

## First Isolation of *Acinetobacter radioresistens* from Golden grey mullet (*Liza aurata*) in Turkey<sup>1\*</sup>

Mustafa TÜRE<sup>1\*</sup>

Ayça ALTUNTAŞ<sup>1</sup>

İlyas KUTLU<sup>1</sup>

<sup>1\*</sup>Department of Fisheries Health, Central Fisheries Research Institute, Trabzon, Turkey. [ID: https://orcid.org/0000-0001-9878-826X](https://orcid.org/0000-0001-9878-826X)

<sup>1</sup>Department of Fisheries Health, Central Fisheries Research Institute, Trabzon, Turkey.

[ID: https://orcid.org/0000-0002-2916-8195](https://orcid.org/0000-0002-2916-8195)

[ID: https://orcid.org/0000-0002-6096-6478](https://orcid.org/0000-0002-6096-6478)

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**Abstract:** In this study, the bacteriological examination was performed in golden grey mullet (*Liza aurata*) with suspected infection which kept in the hatchery facility of Central Fisheries Research Institute (CFRI), Turkey. During the disease outbreak, 35% of the grey mullet (400–420g) have died. The most important external clinical signs were anorexia, lethargy, dark pigmentation, and hemorrhage at the base of the pectoral and anal fins. Bacteria were isolated from head-kidney and liver. The pure bacteria was identified as *Acinetobacter radioresistens* by rapid test kit (API 20NE, Profile: 0400032). The antibacterial susceptibility of bacteria against five different antibiotics was determined by the disk diffusion method. The bacteria were sensitive to enrofloxacin, trimethoprim+sulfamethoxazole, oxytetracycline, whereat resistant to florfenicol and erythromycin. The most effective antibiotic was enrofloxacin. When symptoms persist, enrofloxacin was administered (10 mg/kg/2 days, active ingredient) intraperitoneally. Fish were recovered after antibiotic treatment. In this study, an infection caused by *Acinetobacter radioresistens* was reported in golden grey mullet. Overall, to our knowledge, this study reports the first *Acinetobacter* infection in cultured golden grey mullet.

**Keywords:** Fish, golden grey mullet, fish diseases, *Acinetobacter radioresistens*.

### *Acinetobacter radioresistens*'in Türkiye'de altınbaş kefalardan (*Liza aurata*) ilk izolasyonu

**Öz:** Bu çalışmada, Türkiye'de, Su Ürünleri Merkez Araştırma Enstitüsü (SUMAE)'ne ait kuluçkahanede yetiştirilen altınbaş kefaller (*Liza aurata*), hastalık şüphesi sonrası bakteriyolojik olarak araştırılmıştır. Salgın esnasında, 400-420 g arasında bulunan kefallerin %35'i ölmüştür. En önemli klinik belirtiler, iştahsızlık, uyuşukluk, kararma ve anal ve göğüs yüzgeci kaidesinde kanamalıdır. Bakteri, balıkların karaciğer ve ön-böbreklerinden izole edilmiştir. Saflaştırılan bakteriler, hızlı test kiti (API 20NE, Profile: 0400032) ile *Acinetobacter radioresistens* olarak isimlendirilmiştir. Bakterinin antimikrobiyal duyarlılığı beş farklı antibiyotiğe karşı disk difüzyon metodu ile belirlenmiştir. Bakteri, enrofloksasin, trimetoprim-sulfametoksazol ve oksitetrasiklin antibiyotiklerine karşı duyarlı, florfenikol ve eritromisin antibiyotiklerine karşı ise dirençli olarak bulunmuştur. En etkili antibiyotik enrofloksasindir. Semptomlar devam ederken, balıklar periton-içi enrofloksasin uygulaması (10 mg/kg/2 gün) ile tedavi edilmişlerdir. Bu çalışmada, altınbaş kefallerde *Acinetobacter radioresistens*'in sebep olduğu bir enfeksiyon rapor edilmiştir. Sonuç olarak, bizim bilgimize göre, kültürü yapılan kefallerde rapor edilen ilk *Acinetobacter* enfeksiyonudur.

**Anahtar sözcükler:** Balık, altınbaş kefal, balık hastalıkları, *Acinetobacter radioresistens*.

## INTRODUCTION

Bacterial fish diseases are one of the major problems in aquaculture and can lead to severe economic losses in many countries. Sustainability and success in aquaculture depend on prevention of fish diseases and effective fish health management (Toranzo et al., 2005; Kayış et al., 2017; Türe & Kutlu, 2018; Balta & Dengiz Balta, 2016). *Acinetobacter* sp. is a bacterium belonging to the family Moraxellaceae. *Acinetobacter radioresistens* is a bacteria which aerobic, Gram-negative, short-rod, non-motile, oxidase-negative and catalase-positive. Acinetobacteriosis are frequently encountered in nosocomial infections, in particular in infections in intensive care units (Coyne et al., 2011). It is also frequently found in the intestinal flora of fish, on gills and skin of aquatic animals. Because of this reason, bacteria can lead to diseases transmission easily (Austin & Austin, 2007). The first *Acinetobacter* infection was observed in mature Atlantic salmon (*Salmo salar*) in Norway. Affected fish had anorexia, dark pigmentation, hemorrhage and edema at the base of the pectoral, pelvic, and anal fins (Austin & Austin, 2007). Acinetobacter outbreak with high mortality was reported in different rainbow trout (*Oncorhynchus mykiss*) farms in Turkey in 2008 (Yonar et al., 2010).

Mulletts (Mugilidae) are omnivorous fishes living in schools, mostly in coastal regions of tropical and subtropical seas. It is represented by about 13 genera and 70 species in world seas (McDowal, 1988). Majority of the *Mugilidae* species live in the Mediterranean and the Black Sea. Eight *Mugilidae* species live in Turkish waters: *Liza aurata*, *Liza saliens*, *Chelon labrosus*, *Liza ramada*, *Oedalichilus labeo*, *Liza abu*, *Liza carinata*, and *Liza haematocheila*. The members of the Mugilidae family are euryhaline species tolerated the salinities of 0‰-60‰. Their also tolerances to temperature changes extremely high and can survive the temperatures between 3 and 30°C (Shapiro, 1998). Mullet production in Turkey is constantly decreasing trend in recent years; the production in 2017 (2313 tons) was approximately 1% of total fisheries production (269, 676 tons) (TSI, 2018).

In the present study, the first *Acinetobacter* infection in golden grey mullet (*Liza aurata*) was reported. Biochemical properties of the bacteria were also described

## MATERIAL and METHOD

**Bacterial examination:** The project was approved by the Local Ethical Committee of the CFRI, (Protocol No: 2017/2). The disease outbreak occurred during the Autumn of 2018 in golden grey mullet which kept in an aquaculture facility of Central Fisheries Research Institute (CFRI), Trabzon, Turkey. During the disease outbreak, ten fish died and moribund grey mullet were sampled for bacterial examination. Size of the affected fish was ranged between

400 g and 420 g. The water temperature was 18°C during the infection. Liver and head-kidney of affected fish were aseptically streaked on Tryptic Soy Agar (TSA, Merck) and incubated at 22 °C for 2 days. After that, typical colonies were selected from the plate and streaked onto the same media to check the purity of bacteria. The pure colonies were biochemically characterized by following biochemical tests: Gram staining, motility, cytochrome oxidase, and catalase. Analytical Profile Index (API 20 NE test, Biomerieux) was performed to biochemically identify bacteria species (Altınok et al., 2007).

**Antimicrobial Susceptibility Test:** The antibacterial susceptibility of bacteria was evaluated against five different antibiotics. Antimicrobial susceptibility test was performed by the disk diffusion method using commercial discs (Oxoid) on Mueller Hinton Agar (MHA, Oxoid) plates. The test was performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2014). The commercial antibiotic discs used in this study are florfenicol (FFC, 30 µg), enrofloxacin (ENR, 5 µg), oxytetracycline (OT; 30 µg), erythromycin (E, 15 µg) and trimethoprim-sulfamethoxazole (SXT; 25 µg). The plates were incubated at 30°C for 22h. The isolate was characterized as susceptible or resistant to the antibiotics.

## RESULTS

Mass mortalities occurred in adult golden grey mullet and mortality reached 35% during the Autumn of 2018. Externally, affected fish had anorexia, lethargy, dark pigmentation and hemorrhage at the base of the pectoral, and anal fins. Surprisingly diseased fish did not have any internal abnormality. Bacteria were isolated from 8 out of 10 examined fish. Short and rod-shaped bacteria were gram-negative, nonmotile and nonoxidative while catalase test result was positive. Bacteria were characterized as *Acinetobacter radioresistens* by API 20 NE test (Profil: 0400032, % ID: 96,7). Comparison of some biochemical characteristics of *Acinetobacter radioresistens* isolated from golden grey mullet in this study with the described by Austin & Austin (2007) were shown in Table 1.

Based on antimicrobial susceptibility test, *Acinetobacter* sp. was susceptible to enrofloxacin, trimethoprim+sulfamethoxazole, oxytetracycline while resistant to florfenicol and erythromycin. The most effective antibiotic was enrofloxacin. When symptoms persist, enrofloxacin was administered (10 mg/kg/2 days, active ingredient) intraperitoneally. Fish were recovered after antibiotic treatment.

## DISCUSSION

Bacteria belonging to the genus *Acinetobacter* are Gram-negative, aerobic, non-motile, catalase positive, oxidase negative and short-rods. *Acinetobacter* species are naturally found in soil, water, marine, and freshwater fish. These bacteria are recognized as emerging opportunistic pathogens of fish farmed in the worldwide (Doughari et al., 2011; Kozinska et al., 2014). The present study reports the first *Acinetobacter radioresistens* infection that causes high mortality in golden grey mullet.

**Table 1.** Comparison of some phenotypic characteristics of *Acinetobacter* sp. isolated from golden grey mullet in this study with the previously published study.

Biochemical features	A	B
Gram stain	-	-
Motility	-	-
Pigmentation	-	-
Cytochrome oxidase	+	-
Catalase	+	+
Reduction of nitrates	-	-
β-galactosidase	-	-
H <sub>2</sub> S	-	-
Indol production	-	-
Arginine dihydrolase	-	-
Hydrolysis gelatin	-	-
Urease	-	-
Galactose	+	?
Maltose	+	-
Mannose	+	-
Glucose	-	-
Arabinose	-	-
Lactose	-	-
Assimilation capric acid	?	+
Assimilation adipic acid	?	+
Hydrolysis esculin	?	+

Phenotypic features of bacteria were obtained from, A: Austin & Austin, (2007), B: In this study, ?: It was not done.

In this study, the pathogenic bacteria were identified as *Acinetobacter radioresistens* based on phenotypic characterization. In a previous study, phenotypic features of *Acinetobacter* sp. were described by Austin & Austin (2007). In contrast to the results detailed in our study, they described that *Acinetobacter* sp. with biochemical characteristics showed cytochrome oxidase, maltose, and mannose positive reactions. In another study, biochemical characteristics of *Acinetobacter* isolates tested by using API 20 NE system were similar to our results (Kozinska et al., 2014).

*Acinetobacter* strains are generally known as microorganisms transmitting the antibiotic resistance genes (Kozinska et al., 2014). In a previous study, antibiotic susceptibility tests revealed that *Acinetobacter* strains were sensitive to norfloxacin, and gentamicin. On the other hand, some of the strains were found resistant to cephalothin, oxytetracycline, sulfamethoxazole, chloramphenicol and amoxicillin (Yonar et al., 2010). The antibiotic susceptibility may vary depending on many factors including bacterial species, isolation area, and origin. However, in our study, the bacteria was found as sensitive to enrofloxacin, trimethoprim+sulfamethoxazole, oxytetracycline, and resistant to florfenicol and erythromycin. When symptoms

persist, enrofloxacin administration surprisingly helped fish to recover.

Studies on bacteria associated with a disease outbreak in grey mullet from Turkey and different countries are very limited. The mortality records of wild and cultured mullet populations are rare. Mortalities generally are occurred due to environmental stress such as temperature, salinity, and pollution. A epizootics with up to 10% mortality were recorded on some grey mullet farms in Taiwan. In the mentioned investigation, *Lactococcus garvieae* was reported as a causative agent (Chen et al., 2002). In another study, the disease outbreaks in grey mullet were investigated. *Vibrio harveyi*, *Vibrio fischeri*, and *Pseudoalteromonas* were the main bacterial pathogens found in the mullet outbreaks (Lopes et al., 2014).

In the present study, the bacterial strain was identified to species level based on the biochemical method. According to the API 20NE result, bacteria were identified as *Acinetobacter radioresistens*. In conclusion, an infection caused by *Acinetobacter radioresistens* were reported in golden grey mullet. Overall, this study reports the first *Acinetobacter* infection in cultured golden grey mullet in Turkey.

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**\*Corresponding author's:**

Mustafa TÜRE

Su Ürünleri Merkez Araştırma Enstitüsü, Vali Adil Yazar C. Yomra, Trabzon, Turkey.

✉E-mail: mustafa.ture@tarimorman.gov.tr

ORCID: <https://orcid.org/0000-0001-9878-826X>