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# Antibiotic susceptibility and resistance genes of *Escherichia coli* isolates from diseased rainbow trouts (*Oncorhynchus mykiss*)

## Susceptibilidad a los antibióticos y genes de resistencia de aislados de *Escherichia coli* procedentes de truchas arco iris (*Oncorhynchus mykiss*) enfermas

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### ABSTRACT

It was aimed to isolate *Escherichia coli* from infected trouts in different farms, and to investigate antibiotic susceptibility profiles and antibiotic resistance genes of these isolates. Identification processes were carried out according to ISO 6887-3:2017 and ISO 16654:2001 guidelines. Antimicrobial susceptibility was tested according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Extended-spectrum beta-lactamase (ESBL) resistant strains were investigated by the Modified Double Disc Synergy Test (MDDST) method. The specific regions of 15 genes were analyzed by the real-time PCR system. As a result, 24 isolations were performed from different tissues belonging to eight out of 108 diseased trouts. The highest phenotypical resistance status was found against penicillins (ampicillin 100%, amoxicillin 91.67%) and first-generation cephalosporins (cefazolin 100%). Phenotypic resistance rates of amoxicillin-clavulanate, nalidixic acid, and erythromycin were 83,33%, tetracycline was 75%, ceftazidime, ceftriaxone, cefotaxime, cefepime, and ciprofloxacin were 66,67%, trimethoprim-sulfamethoxazole was 50%, and chloramphenicol and gentamycin were 33.33%. Phenotypical resistances for amikacin and imipenem were detected at the level of 16.67%. In addition, ESBL production was detected phenotypically in 12 (50%) out of 24 *E. coli* isolates. The highest antimicrobial resistance gene rate was 58.33% for *tetA*. Gene regions of *sulI*, *ermB*, *ermF*, *qnrB*, *sulII*, *qnrS*, and *tetB* were detected at 50%, 50%, 50%, 33.33%, 25%,16.67%, and 16.67% respectively. None of the isolates included the gene region of the *qnrA*, *qnrC*, *qnrD*, and *qepA*. ESBL-producing genes, *blaTEM*, *blaCTX*, and *blaSHV* were detected at 33.33%, 33.33%, and 16.67% respectively. In conclusion, *E. coli* contamination of the water can cause infections among fish and increase the agent's antimicrobial resistance. Resistant strains of *E. coli* cannot only cause financial damage to create yield loss but also can threaten human health by causing infections throughout the food chain.

**Key words:** Aquaculture; antibiotic susceptibility; *E. coli*; isolation; resistance genes

### RESUMEN

Con el objetivo de aislar *Escherichia coli* de truchas infectadas en diferentes granjas, e investigar los perfiles de susceptibilidad a los antibióticos y los genes de resistencia a los antibióticos de estos aislados. Los procesos de identificación se llevaron a cabo de acuerdo con las directrices ISO 6887-3:2017 e ISO 16654:2001. La susceptibilidad antimicrobiana se probó de acuerdo con las directrices del Instituto de Normas Clínicas y de Laboratorio (CLSI). Las cepas resistentes a betalactamasas de espectro extendido (EBSL) se investigaron mediante el método de prueba de sinergia de doble disco modificado (MDDST). Se analizaron las regiones específicas de 15 genes mediante el sistema PCR en tiempo real. Como resultado, se realizaron 24 aislamientos a partir de diferentes tejidos pertenecientes a ocho de las 108 truchas enfermas. El mayor estado de resistencia fenotípica se encontró frente a penicilinas (ampicilina 100%, amoxicilina 91,67%) y cefalosporinas de primera generación (cefazolina 100%). La tasa de resistencia fenotípica a la amoxicilina-clavulánico, el ácido nalidíxico y la eritromicina fue del 83,33%, la de la tetraciclina del 75%, la de la ceftazidima, la ceftriaxona, la cefotaxima, la cefepima y la ciprofloxacina del 66,67%, la del trimetoprim-sulfametoxazol del 50%, y la del cloranfenicol y la gentamicina del 33,33%. La resistencia fenotípica a la amicaína y al imipenem se detectó a un nivel del 16,67%. Además, se detectó fenotípicamente la producción de ESBL en 12 (50%) de los 24 aislados de *E. coli*. La tasa más alta de genes resistentes a los antimicrobianos fue del 58,33% para *tetA*. Las regiones génicas de *sulI*, *ermB*, *ermF*, *qnrB*, *sulII*, *qnrS* y *tetB* se detectaron en un 50%, 50%, 50%, 33,33%, 25%,16,67% y 16,67% respectivamente. Ninguno de los aislados incluía la región génica de *qnrA*, *qnrC*, *qnrD* y *qepA*. Los genes productores de ESBL, *blaTEM*, *blaCTX* y *blaSHV* se detectaron en un 33,33%, 33,33% y 16,67% respectivamente. En conclusión, la contaminación del agua por *E. coli* puede causar infecciones entre los peces y aumentar la resistencia antimicrobiana del agente. Las cepas resistentes de *E. coli* no sólo pueden causar perjuicios económicos al crear pérdidas de rendimiento, sino que también pueden amenazar la salud humana al provocar infecciones en toda la cadena alimentaria.

**Palabras clave:** Acuicultura; sensibilidad a los antibióticos; *E. coli*; aislamiento; genes de resistencia

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## INTRODUCTION

With the increasing demand for seafood, intensive fishing activities are increasing daily. On the other hand, with the gradual decrease of physical and biological capacity due to worsening environmental conditions, and increases in fishing costs, it has been understood that the fish production that can be obtained through conventional fishing will not increase at the needed pace. However, aquaculture can meet this high demand that traditional fisheries cannot reach. Additionally, it is developing in the world and creates an important trade opportunity for countries and human livelihood [1].

Thanks to a similar increase in national demand, there have been great developments in aquaculture in the 29 trout production facilities located in Karkamış Dam Lake in the Birecik district of Şanlıurfa province in recent years. Current development has driven capacity increases and accordingly, increases in fish production. With support from the Ministry of Agriculture and Forestry for fish farming, the total production capacity of aquaculture facilities within the borders of Karkamış Dam Lake reaches 16.012 tons/year as of February 20, 2015 [2].

Bacterial pathogens such as *Escherichia coli*, *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., *Yersinia enterecolitica*, *Listeria monocytogenes,* and *Vibrio* spp. are responsible for 75% of fish-borne food infections in humans. For this reason, in aquaculture, various antibiotics are applied in the form of oral or premixed by adding to the feed, both for fighting and protection against such pathogens. While most oxytetracycline is used for this purpose in the world, florfenicol, sulfadiazine + trimethoprim, enrofloxacin, and amoxicillin are used as licensed antibiotics in Türkiye [3, 4]. If infected fish are not treated with antibiotics, mortality rates can reach 60% to 80% cumulatively in farms and may cause serious losses in production [5].

He *et al*. [6] stated that using low doses of antibiotics as growth promoters is banned in most countries because it increases the development of antibacterial resistance, and that illegal usage may be too large to be ignored. They showed that by modeling in zebrafish, using low doses of antibiotics causes immunosuppression in fish over a long time and negatively affects the intestinal microbiota of fish resulting in increased susceptibility to pathogens. In Türkiye, antibiotics used in fish are sold and applied with a veterinarian's prescription. In addition, according to the "Medicated Feed Communiqué" of the Ministry of Agriculture and Forestry numbered 2005-12, which entered into force in 2005, it is forbidden to add antibiotics and pharmaceutical substances to feed as feed additives (as protection against pathogens or growth promoters). In case of an outbreak of any disease in the animals, and if the medication will be used on the animals with the feed, it is allowed to produce medicated feed only in feed factories.

Factors such as the use of antibiotics in wrong doses, non-compliance with dose times, immediate empirical treatment, and illegal antibiotics can cause high selective pressure to resistance to antibiotics in some pathogens [7]. It is thought that transmission of resistance genes may be realized if these pathogens encounter other bacterial agents. This situation may increase the resistance profiles of the bacteria. For the reason of formed antibacterial resistance the doses of antibiotics used against bacterial diseases in people are either increased or new types of antibiotics are used [8, 9, 10, 11].

The researchers stated that the physicochemical properties of the water in the dams where fish production activities are carried out may change according to the seasons, and urban, agricultural and industrial wastes are the most important factors affecting water quality. They stated that pollutant elements could be physical, chemical, or biological according to their sources, and in this context, "many parameters such as temperature, pH, dissolved oxygen content, electrical conductivity, turbidity, nitrite, nitrate, phosphate, biological oxygen demand, and chemical oxygen demand can be accepted as criteria", especially in surface waters in determining water quality and pollution level. In the water analyses, researchers conducted between January 2015 and December 2015 in Karkamış Dam Lake, they reported that the surface water and the water column between 0–8 m depths are in class I, high-quality water class according to the classes and quality criteria of the surface water quality management regulation in terms of general chemical and physicochemical parameters of continental surface water resources [12].

In this study, it was aimed to investigate the presence of *E. coli* in tissues such as muscle, liver, kidney, and gill of infected trouts detected in trout farms in Karkamış Dam Lake and to evaluate the antibiotic susceptibility profiles of *E. coli* isolates from these different tissues of infected fish.

## MATERIALS AND METHODS

### Collection and preparation of samples

This study was carried out with 108 infected fish provided in nine different trout farms in Karkamış Dam Lake between May 2022 and March 2023. Nine different farms were visited every two months. Two fish samples were taken from each farm on each visit. The fish were taken into sterile sample containers and placed in refrigerated isothermal boxes and quickly delivered to the veterinary clinic. In the clinic, 4 different tissue samples (Liver, kidney, muscle, and gill) were taken from each fish by aseptic surgical techniques, and approximately 20 g of tissue samples were placed in stomacher bags containing 200 mL of transport medium (buffered peptone water) and delivered to the laboratory [13].

### Bacterial identification

*Escherichia coli* identification and microbiological analyses in fish samples were performed according to ISO 6887-3:2017 [14] and ISO 16654:2001 [15] guidelines. The stomacher bags delivered to the laboratory were incubated in an incubator (Panasonic, MCO-18AC-PE, Japan) at 41.5°C for 6 hours (h). After incubation, the bag contents were homogenized using a homogenizer. After taking the amount of 200 µL sample from each bag contents using a filtered pipette tip, the samples were inoculated on Sorbitol-MacConkey (SMAC) agar (Becton Dickinson GmbH), and these petri dishes were incubated at 37°C for 24 h. *E. coli* colonies that do not ferment sorbitol and were negative for slide agglutination using the *E. coli* O157 latex test kit (Oxoid) were inoculated on Levine Eosin Methylene Blue agar (L-EMB) (Merck) and incubated for 24 h at 37°C. Indole, citrate, and Voges-Proskauer tests were also used for biochemically characterizing [16].

### Antimicrobial susceptibility tests

Antimicrobial susceptibility tests were performed using the Kirby-Bauer disc diffusion method. First, the bacterium was swabbed on Mueller Hinton Agars (Oxoid), then antimicrobial discs were placed on the agar surface carefully, and then the plates were incubated at 37°C for 24 h. Inhibition zones formed around the discs after incubation were measured and the results were evaluated according to the

Clinical and Laboratory Standards Institute (CLSI) guidelines [17]. Ampicillin, amoxicillin (penicillins), amoxicillin–clavulanate (penicillins + beta–lactamase inhibitors), cefazolin (1st generation cephalosporins), ceftazidime, ceftriaxone, cefotaxime (3rd generation cephalosporins), cefepime (4th generation cephalosporins), nalidixic acid, ciprofloxacin (quinolones), tetracycline, trimethoprim-sulfamethoxazole (folate pathway inhibitors), amikacin, gentamicin (aminoglycosides), imipenem (carbapenems) and erythromycin (macrolides) discs (Oxoid) were used as antimicrobial agents and their antimicrobial susceptibility was tested.

### Phenotypic detection of ESBL production

Isolates were tested to determine whether they produce extended-spectrum beta–lactamases (ESBL) on Mueller–Hinton agar plates using the MDDST method [18, 19]. An amoxicillin–clavulanate disc (20/10 µg) and four cephalosporins were used for the determination and evaluation of ESBL according to the CLSI guidelines [17, 18, 19].

### Investigation of antimicrobial resistance genes

After 24 h of incubation at 37°C on L–EMB agar, *E. coli* colonies were placed in a sterile microcentrifuge tube (Isolab, Germany) containing PBS and adjusted to 0.5 McFarland standard. DNA isolations were made from 200 µL of this mixture using the High Pure PCR template preparation kit (Roche Diagnostics GmBH, Mannheim, Germany) according to the manufacturer's instructions. DNAs were stored in deep freeze (Nüve, DF–590, Türkiye) at -80°C until the qPCR. *BlaTEM*, *blaSHV*, *blaCTX* (ESBL production genes), *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA* (quinolone resistance genes), *tetA*, *tetB* (tetracycline resistance genes), *sulI*, *sulII* (sulphanamide resistance genes), and *ermB* and *ermF* (erythromycin resistance genes) were detected using the LightCycler 480 real–time PCR system (Roche, Switzerland) with specific primers by following the manufacturer's instructions of LightCycler 480 SYBR Green I Master kit (Roche Diagnostics GmBH, Mannheim, Germany). The total reaction volume was 20 µL and the template DNA added 5 µL [20, 21, 22].

## RESULTS AND DISCUSSION

Twenty–four *E. coli* isolates were detected in different tissues of eight infected fish from the farms except numbers eight and nine (TABLE I). The phenotypically antibiotic resistance and ESBL distribution of the isolates from tissues is given in TABLE I.

While the highest resistance was found against penicillin and first–generation cephalosporins, resistance to imipenem was detected at 16.67%. It was noticed that the rates of resistance to tetracyclines and quinolones, which are frequently used in fisheries, were high (TABLE II). In addition, phenotypically extended–spectrum beta–lactamase production was detected in 12 (50%) of the 24 *E. coli* isolates (TABLE II).

The distribution of antimicrobial resistance genes from all *E. coli* isolates obtained by culture from different tissues is given in TABLE III.

The highest antimicrobial resistance gene rate among the isolates included in this study was 58.33% for the tetracycline (*tetA*). Sulfonamide resistance gene *sul* and Erythromycin resistance genes

***TABLE I***
**Distribution of Phenotypical Antibiotic Resistance**

| Nº | Tissues | Farm Nº | Fish Nº | AM | AX | AMC | CZ | CAZ | CRO | CTX | CPM | C | NA | CIP | TE | SXT | AMK | GEN | IPM | E | ESBL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | L | 1 | 1 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | + |
| 2 | K | 1 | 1 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | + |
| 3 | M | 1 | 1 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | + |
| 4 | G | 1 | 1 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | + |
| 5 | K | 2 | 2 | R | R | R | R | S | S | S | S | S | R | S | S | S | S | S | S | R | – |
| 6 | M | 2 | 2 | R | R | R | R | S | S | S | S | S | R | S | S | S | S | S | S | R | – |
| 7 | L | 2 | 3 | R | R | R | R | S | S | S | S | S | R | S | S | S | S | S | S | R | – |
| 8 | G | 2 | 3 | R | R | R | R | S | S | S | S | S | R | S | S | S | S | S | S | R | – |
| 9 | L | 3 | 4 | R | R | R | R | R | R | R | R | S | R | R | R | R | S | S | S | R | + |
| 10 | K | 3 | 4 | R | R | R | R | R | R | R | R | S | R | R | R | R | S | S | S | R | + |
| 11 | M | 3 | 4 | R | R | R | R | R | R | R | R | S | R | R | R | R | S | S | S | R | + |
| 12 | G | 3 | 4 | R | R | R | R | R | R | R | R | S | R | R | R | R | S | S | S | R | + |
| 13 | L | 4 | 5 | R | R | R | R | R | R | R | R | S | R | R | R | S | S | S | S | R | – |
| 14 | K | 4 | 5 | R | R | R | R | R | R | R | R | S | R | R | R | S | S | S | S | R | – |
| 15 | M | 4 | 5 | R | R | R | R | R | R | R | R | S | R | R | R | S | S | S | S | R | – |
| 16 | G | 4 | 5 | R | R | R | R | R | R | R | R | S | R | R | R | S | S | S | S | R | – |
| 17 | K | 5 | 6 | R | S | S | R | S | S | S | S | S | S | S | R | R | S | S | S | S | – |
| 18 | G | 5 | 6 | R | S | S | R | S | S | S | S | S | S | S | R | R | S | S | S | S | – |
| 19 | L | 6 | 7 | R | R | S | R | S | S | S | S | S | S | S | S | R | S | S | S | S | – |
| 20 | G | 6 | 7 | R | R | S | R | S | S | S | S | S | S | S | S | R | S | S | S | S | – |
| 21 | L | 7 | 8 | R | R | R | R | R | R | R | R | R | R | R | R | S | S | R | S | R | + |
| 22 | K | 7 | 8 | R | R | R | R | R | R | R | R | R | R | R | R | S | S | R | S | R | + |
| 23 | M | 7 | 8 | R | R | R | R | R | R | R | R | R | R | R | R | S | S | R | S | R | + |
| 24 | G | 7 | 8 | R | R | R | R | R | R | R | R | R | R | R | R | S | S | R | S | R | + |

L: Liver, K: Kidney, M: Muscle, G: Gill, R: Resistant, S: Sensitive, AM: Ampicillin, AX: Amoxicillin, AMC: Amoxicillin–clavulanate, CZ: Cefazolin, CAZ: Ceftazidime, CRO: Ceftriaxone, CTX: Cefotaxime, CPM: Cefepime, C: Chloramphenicol, NA: Nalidixic acid, CIP: Ciprofloxacin, TE: Tetracycline, SXT: Trimethoprim-sulfamethoxazole, AMK: Amikacin, GEN: Gentamicin, IPM: Imipenem, E: Erythromycin, ESBL: Production of Extended Spectrum β–lactamases

***TABLE II***
**Antibiotic resistance rates of the isolates**

| Antimicrobial Groups | Antimicrobials | N° Resistant Strains | Resistance Ratio |
|---|---|---|---|
| Penicillins | Ampicillin | 24 | 100.00 |
| | Amoxicillin | 22 | 91.67 |
| Penicillins + beta–lactamase inhibitors | Amoxicillin–clavulanate | 20 | 83.33 |
| 1st generation cephalosporin | Cefazolin | 24 | 100.00 |
| 3rd generation cephalosporins | Ceftazidime | 16 | 66.67 |
| | Ceftriaxone | 16 | 66.67 |
| | Cefotaxime | 16 | 66.67 |
| 4th generation cephalosporins | Cefepime | 16 | 66.67 |
| Phenicols | Chloramphenicol | 8 | 33.33 |
| Quinolones | Nalidixic acid | 20 | 83.33 |
| | Ciprofloxacin | 16 | 66.67 |
| Tetracyclines | Tetracycline | 18 | 75.00 |
| Folate pathway inhibitors | Trimethoprim-Sulfamethoxazole | 12 | 50.00 |
| Aminoglycosides | Amikacin | 4 | 16.67 |
| | Gentamicin | 8 | 33.33 |
| Carbapenems | Imipenem | 4 | 16.67 |
| Macrolides | Erythromycin | 20 | 83.33 |
| Modified Double Disc Synergy Test (MDDST) | | 12 | 50.00 |

*ermB* and *ermF* were detected in 50% of the isolates. *QnrB* associated with quinolone resistance, and *blaTEM* and *blaCTX* responsible for the production of extended–spectrum beta–lactamases were detected in 33.33% of the isolates (TABLE IV).

***TABLE IV***
**Percentages of the resistance genes of the isolates**

| Effected Antimicrobial | Genes | Positivity | Percentage (%) |
|---|---|---|---|
| Extended–spectrum beta–lactamase (ESBL) producing genes [21] | *blaTEM* | 8 | 33.33 |
| | *blaSHV* | 4 | 16.67 |
| | *blaCTX* | 8 | 33.33 |
| Quinolone resistance genes [20] | *qnrA* | 0 | 0.00 |
| | *qnrB* | 8 | 33.33 |
| | *qnrC* | 0 | 0.00 |
| | *qnrD* | 0 | 0.00 |
| | *qnrS* | 4 | 16.67 |
| | *qepA* | 0 | 0.00 |
| Tetracycline resistance genes [22] | *tetA* | 14 | 58.33 |
| | *tetB* | 4 | 16.67 |
| Sulphonamide resistance genes [22] | *sulI* | 12 | 50.00 |
| | *sulII* | 6 | 25.00 |
| Erythromycin resistance genes [22] | *ermB* | 12 | 50.00 |
| | *ermF* | 12 | 50.00 |

***TABLE III***
**Distribution of resistance genes of the isolates**

| N° | Tissue | Farm N° | Fish N° | blaTEM | blaSHV | blaCTX | qnrA | qnrB | qnrC | qnrD | qnrS | qepA | tetA | tetB | sulI | sulII | ermB | ermF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | L | 1 | 1 | + | + | + | | + | | | | | + | | + | + | + | |
| 2 | K | 1 | 1 | + | + | + | | + | | | | | + | | + | + | + | |
| 3 | M | 1 | 1 | + | + | + | | + | | | | | + | | + | + | + | |
| 4 | G | 1 | 1 | + | + | + | | + | | | | | + | | + | + | + | |
| 5 | K | 2 | 2 | | | | | | | | + | | | | | | + | + |
| 6 | M | 2 | 2 | | | | | | | | + | | | | | | + | + |
| 7 | L | 2 | 3 | | | | | | | | + | | | | | | + | + |
| 8 | G | 2 | 3 | | | | | | | | + | | | | | | + | + |
| 9 | L | 3 | 4 | + | | | | + | | | | | + | | + | | | + |
| 10 | K | 3 | 4 | + | | | | + | | | | | + | | + | | | + |
| 11 | M | 3 | 4 | + | | | | + | | | | | + | | + | | | + |
| 12 | G | 3 | 4 | + | | | | + | | | | | + | | + | | | + |
| 13 | L | 4 | 5 | | | | | | | | | | | + | | | | + |
| 14 | K | 4 | 5 | | | | | | | | | | | + | | | | + |
| 15 | M | 4 | 5 | | | | | | | | | | | + | | | | + |
| 16 | G | 4 | 5 | | | | | | | | | | | + | | | | + |
| 17 | K | 5 | 6 | | | | | | | | | | + | | + | + | | |
| 18 | G | 5 | 6 | | | | | | | | | | + | | + | | | |
| 19 | L | 6 | 7 | | | | | | | | | | | | + | | | |
| 20 | G | 6 | 7 | | | | | | | | | | | | + | + | | |
| 21 | L | 7 | 8 | | | + | | | | | | | + | | | | + | |
| 22 | K | 7 | 8 | | | + | | | | | | | + | | | | + | |
| 23 | M | 7 | 8 | | | + | | | | | | | + | | | | + | |
| 24 | G | 7 | 8 | | | + | | | | | | | + | | | | + | |

M: Muscle, L: Liver, K: Kidney, G: Gill

In recent years, interest in aquaculture has increased in many different parts of the world. On the other hand, due to the deficiencies in biosafety principles, especially in developing countries, the use of antimicrobials especially for poultry is increasing for the treatment of diseases of fish, and this causes bacteria to develop resistance to these antimicrobials [23]

When the antibiotic resistance results of the bacteria isolated from some fish species in Iskenderun Bay were examined by Matyar *et al.* [24] the resistance to IPM could not be determined in bacteria isolated from the gills, while this rate was reported as 5.3% in intestinal isolates. In the same study, 12.9% of bacteria isolated from gills were resistant to TE, while this rate was 5.3% in intestinal isolates, while SXT resistance was 3.2% in gill isolates and 9.3% in intestinal isolates. In this study, high resistance was observed against penicillin and first-generation cephalosporins.

The resistance rates detected in the study on the antibiotic resistance levels of *E. coli* strains isolated from Giresun Batlama Deresi were found lower than in this current study. In the study, ampicillin 59%, tetracycline 50.8%, nalidixic acid 44.4%, erythromycin 42.9%, chloramphenicol 38.1%, cefazolin 36%, cefuroxime 35.9% and cefotaxime 28.4%, were found respectively. Value (CAD) rate was found 73.28% [25].

Gufe *et al.* [26] investigated the antibiotic susceptibility levels in isolated bacteria from 36 fish samples collected from the public market. While all isolates were susceptible to gentamicin, lincomycin (100%), ampicillin (81%), penicillin (67%), erythromycin (65%), tetracycline (63%), neomycin (61%), cloxacillin (43%), kanamycin (24%) and sulfamethoxazole (13%) antibiotic resistance rates were observed. The detected ampicillin resistance rate was 81%, lower than the current study (100%). This shows that due to the resistance developed against penicillin derivatives in the fish farms where the study was conducted, alternative antibiotics should be used as alternatives to such drugs.

In their study conducted by Zhang *et al*. [27] they detected resistance genes such as *blaTEM*, *qnr*, *sul*, and *tetA*, as well as resistance genes such as *blaTEM*, *qnr*, *sul*, and *tetA*, in seven fish they detected in fish farms, as well as a resistance gene against colistin, an antimicrobial used in the treatment, especially in the case of carbapenem resistance, and that these strains can be quite resistant.

Ryu *et al*. [28] reported that they detected 6.7% of *E. coli* in commercially sold fish collected in South Korea and they found more than 30% resistance to tetracycline in their origins. When they examined the resistance genes, they reported that *blaTEM* was detected at a rate of 21% and *tetD* at a rate of 41%.

In their study in Lebanon, Hassuna *et al.* [19] reported that when they examined the *E. coli* strains of six fish with the Whole-Genome Sequencing method, they detected *blaTEM*, *erm*, *sul*, and *tetA* resistance, and they also detected *mcr* resistance, which may cause colistin resistance, in the isolates of these fish. All these study data support current study data. It was observed that different genes that can affect many antimicrobial groups in *E. coli* strains detected in the study were produced by these strains.

In this study, phenotypic resistance profiles were detected at various rates against all antibiotics tested. Contrary to Matyar *et al.* [24], the same phenotypic profiles were detected among the tissue isolates of the fish from which bacteria were isolated, and no differences were observed. In general, high rates of phenotypic antibiotic resistance profiles were detected against antibiotics licensed for fish diseases and antibiotics that have been in use for a long time all over the world, while low rates of phenotypic antibiotic resistance profiles were detected against relatively newly discovered antibiotics, and not licensed for use in fish diseases (TABLE II). This was considered a possible result of a combination of antibiotic misuse, antibiotics in use for long periods, and the ability of bacteria to develop resistance [11]. It is seen that the phenotypic resistance profiles detected vary between farms. This difference may be due to the management of the farms, the variety of antibiotic drugs used for treatment, differences in doses and duration of use, incorrect antibiotic use, differences in the origin of the isolates, and possible gene transfers through various means. Especially in farm numbered one, the presence of isolates resistant to all antibiotics tested in this study is noteworthy.

The rates of resistance genes detected against the antibiotics investigated in this study are compatible with phenotypic resistance rates. However, this agreement is not one hundred percent. The greatest agreement is between the rates of tetracycline resistance genes and the rates of phenotypic resistance to tetracycline. In their studies on the development of antibiotic resistance, researchers address hereditary resistance. They reported that for differentiation in phenotypic resistance, either mutation must occur or antibiotic-resistance genes must be acquired through gene transfer. However, researchers have reported that phenotypic resistance can in some cases be acquired without any genetic modification, that it may be associated with specific processes such as growth in biofilms, a stationary growth phase or persistence, drug indifference, and changes in bacterial permeability, and that phenotypic resistance to antibiotics is a complex phenomenon that depends on the metabolic state of bacterial populations [11, 29].

ESBL gene regions were detected in fish isolates numbered one, four, and eight. In addition, it was determined that the isolates belonging to fish numbered one contained all three ESBL gene regions. The detection of different molecular class ESBL gene regions in this study suggests the possible presence of horizontal gene transfer. All isolates included ESBL gene regions in this study were determined to produce ESBL phenotypically. This high concordance is similar to a study conducted previously [30].

## CONCLUSIONS

According to the findings of this study, these bacteria can be detected in fish farms in Türkiye and they have the potential to produce serious antimicrobial resistance genes. Also, the detection of *E. coli* in fish samples could be accepted as an indicator of fecal contamination. It was thought that both findings, the presence of the contamination and the antimicrobial resistance genes, could be dependent on the lack of infrastructure, management, and regulation in fish farms, and erroneous antimicrobial usage.

### Recommendations

The lack of follow-up data on the strains that can be detected in these farms, their resistance profiles, and the status of genes that can cause resistance is noteworthy. It is predicted that in the future, new gene editing technologies such as CRISPR and new drugs to be produced with nanotechnology will play an important role in the treatment of infections caused by bacterial strains resistant to existing antimicrobials. However, in order to use the currently available antimicrobials, erroneous antimicrobial use should be avoided and

these farms should be followed with official protocols. Since the resistance genes of these bacteria have the potential to reach humans through water and fish, they can pose a high risk to animal and human health. It is advised that these farms should be kept in mind within the framework of a single health perspective and they should be followed up, and similar studies should be carried out in other farms.

**Conflict of Interest**

The authors declared no conflict of interest.

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