et al 2005, Tel et al 2007, Erdem et al 2010, Akaylı et al 2011, Boran et al 2013, Capkin et al 2015, Türe and Alp 2016, Şık et al 2020, Kayış et al 2021, Tanrıkul and Dinçtürk 2021, Ünver and Bakıcı 2021, Yardımcı and Turgay 2021, Telli et al 2022, Çilli et al 2023). For instance, Durmaz and Türk (2009) examined 73 fish samples and 22 water samples collected from trout farms and isolated *Aeromonas* spp. from 52 of the 95 samples (41.0%). Among these isolates, 21 (40.3%) were identified as *A. hydrophila*, 24 (46.1%) as *A. caviae*, and 7 (13.4%) as *A. sobria*. They reported isolating *Aeromonas* spp. from 34 fish samples (35.6%) and 18 water samples (59%), with *A. hydrophila* being the most frequently isolated species from both sources. Additionally, Akşit and Kum (2008) reported a 16.22% isolation rate of *A. salmonicida* in a study conducted in four trout farms in the provinces of Muğla, Aydın and Denizli. A study in Ankara, which analyzed a total of 78 raw fish samples (30

Table 1. The MALDI-TOF MS analysis results of 21 isolates grown on Aeromonas selective agar

Fish number	Fish species	MALDI-TOF MS results	Liver	Kidney	Spleen
1, 4, 7, 9, 10, 11, 13, 14, 18	Chub mackerel (*Scomber japonicus* Houttuyn, 1782)	*Pseudomonas* spp.	+	+	+
22, 32, 36	Sardines (*Sardina pilchardus* Walbaum, 1792)	*Pseudomonas* spp.	+	+	+
41	Axillary seabream (*Pagellus acarne* Risso, 1826)	*Shewanella* spp. and *Aeromonas molluscorum*	+	+	-
42	Axillary seabream (*Pagellus acarne* Risso, 1826)	*Shewanella* spp. and *Pseudomonas* spp.	+	-	-
43	Axillary seabream (*Pagellus acarne* Risso, 1826)	*Pseudomonas* spp.	+	-	+
47	Common pandora (*Pagellus erythrinus* Linnaeus, 1758)	*Pseudomonas* spp.	+	-	+
50,52	Common pandora (*Pagellus erythrinus* Linnaeus, 1758)	*Pseudomonas* spp.	+	-	-
80	Goldband goatfish (*Upeneus moluccensis* Bleeker, 1855)	*Pseudomonas* spp.	+	+	+

freshwater and 48 marine), found a significantly higher isolation rate of motile *Aeromonas* species in marine fish (93.7%) compared to freshwater fish (10%) (Yücel and Balcı 2010). Özer et al (2009) reported that they isolated motile *Aeromonas* strains from 48 out of 259 rainbow trout (*Oncorhynchus mykiss,* Walbaum) samples (18.53%) and from 6 out of 56 water samples (10.71%) collected from seven commercial farms located in Mersin, in the Eastern Mediterranean region. Among these isolates, 20 (91.3%) were identified as *A. hydrophila* and 2 (8.7%) as *A. sobria*. Notably, Karatas et al (2005) first isolated *A. salmonicida achromogenes* in 2002 from sea bass raised along the Black Sea coast, reporting that this *Aeromonas* species caused bacterial infections leading to cumulative mortality rates of up to 20% in sea bass. Furthermore, Uzun and Ogut (2015) identified *Aeromonas veronii* biovar sobria as the predominant bacterial pathogen with an isolation rate of 65.2% in their study conducted in two separate sea bass farms in the Black Sea.

Although the prevalence of *Aeromonas* species in fish farming in Türkiye and the associated economic impacts have been well documented, there is still a lack of sufficient studies investigating the presence of *Aeromonas* spp. in marine fish and natural habitats (Uzel and Uçar 2000, Matyar et al 2010, Uğur et al 2012, Akkan et al 2013, Erdem et al 2017). Uzel and Uçar (2000) reported isolating *A. hydrophila* from 4 out of 14 seawater samples (28.5%), 3 out of 10 mussels (30%), 1 out of 4 stream samples (25%), and 1 out of 10 fish samples (including anchovy, horse mackerel, coral fish, scorpion fish, mullet, bogue, red mullet, sardine, bonito, and trout) (10%) collected from the Izmir region between January 1995 and January 1996. Uğur et al (2012) reported that 15 out of 46 Gram-negative bacteria (32.60%) isolated from seawater samples collected from nine different locations along the Bodrum coast of Muğla province were identified as *Aeromonas* spp. Matyar et al (2010) found a total of 60 *Aeromonas* spp. from 40 seawater samples collected from three different areas along the southeastern coast of İskenderun Bay, Türkiye, in the northeastern Mediterranean Sea. Of these isolates, 57 (95%) were identified as *A. hydrophila*, while only 3 (5%) were identified as *A. caviae*. Korun et al (2019) isolated *Shewanella putrefaciens*, *A. sobria* and *A. veronii* from three freshly dead silver eels caught by local fishermen in Antalya Bay. However, there are no studies specifically investigating the prevalence of *Aeromonas* spp. in fish species caught for human consumption by local fishermen in the Antalya Bay of the Mediterranean Sea, nor in seawater, which considered to be a reservoir for bacterial pathogens affecting marine organisms and humans. Similarly, there are few reports on the presence of *Aeromonas* spp. in seawater and wild marine fish from other Mediterranean countries (Alduina et al 2020, Sucato et al 2021, Eid et al 2022, Gambino et al 2022). Eid et al (2022) collected 100 healthy mullet fish and 25 water samples from the Mediterranean Sea in Egypt and reported that *Aeromonas* spp. were isolated from 44 of the fish (44%) and 9 of the water samples (36%). They identified *A. hydrophila* as the most frequently isolated *Aeromonas* species isolated from fish (53.85%). *A. sobria*, *A. caviae*, and *A. schubertii* followed with 26.92%, 16.67% and 2.56%, respectively. They also isolated the same species from the water samples, except for *A. schubertii*. Gambino et al (2022) reported that the highest proportions of *Vibrio*

spp. (44.8%) and *Aeromonas* spp. (31%) were isolated from seawater samples collected from various shores of Sicily.

In this study, *Aeromonas* spp. was isolated from only one wild fish specimen (*Pagellus acarne* Risso, 1826) collected from Antalya Bay in the Levantine Sea, which is located in the eastern Mediterranean, representing 1.25% of the total sample size (1 out of 80 specimens). Additionally, no *Aeromonas* spp. were detected in the seawater samples. Several factors may explain the lower isolation rate of *Aeromonas* spp. observed in this study compared to other reports: I. The limited number of fish caught due to the inability of local fishermen to venture far into the open sea from the coastal areas of Antalya Bay, resulting in a smaller sample size for this study; II. The lack of widespread aquaculture in this region, in contrast to other areas; III. The adaptation of *Aeromonas* species mainly to freshwater environments and their inability to survive in salt concentrations above 3% NaCl; IV. The presence of various microorganisms, such as halophilic bacteria like *Vibrio* spp. and marine heterotrophic bacteria, which compete with *Aeromonas* spp. for resources and space in marine environments; V. The limited availability of nutrients necessary for the proliferation of *Aeromonas* spp. in marine habitats; and VI. Other physical conditions present in marine environments, such as temperature and oxygen levels, present in marine environments, which may also influence isolation rates (Cavicchioli et al 2003, Khan et al 2008, Silva et al 2014, Setiaji et al 2020, Abdella et al 2024).

In this study, *A. molluscorum* was isolated from the liver and kidney of one wild fish specimen (*Pagellus acarne* Risso, 1826). No previous reports of *A. molluscorum* isolation from fish were found. Most research on *Aeromonas* species in aquatic environments has focused on other species that are common pathogens affecting fish, such as *A. hydrophila*, *A. veronii*, and *A. caviae*. It has been reported that *A. molluscorum* has been isolated from bivalve molluscs (Igbinosa et al 2012). It is considered to be of no clinical significance (Janda and Abbott 2010). In this study, *Shewanella* spp. were also isolated from the same fish specimen along with *A. molluscorum*.

Antimicrobial resistance detected in seawater and marine organisms consistently raises concerns. Antibiotic resistance has emerged as one of the most critical public health challenges globally, affecting not only developing countries but also developed nations. This resistance leads to serious infectious diseases and prolonged hospitalizations, increased healthcare costs, higher spending on second-line drugs, increased antibiotic use, and treatment failures, resulting in significant economic losses (Dadgostar 2019, Kraemer et al 2019).

The emergence of antimicrobial resistance is significantly influenced by the inappropriate and excessive use of various antibacterial agents in the healthcare and agricultural industries, as well as the uncontrolled discharge of waste in numerous locations worldwide (Dadgostar 2019). In addition, bacteria can acquire antibiotic resistance through natural mutations and the horizontal transfer of resistance genes, which can also be induced by the presence of antimicrobials in the environment (Gwenzi et al 2018, Dadgostar 2019, Kraemer et al 2019). It has been reported that antibiotic resistance genes are commonly found in antibiotic-resistant bacteria in the environment, and even in those that have never undergone antibiotic treatment (Alduina 2020). Furthermore, it has been noted that a significant number of antibiotic resistance genes associated with human disease have an environmental origin (Waseem et al 2018). The term "hotspot environment" is used to describe a setting in which bacteria are continuously and intensively exposed to antibiotics. Such environments include hospitals, farms, aquaculture facilities and wastewater treatment plants. In these settings, the abundance of nutrients leads to increased reproduction rates of the exposed bacteria (Gwenzi et al 2018, Kraemer et al 2019). As a result, wastes discharged from these "hotspots" have the potential to carry antimicrobial agents and resistant pathogens into the sewer system and ultimately into aquatic ecosystems via wastewater (Bondarczuk and Piotrowska-Seget 2019).

Numerous studies have documented the detection of antimicrobial-resistant pathogens and related genetic elements in a range of aqueous environments, including surface waters (Stoll et al 2012, Sucato et al 2021), wastewater (Matyar 2016, Gwenzi et al 2018), and coastal waters (Belding and Boopathy 2018, Gambino et al 2022), as well as in the surrounding environments and associated animals. As a result, aquatic environments are regarded as reservoirs of antibiotic resistance, with seawater playing a crucial role in facilitating the transfer of resistance genes between bacterial species (Su et al 2020). MDR bacteria and antibiotic resistance genes can serve as key indicators of anthropogenic contamination of the environment, particularly in coastal waters where human activities predominate and sewage and wastewater treatment plants are present (Zhang et al 2020).

Although the antibiotic resistance of *Aeromonas* spp. isolates obtained from retail, cultured, and frozen fish in Türkiye has been comprehensively studied (Kırkan et al 2003, Yücel et al 2005, Tel et al 2007, Akşit and Kum 2008, Durmaz and Türk 2009, Özer et al 2009, Korun and Toprak 2010, Capkin et al 2015, Uzun and Ogut 2015, Türe and Alp 2016, Balta 2020, Filik et al 2021, Kayış et al 2021, Telli et al 2022), there is a lack of sufficient information on antibiotic resistance

in *Aeromonas* spp. isolated from fish and seawater in natural environments (Matyar et al 2008, Matyar et al 2010, Akkan et al 2013, Onuk et al 2015, Onuk et al 2017). However, research has documented the occurrence of drug-resistant pathogens and associated genes in diverse aquatic ecosystems, including coastal marine environments, wastewater, and surface waters, as well as in their surrounding environments and animals (Matyar et al 2010, Onuk et al 2015, Onuk et al 2017). Furthermore, regional differences in antibiotic resistance levels have been documented, which correlate with proximity to populated areas and industrial sites (Matyar et al 2010).

Durmaz and Türk (2009) reported that all 52 motile *Aeromonas* isolates obtained from trout farms, as well as fish and water samples, exhibited severe resistance to ciprofloxacin and enrofloxacin (between 96.1% and 100%), high resistance to amikacin (92.3%) and moderate resistance to oxolinic acid, flumequine, cefoperazone/sulbactam, imipenem, mezlocillin, piperacillin, and cefotaxime (between 76.9% and 84.6%). They found low susceptibility (between 26.9% and 61.5%) to neomycin, nalidixic acid, sulfonamides, nitrofurantoin, and trimethoprim. They also reported high resistance (between 80.8% and 96.2%) to oxytetracycline, streptomycin and carbenicillin. In a study conducted by Onuk et al (2017) to determine the antimicrobial susceptibility profiles of 45 *Aeromonas* isolates obtained from fish and aquaculture waters in Türkiye, the highest susceptibility rates were found for gentamicin (100%), ciprofloxacin (91.1%) and florfenicol (91.1%), while the highest resistance rates were found for amoxicillin (82.2%) and ampicillin (77.8%). Filik et al (2021) investigated the antibiotic resistance profiles of a total of 20 *A. hydrophila* strains isolated from various regions and different fish species in Türkiye, reporting 100% resistance to oxacillin, ampicillin, vancomycin and penicillin G; 95.23% resistance to clindamycin and tylosin; 90.47% resistance to oxytetracycline and ciprofloxacin; and 80.95% resistance to cephalothin and oxolinic acid, with 61.90% resistance to nitrofurantoin. According to the results of the MAR index, *A. hydrophila* strains exhibited multi-drug resistance to 14 different antibiotics, including sulfadiazine, oxytetracycline, oxacillin, apramycin, clindamycin, tylosin, cephalothin, pristinamycin, nitrofurantoin, sulfamethoxazole/trimethoprim, oxolinic acid, ampicillin, vancomycin and penicillin G. Matyar et al (2010) found the highest resistance rates to cefazolin and trimethoprim-sulfamethoxazole (66.6%) among *Aeromonas* isolates obtained from water samples collected from three different regions in the İskenderun Bay. They reported lower resistance levels to gentamicin (13.3%), chloramphenicol (13.3%), nalidixic acid (13.3%) and amikacin (3.3%).

There are only a few reports on the presence of resistant *Aeromonas* spp. in marine organisms and seawater in Mediterranean countries (Alduina et al 2020, Eid et al 2022, Gambino et al 2022). In a study by Eid et al (2022) investigating the presence of extensively drug-resistant (XDR) *Aeromonas* spp. in mullet fish and seawater in Egypt, it was reported that all isolates tested (100%) demonstrated resistance to ampicillin, penicillin and sulfamethoxazole/trimethoprim, followed by oxytetracycline (90%), streptomycin (63.33%), norfloxacin, amikacin, nalidixic acid and chloramphenicol (<25%), with none of the isolates showing resistance to cefotaxime. Moreover, the authors stated that 90% of the isolates exhibited MDR, while 26.67% demonstrated XDR. Gambino et al (2022) reported high levels of resistance to cefazolin (89.6%), streptomycin (31%), amoxicillin/clavulanic acid (37.9%), ceftriaxone (13.2%) and sulfamethoxazole/trimethoprim (17.2%) in bacteria isolated from seawater collected from different coasts of Sicily. In the present study, it was determined that the isolated *A. molluscorum* strain was only resistant to ampicillin and cefazolin. Based on the calculation of the MAR index of the isolate as 0.16, it was concluded that the Antalya Bay is a low-risk area where antibiotics are either not used or rarely used.

Conclusion

The data obtained in this study highlight that there is no significant risk of MDR *Aeromonas* spp. in fish or seawater from natural habitats in Antalya Bay, which could pose a risk to humans through foodborne infections or direct contact. The absence of high levels of antibiotic resistance in the fish caught in Antalya Bay may be related to the lower fish population in the region compared to areas with intensive aquaculture, resulting in less antibiotic use. In addition, Antalya's status as a tourist city, attracting approximately 10 million foreign visitors annually, along with effective waste management practices—including the conversion of certain industrial wastes into energy and the implementation of comprehensive wastewater treatment processes—are critical factors that are likely to influence the antibiotic resistance profile. On the other hand, due to the limited sample size in this study, further investigations should focus on larger populations, other marine pathogens and deeper-sea fish in Antalya Bay to gain a more comprehensive understanding of antibiotic resistance dynamics.

Declarations

Competing Interests

The authors declares that there are no conflict of interest related to the publication of this article.

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Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

Ethical Statement

This research was carried out with the approval of Burdur Mehmet Akif Ersoy University Experimental Animals Local Ethics Committee (MAKUHAYDEK/29.03.2023 dated and 1066 numbered decision).

Author Contributions

Motivation/Concept: ESB; Design: ESB; Control/Supervision: ESB; Data Collection and Processing: ESB, OK; Analysis and Interpretation: ESB; Literature Review: ESB, OK; Writing the Article: ESB; Critical Review: ESB

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