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Antibiotic resistance phenotypes and genes of *Escherichia coli* isolates from rainbow trout (*Oncorhynchus mykiss*) sold in retail settings in Kerman, Iran

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Abstract

Antibiotics are widely used to treat infectious diseases in humans and animals. However, the indiscriminate use of antibiotics contributes to the emergence of antibiotic-resistant bacteria, making infections more difficult to treat. In this study, we randomly collected thirty-six rainbow trout fish from various retail stores in Kerman city, located in the southeast of Iran. Skin samples were obtained from each fish using a swab, and *Escherichia coli* (*E. coli*) isolates were identified and screened for antimicrobial resistance (AMR) phenotypes, as well as related genes, using microbiological culturing, disc diffusion, and conventional PCR methods. Our findings showed that the prevalence of phenotypic resistance against the tested antibiotics was high, with erythromycin (88.57%), florfenicol (77.14%), oxytetracycline (74.28%), trimethoprim-sulphamethoxazole (71.42%), trimethoprim (65.71%), chloramphenicol (62.85%), flumequine (60%), ciprofloxacin (54.28%), and tetracycline (54.28%) having the highest resistance rates. Moreover, 8.57% of the *E. coli* isolates were found to be ESBL-producing strains, and 74.28% of the isolates were multi-drug resistant (MDR). The highest frequencies of antibiotic resistance genes were 5.71 % for bla_{TEM}, 14.28% for *qnrA*, 17.14% for *sul1*, and 20% for *sul2*. *E. coli* is a mesophilic bacteria and is not naturally present in fish. Fishes have mostly psychrophilic bacteria in their microflora. The origin of *E. coli* on the skin of fish is water contaminated with human and animal feces, so the antibiotic resistance of this bacterium has an indirect relationship with aquaculture. Our study showed that *E. coli* isolates from the skin of rainbow trout has a high level of antibiotic resistance, which may be a risk to public health. Therefore, it is very important to control the use of antibiotics in fish farming to reduce the selection pressure to emergence and spread of antibiotic-resistant bacteria.

Key words: *Escherichia coli*, Antibiotic resistance, Fish, Kerman

Introduction

E. coli is one the most important members of gut microflora in human and warm-blood animals but not naturally present in fish microbiota that can be acquired from sewage-contaminated aquatic environments (Chigor et al, 2020).

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E. coli is an indicator of fecal contamination of water and aquatics and this contamination is an important problem in aquaculture. Consumption of contaminated fish may lead to food-borne infections such as diarrhea (Cardozo et al, 2018), as an important problem in public health. So, this research focuses on this bacterium and its characteristics. Microorganisms can be present in fish hosts by attaching to the skin, passing through the gills and intestines (Tiralongo et al, 2020); fish skin is in contact with water contaminated with feces and fecal bacteria (such as *E. coli*), so the skin can be one of the suitable samples of study.

E. coli has the potential to disseminate some factors such as antibiotic resistance among various hosts. Antimicrobial resistance (AMR) occurs when microorganisms develop the ability to evade the effects of antimicrobial agents. Several factors such as socio-economic conditions, ecological factors influencing infections, and misuse of antibiotics can contribute to the spread of antibiotic resistance among humans, animals, and the environment (Wendlandt et al, 2015). The indiscriminate use of antibiotics exerts selective pressures in favor of resistant microorganisms. (Berendonk et al, 2015; Zhang et al, 2018). These microbes commonly utilize four fundamental mechanisms of resistance including: (i) limiting the uptake of a drug, (ii) modification of an antibiotic target, (iii) inactivating an antimicrobial agent, and (iv) use of active drug efflux pumps (Reygaert, 2018).

The molecular basis of antimicrobial resistance mechanisms in bacteria is closely related to the presence of antibiotic resistance genes or ARGs (Jian et al, 2021). There are two main types of ARGs, including intrinsic (chromosomally) and acquired (via mutations or horizontal gene transfer). Most ARGs are transferred between bacterial strains of the same or different genera. This transfer occurs through mobile genetic elements, such as plasmids or transposons, via conjugation, transformation, or transduction (Durão et al, 2018; Fang et al, 2019).

In aquaculture, antibiotics are commonly used to treat a wide range of infections (Done et al, 2015); on average, 500 grams of antibiotics, such as β-lactams, tetracyclines, sulfonamides, aminoglycosides, amphenicols, and nitrofuran, are used for each ton of produced rainbow trout (Jara et al, 2021). Chloramphenicol, oxytetracycline, and erythromycin are among the antibiotics that are frequently used to eliminate bacterial pathogens in aquaculture (Schar et al, 2020). However, the use of antibiotics can lead to the development of a problem known as antibiotic residues, which can easily spread in aquatic environments (Xu et al, 2015) and affect the microflora of aquatic or environmental bacteria in water (Huang et al, 2017; Fang et al, 2019). This can result in the emergence of antibiotic-resistant bacteria, which is a cause for a global concern.

There are various phenotypic and genotypic methods for identifying antibiotic-resistant bacteria. Five commonly used techniques include dilution methods (both broth and agar dilution), antimicrobial gradient method, disk diffusion test, chromogenic agar media, and colorimetric tests. Additionally, there are several molecular-based methods for detection of resistance, such as polymerase chain reaction (PCR), real-time PCR (quantitative PCR or qPCR), DNA microarrays, and whole-genome sequencing (WGS) for antimicrobial susceptibility testing (Gajic et al, 2022).

As previously mentioned, the objective of the present study was to investigate the antibiotic resistance phenotypes and genes of *E. coli* isolates obtained from retail rainbow trout fish samples in Kerman city, Iran.

Materials and Methods

Sampling, culture and *E. coli* isolations

A total of 36 rainbow trout fish were randomly collected from five different retail fish stores located in Kerman province, South-eastern Iran, between February 2020 and February 2021 at two-week intervals. The fishes were stored in polyethylene bags with an ice pack and transported to the microbiology laboratory at Shahid Bahonar University of Kerman. A skin sample was taken from each fish using a swab, which was then cultured on MacConkey agar (Merck, Germany) and incubated aerobically at 37°C for 18 to 24 hours. Suspected colonies of *E. coli* (i.e., those that appeared round, smooth, and pink) were selected for biochemical confirmation using IMViC tests (indole, methyl red, Voges Proskauer, and citrate). Finally, 35 *E. coli* isolates were confirmed and used for subsequent analysis (Markey et al, 2013).

Phenotypic assessment of antibiotic resistance in *E. coli* strains

Antimicrobial susceptibility testing was performed using the disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines 2021 (CLSI, 2021). The antibiotics tested were chosen based on those commonly used in farm fishes, including florfenicol (FF; 30 µg), erythromycin (E; 30 µg), flumequine (FLM; 30 µg), oxytetracycline (T; 30 µg), ciprofloxacin (CP; 30µg), chloramphenicol (C; 30 µg), trimethoprim-sulfamethoxazole (SXT; 30 µg), tetracycline (TE; 30 µg), trimethoprim (TMP; 30 µg), amoxicillin (AMX; 30 µg), cefotaxime (CTX; 30 µg), nitrofurantoin (FM; 30 µg), and ceftazidime (CAZ; 30µg).

A suspension of each confirmed *E. coli* isolate was prepared with a turbidity similar to that of a 0.5 McFarland standard. A sterile swab was then inserted into the suspension and used to culture a lawn of bacteria on the surface of Muller-Hinton (MH) agar. After 3 to 5 minutes, antibiotic discs were placed on the agar surface. The plates were incubated at 37°C for 24 hours, and the zone diameter of growth inhibition was measured in millimeters. The microorganisms were labeled as sensitive (S), resistant (R), or intermediate (I) based on the 2021 guidelines from the CLSI (CLSI, 2021).

The double disc diffusion method was employed to identify strains that produce extended-spectrum β-lactamases (ESBLs). In this method, ESBL-producers were identified based on a ≥5 mm increase in the zone of growth inhibition diameter around the cefotaxime-clavulanate and/or ceftazidime-clavulanate discs as compared to the zone of growth inhibition diameter around the cefotaxime and/or ceftazidime discs, respectively (CLSI, 2021).

Genotypic assessment of antibiotic resistance

The total genomic DNA of confirmed *E. coli* strains was extracted using the boiling technique. A single, confirmed *E. coli* colony was suspended in 400 µl of sterile distilled water and heated to 98-100°C for 10-15 minutes in a heating block (Eppendorf, Germany). The suspension was then refrigerated on ice for 10 minutes and centrifuged at 13,000 × g for 1 minute. The resulting supernatant was used as the DNA template in PCR (Jajarmi et al, 2021).

The study examined the presence of antimicrobial genes, including bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, bla_{OXA}, *sulI*, *sulII*, *dhfrI*, *dhfrV*, *cat1*, *qnrA*, *qnrB*, *tetA* and *tetB*, *aadA*, *floR*. The clinical *E. coli* strains including 17DN for *sulI* and *sulII*; 21DN for *qnrB*; 25DN for *tetA*, *tetB* and *cat1*; 170DN for *dhfrI* and *dhfrV* were used as positive controls that all were supplied by Dr. Reza Ghanbarpour from Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman. Also, the *E. coli* strains ATCC 35218 (for bla_{TEM}) and ATCC 700603 (for bla_{SHV} and $bla_{\text{CTX-M}}$) were used as standard positive controls for β-lactamase genes. Distilled water was used as the negative control.

PCR was conducted in a total volume of 25 µL, including 12 µL of 2x Master Mix (Parstous, Iran), 0.5 µM of each primer, 3 µL of DNA template, and distilled water to reach the final volume. The thermal cycling program included an initial denaturation at 95°C for 15 minutes, followed by 30 to 35 cycles at 95°C for 1 minute (denaturation), 54-65°C for 1 minute (annealing), and 72°C for 1 minute (extension). The final extension was performed for 10 minutes at 72°C. Table 1 displays the specific DNA sequences, sizes for PCR products, the number of cycles and annealing temperatures for each program.

Data for the presence or absence of phylogenetic groups and antibiotic resistance in each isolate were entered into Excel (Microsoft 2016) and SPSS (SPSS 24; IBM) for descriptive statistical analysis.

Table 1: Sequences of primers used in this study

Target	Sequence (5'-3')	Size (bp)	Anealing	Reference
bla$_{TEM}$	F- AAA ATT CTT GAA GAC G R- TTA CCA ATG CTT AAT CA	1080	50°C	(Sharma et al., 2010)
bla$_{SHV}$	F- TTA ACT CCC TGT TAG CCA R- GAT TTG CTG ATT TCG CCC	768	50°C	(Sharma et al., 2010)
bla$_{CTX-M}$	F- CGC TTT GCG ATG TGC AG R- ACC GCG ATA TCG TTG GT	550	60°C	(Messai et al., 2006)
bla$_{OXA}$	F- TCA ACT TTC AAG ATC GCA R- GTG TGT TTA GAA TGG TGA	591	48°C	(Colom et al., 2003)
sulI	F- TTC GGC ATT CTG AAT CTC AC R- ATG ATC TAA CCC TCG GTC TC	822	58°C	(Vickers, 2017)
sulII	F- GCG CTC AAG GCA GAT GGC ATT R- GCG TTT GAT ACC GGC ACC CGT	293	69°C	(Kerrn et al., 2002)
dhfrI	F- AAG AAT GGA GTT ATC GGG AAT G R- GGG TAA AAA CTG GCC TAA AAT TG	391	55°C	(Vickers, 2017)
dhfrV	F- CTG CAA AAG CGA AAA ACG G R- AGC AAT AGT TAA TGT TTG AGC TAA AG	432	58°C	(Vickers, 2017)
tetA	F- GTG AAA CCC AAC ATA CCC C R- GAA GGC AAG CAG GAT GTA G	887	50°C	(Vickers, 2017)
tetB	F- CCT TAT CAT GCC AGT CTT GC R- ACT GCC GTT TTT TCG CC	773	50°C	(Vickers, 2017)
cat1	F- AGT TGC TCA ATG TAC CTA TAA CC R- TTG TAA TTC ATT AAG CAT TCT GCC	547	56°C	(Vickers, 2017)
qnrA	F- AGA GGA TTT CTC ACG CCA GG R- TGC CAG GCA CAG ATC TTG AC	580	54°C	(Cattoir et al., 2007)
qnrB	F- GGM ATH GAA ATT CGC CAC TG R- TTT GCY GYY CGC CAG TCG AA	264	54°C	(Cattoir et al., 2007)
floR	F- TAT CTC CCT GTC GTT CCA G R- AGA ACT CGC CGA TCA ATG	399	56°C	(Vickers, 2017)
aadA	F- TGA TTT GCT GGT TAC GGT GAC R- CGC TAT GTT CTC TTG CTT TTG	284	58°C	(Vickers, 2017)

Results

Phenotypic antimicrobial resistance

Three isolates (8.57%; 95% CI: 1.80-23.06%) were ESBL-producers, and four isolates (11.42%; 95% CI: 3.20-26.74%) were resistant to only one antibiotic. The majority of the isolates (27 isolates; 77.14%, 95% CI: 59.86-89.58%) were resistant to more than one antibiotic (Table 4), with 74.28% of the isolates being resistant to three or more antibiotics from different antimicrobial families (multi-drug resistant or MDR); The most common resistance pattern was the

FF/E/FLM/T/CP/C/SXT/TE/TMP/AMX/CTX profile, which was detected in six (17.14%; 95% CI: 6.56-33.65%) isolates (Table 4).

The results indicate a high prevalence of resistance to the antibiotics erythromycin (88.57%; 95% CI: 73.26-96.80%) and florfenicol (77.14%; 95% CI: 59.86-89.58%), as shown in Table 3 and Figure 1. Conversely, nitrofurantoin (8.57%; 95% CI: 1.80-23.06%) and ceftazidime (17.14%; 95% CI: 6.56-33.65%) exhibited the lowest rates of antibiotic resistance (Table 3 and Figure 1).

Antimicrobial Resistance Genes

Out of 35 *E. coli* isolates, the highest frequencies of antibiotic resistance genes were 5.71 % for bla_{TEM}, 14.28% for *qnrA*, 17.14% for *sul1*, and 20% for *sul2*, followed by 5.71 % for bla_{TEM} and 2.85 % for $bla_{\text{CTX-M}}$, bla_{SHV}, *tetB*, and *dhfr1*. The remaining studied genes were not detected in any of the strains (Table 2 and Figure 2).

Table 2: Prevalence of each antimicrobial resistance gene and phenotype among *E. coli* isolates

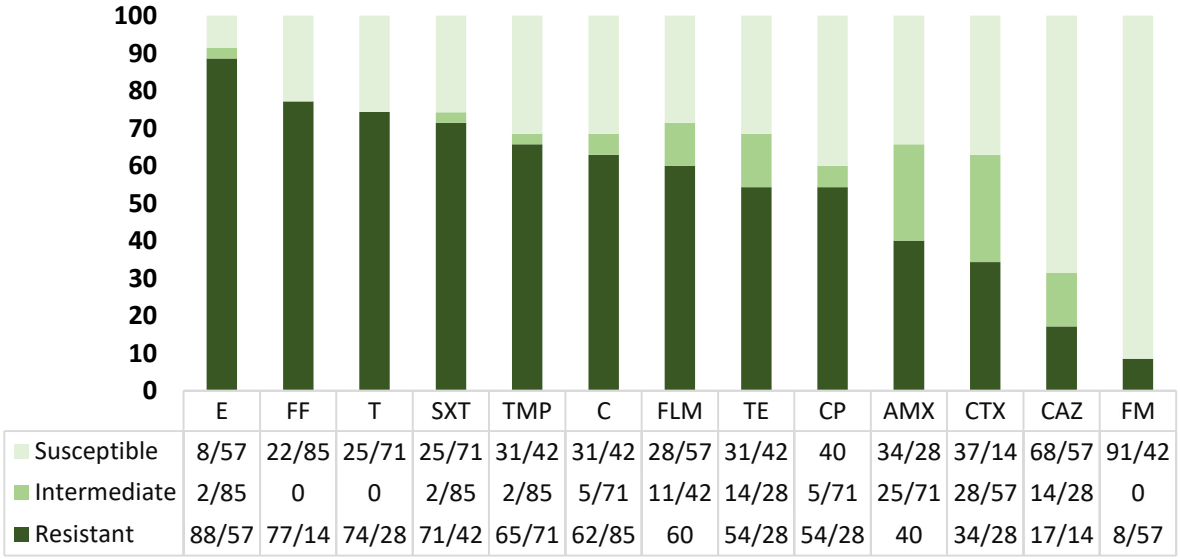
Antibiotic family	AR Phenotype	No.	Prevalence	AR Gene	No.	Prevalence
β-Lactam	AMX	14	40 %	bla_{TEM}	2	5.71 %
	CAZ	6	17.14 %	bla_{SHV}	1	2.85 %
	CTX	12	34.28 %	$bla_{\text{CTX-M}}$	1	2.85 %
	-	-	-	bla_{OXA}	-	-
Tetracycline	TE	19	54.28 %	*tetA*	-	-
	T	26	74.28 %	*tetB*	1	2.85 %
Amphenicol	FF	27	77.14 %	*cat1*	-	-
	C	22	62.85 %	*floR*	-	-
Fluoroquinolones	CP	19	54.28 %	*qnrA*	5	14.28 %
	FLM	21	60 %	*qnrB*	-	-
Sulphonamide	SXT	25	71.42 %	*sul1*	6	17.14 %
	-	-	-	*sul2*	7	20 %
Trimethoprim	TMP	23	65.71 %	*dhfr1*	1	2.85 %
	-	-	-	*dhfrV*	-	-
Aminoglycoside	-	-	-	*aadA*	-	-
Macrolides	E	31	88.57 %	-	-	
Others	FM	3	8.57 %	-	-	-

Table 3: Number (%) of AR gene-positive isolates among positive isolates for AR phenotypes

Antimicrobial resistance phenotype	Antimicrobial resistance genes								
	sul1	*sul2*	*qnrA*	$bla_{\text{CTX-M}}$	bla_{TEM}	bla_{SHV}	*tetB*	*dhfr1*	ESBL+
AMX	2(14.28)	0	2(14.28)	1(7.14)	0	0	1(7.14)	0	3(21.42)
CAZ	1(16.16)	1(16.16)	0	0	0	0	0	0	2(33.33)
CTX	1(8.33)	4(33.33)	2(16.6)	0	0	0	0	0	3(25)
TE	2(10.52)	4(21.05)	3(15.78)	1(5.26)	0	0	1(5.26)	0	3(15.78)
T	5(19.23)	5(19.23)	3(11.53)	1(3.84)	0	0	1(3.84)	0	3(11.53)
FF	6(22.22)	6(22.22)	3(11.11)	1(3.70)	0	1(3.70)	0	0	3(11.11)
C	5(22.72)	4(18.11)	3(13.63)	1(4.54)	0	0	0	0	3(13.63)
CP	5(26.31)	2(10.52)	1(5.26)	1(5.26)	1(5.26)	0	0	0	3(15.78)
FLM	4(19.04)	3(14.28)	1(4.76)	1(4.76)	1(4.76)	0	0	0	3(14.28)
SXT	5(20)	4(16)	4(16)	1(4)	0	0	1(4)	1(4)	3(12)
TMP	5(21.73)	5(21.73)	4(17.39)	1(4.34)	0	0	1(4.34)	0	3(13.04)
E	6(19.35)	5(16.12)	4(12.90)	1(3.22)	0	1(3.22)	1(3.22)	1(3.22)	3(9.67)
FM	1(33.33)	0	0	0	0	0	0	0	1(33.33)

Table 4: various AR phenotype profiles and distribution pattern of AR gene and ESBL variables among them

Phenotypic resistance pattern	no. (%)	*bla*$_{TEM}$	*bla*$_{SHV}$	*bla*$_{CTX-M}$	*qnrA*	*qnrB*	*sul1*	*sul2*	*dhfr1*	*tetB*
FF/E/FLM/T/CP/C/SXT/TE/ TMP/AMX/CTX/FM/CAZ	3 (8.5)	-	-	-	-	-	1 (4)	1(5.2)	-	-
FF/E/FLM/T/CP/C/SXT/TE/ TMP/AMX/CTX/CAZ	3 (8.5)	-	-	1(4.7)	-	-	-	-	-	-
FF/E/FLM/T/CP/C/SXT/TE/ TMP/AMX/CTX	6 (17.1)	-	-	-	1(5.2)	-	1 (4)	1(5.2)	-	-
FF/E/FLM/T/CP/C/SXT/TE/ TMP/AMX	2 (5.7)	-	-	-	-	-	-	-	-	-
FF/E/FLM/T/CP/C/SXT/TE/ TMP	5 (14.2)	1(3.7)	-	-	2(10.5)	-	-	1(5.2)	-	1(8.3)
FF/E/FLM/T/CP/SXT/TMP	2 (5.7)	-	-	-	1(5.2)	-	2 (8)	1(5.2)	-	-
FF/E/T/C/SXT/TMP	1 (2.8)	-	-	-	-	-	-	-	-	-
FF/E/SXT/TMP	1 (2.8)	-	-	-	-	-	-	-	-	-
FF/E/T/SXT	2 (5.7)	-	-	-	-	-	-	-	-	-
FF/E/T	1 (2.8)	-	-	-	-	-	-	1(5.2)	-	-
FF/E	1 (2.8)	-	-	-	-	-	-	1(5.2)	-	-
E	4 (11.4)	1(3.7)	1(3.2)	-	-	1(4.5)	-	1(5.2)	-	-
No resistance	4 (11.4)	-	-	-	-	-	2 (8)	-	1(4.3)	-
Total	35(100)	2(5.7)	1(2.8)	1(2.8)	4(11.4)	1(4.5)	6(17.1)	7 (2)	1(2.8)	1(2.8)



	E	FF	T	SXT	TMP	C	FLM	TE	CP	AMX	CTX	CAZ	FM
Susceptible	8/57	22/85	25/71	25/71	31/42	31/42	28/57	31/42	40	34/28	37/14	68/57	91/42
Intermediate	2/85	0	0	2/85	2/85	5/71	11/42	14/28	5/71	25/71	28/57	14/28	0
Resistant	88/57	77/14	74/28	71/42	65/71	62/85	60	54/28	54/28	40	34/28	17/14	8/57

Figure 1: Prevalence of susceptible, intermediate and resistant isolates to various antibiotics including florfenicol (FF), erythromycin (E), flumequine (FLM), oxytetracycline (T), ciprofloxacin (CP), chloramphenicol (C), trimethoprim sulphamethoxazole (SXT), tetracycline (TE), trimethoprim (TMP), amoxicillin (AMX), cefotaxim (CTX), nitrofurantoin (FM), ceftazidime (CAZ).

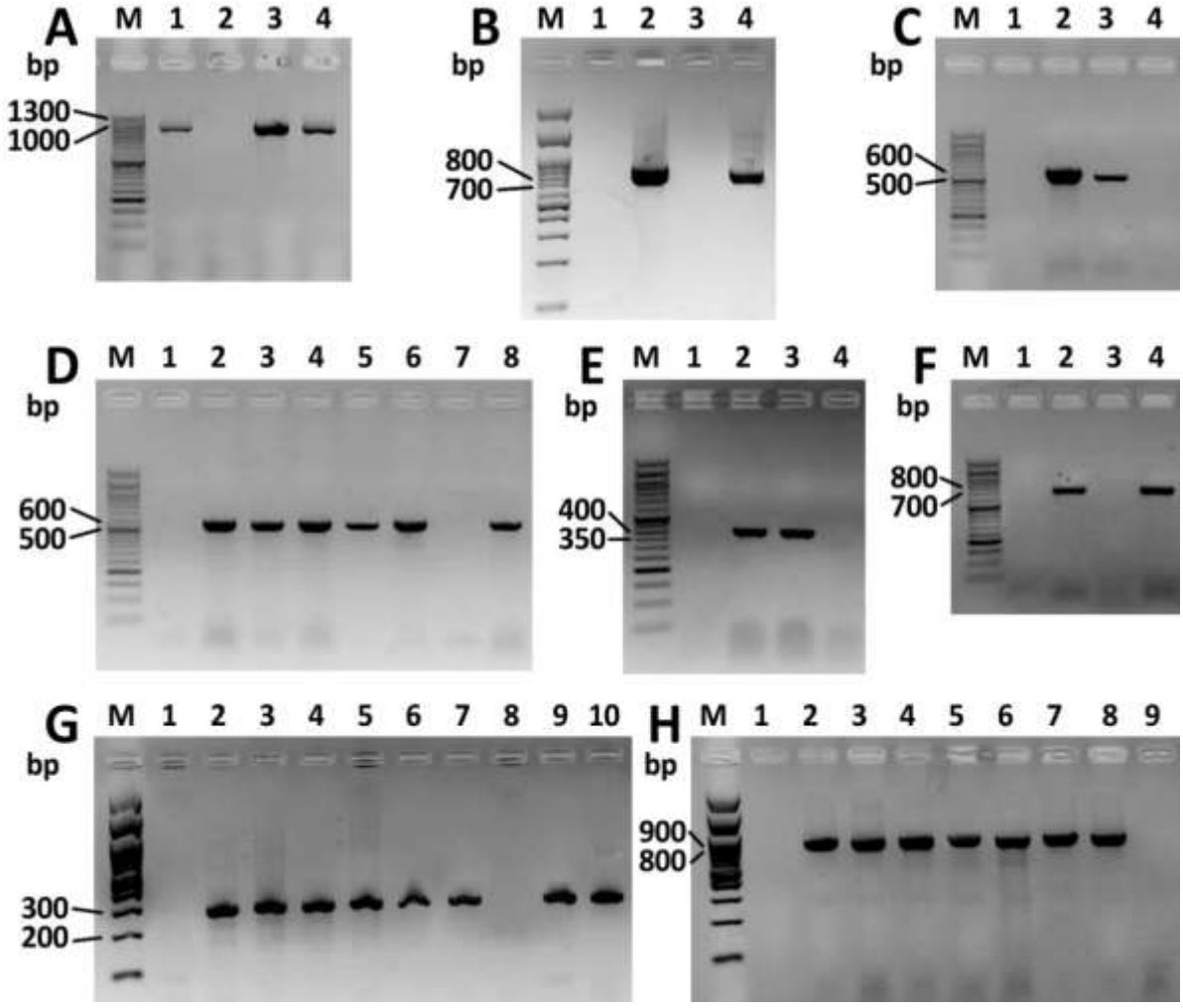


Figure 2: Gel electrophoresis of PCR products. A: M, marker (50 bp); 1, positive control for blaTEM (1080 bp); 2, negative control; 3 and 4, positive samples. B: M, marker (100 bp); 1, negative control; 2, positive control for blaSHV (768 bp); 3, negative sample; 4, positive sample. C: M, marker (50 bp); 1, negative control; 2, positive control for blaCTX-M (550 bp); 3, positive sample; 4, negative sample. D: M, marker (50 bp); 1, negative control; 2, positive control for qnrA (580 bp); 3 to 6 and 8, positive samples; 7, negative sample. E: M, marker (50 bp); 1, negative control; 2, positive control for dhfrI (391 bp); 3, positive sample; 4, negative sample. F: M, marker (50 bp); 1, negative control; 2, positive control for tetB (773 bp); 3, negative sample; 4, positive sample. G: M, marker (100 bp); 1, negative control; 2, positive control for sul2 (293 bp); 3 to 7 and 9 to 10, positive samples; 8, negative sample. H: M, marker (100 bp); 1, negative control; 2, positive control for sul1 (822 bp); 3 to 8, positive samples; 9, negative sample.

Discussion

In this study, the highest prevalence of phenotypic resistance belonged to macrolides familly (erythromycin), with more than 85% of strains, followed by tetracycline, amphenicol, sulphonamide and trimethoprim families. Interestingly, previous studies have mostly shown a low prevalence of erythromycin resistance (Ellis-Iversen et al, 2020; Ishida et al, 2010; Ryu et al, 2012).

The prevalence of ESBL-producing *E. coli* strains was less than one-tenth of the isolates phenotypically. This finding is in contrast to an Indian study, which reported a prevalence of 71.58% in fish (Singh et al, 2020). However, our results are similar to those of a study in Tanzania (Moremi et al, 2016), which found a prevalence of 13.3% of ESBL-producing isolates. The prevalence of resistant *E. coli* strains to β-lactams in our study ranged from 17% to

40% phenotypically. The highest prevalence was related to AMX, followed by CTX and CAZ, respectively. Our results are comparable to those reported by Onmaz et al. (2020) and Odumosu et al., (2021) with prevalence rates ranging from 20% to 50%. In contrast, Brahmi et al. (2018) in Algeria showed a high prevalence of 90% for resistance to CAZ, which was not similar to our study. Several genes have been identified to produce resistance agents against β-lactams. Among these genes, *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$ and *bla*$_{\text{CTX-M}}$ are some of the most frequently reported genetic sequences that encode resistance against β-lactams. In the present study, low frequencies of these genes were observed, while high levels of prevalence have been reported in other countries for *bla*$_{\text{TEM}}$ and *bla*$_{\text{CTX-M}}$-positive isolates from fish, such as China (Liao et al, 2021) and Pakistan (Shah et al, 2012). The results of the present study on the *bla*$_{\text{CTX-M}}$ were similar to those of a study conducted by Ellis-Iversen et al. (2020) on pangasius fillets and prawns in Danish retail imported from Asia (Ellis-Iversen et al, 2020). The order of frequencies observed in our study (*bla*$_{\text{TEM}}$ > *bla*$_{\text{CTX-M}}$) is consistent with findings reported from aquaculture farms and their environment in Zhanjiang, China (Liao et al, 2021). Effluents discharged into aquatic ecosystems, by waste water treatment plants, treating urban and hospital wastewater, have been clearly identified as a source of extended-spectrum β-lactamases producing *E. coli* that are disseminated in aquatic environments (Blaak et al, 2014; Bréchet et al, 2014; Franz et al, 2015).

The results of our study indicate that the prevalence of tetracycline-resistant *E. coli* strains was phenotypically between 54% and 75%. This prevalence is comparable to that reported by studies in other countries, such as Turkey (Onmaz et al, 2020) with 50%. Also, our findings are consistent with a study conducted in Nigeria, which reported a prevalence of 77.8% for oxytetracycline resistance (Odumosu et al, 2021). In this research, two tetracycline-resistance genes, *tetA* and *tetB*, were screened; the frequency of the *tetB* gene in our isolates was higher than that of *tetA*, but much lower than that reported in studies conducted in Japan (Furushita et al, 2003) and Republic of Korea (Ryu et al, 2012) with approximately 100% and 40% frequencies, respectively.

Our study revealed a high prevalence of amphenicol (FF and C)-resistant *E. coli* strains This prevalence was considerably higher than that reported in studies conducted in different countries, including India (Saharan et al, 2020), the Republic of Korea (Ryu et al, 2012) and Mangalore (Kumar et al, 2005). Despite the high prevalence of phenotypic resistance to amphenicols in our study, the frequency of *cat1* and *floR* genes among the isolates was zero. However, Ng et al. (2014) in Malaysia (Ng et al, 2014), Liao et al. (2021) in China (Liao et al, 2021), Ellis-Iversen *et al.* (2020) in Denmark (Ellis-Iversen et al, 2020) reported resistance gene percentages ranging from less than 1% to more than 20%.

The results of our study regarding fluoroquinolone, especially CP, showed frequencies similar to those reported in research conducted in India, ranging between approximately 40% to 60% (Saharan et al, 2020; Singh et al, 2020). In contrast, a study conducted in Egypt on *Oreochromis niloticus* (Nile Tilapia) reported lower antibiotic resistance to CP and FLM, with frequencies of 4.1% and 4.3%, respectively (Abdel-Latif and Sedeek, 2017) which is less than our findings. Among the studied resistance genes associated with fluoroquinolone resistance, *qnrA* was one of the most prevalent genes, consistent with the findings (14.7%) reported by Lima et al. in Brazil (2022); however, our results were higher than those observed by Ishida et al. (5.8%) in Egypt (2010) and Liao *et al.* (6.6 %) in China (Liao et al, 2021).

Among our isolates of *E. coli*, the abundance of resistance phenotype against

SXT was considerable, exceeding 70%. This level of resistance is higher than that reported in the Republic of Korea (less than 10%) and Egypt (less than 50%), but comparable to findings from Turkey (more than 50%) and Algeria (more than 50%) (Brahmi et al, 2018; Ishida et al, 2010; Onmaz et al, 2020; Ryu et al, 2012). Phenotypic resistance to TMP was similar to that of SXT, which is consistent with results reported in Nigeria (Odumosu et al, 2021) and India (Saharan et al, 2020). Among the most common genes responsible for resistance to sulfonamides and trimethoprims, s*ul1*, *sul2* (encoding resistance agents against sulfonamides), *dhfr1* and *dhfrV* (encoding resistance agents against trimethoprims) are highlighted; the frequency of these genes in our study was generally lower than the results reported in Tanzania (Shah et al, 2012); the *sul1* and *sul2* genes had the highest prevalence in our study similar to China (Liao et al, 2021) and Pakistan (Shah et al, 2012).

Nitrofurantoin (FM) was the last antibiotic investigated in our study, and the level of phenotypic resistance against this antibiotic was less than 10%. However, Jagoda et al. (SSSdS et al, 2014) and Miranda and Zemelman (Miranda and Zemelman, 2001) reported a higher frequency of FM resistance among various bacterial agents in fish.

The results of the present study showed that over 70% of the isolates were multidrug-resistant (MDR), which is comparable to the findings in Algeria (Brahmi et al, 2018). The use of β-lactam antimicrobials, aminoglycosides, and fluoroquinolones administered to fish via water or food may contribute to the emergence of MDR bacterial species carrying relevant co-resistance genetic determinants (Cabello et al, 2013; Heuer et al, 2009). Antibiotic resistance genes from MDR bacteria are often transferred to other bacterial strains through plasmid conjugation (Naik et al, 2018). ESBL-producers, in particular, often exhibit MDR to several major antimicrobial groups due to the co-presence of ESBL genes and other AMR genes on the same plasmid; cross-resistance and co-resistance can facilitate the selection of MDR bacteria (Thornber et al, 2020). The rapid increase in MDR bacteria in the food chain poses a serious threat to public health.

The presence of antibiotics in water is the primary factor contributing to the emergence of antibiotic-resistant bacteria in fish and aquatic environments. In addition to antibiotics, other compounds such as herbicides, pesticides, heavy metals, and human waste in water, as well as high-density and high-stress conditions, infections, and global climate warming, also create selective pressures in favor of resistant bacteria (Ben et al, 2019; MacFadden et al, 2018; Thornber et al, 2020). However, data on antibiotics used in aquaculture are limited, as only a few countries monitor the amount of antibiotic usage in aquaculture systems. In Europe, North America, and Japan, regulations on the use of antibiotics in aquaculture are strict (Smith, 2008). For instance, in Canada, only a small amounts of erythromycin, florfenicol, oxytetracycline, and sulfonamides, are approved for use in aquaculture (Thornber et al, 2020). Furthermore, cephalosporins are not approved for use in aquaculture operations in the European Union or North America (Young et al, 2022). The presence of AMR microorganisms in fish and aquatic environments highlights the need for a One Health approach to address the global health crisis of antimicrobial resistance. It is estimated that there are currently 700,000 deaths per year due to antimicrobial resistance, and this number is projected to increase to 10 million by 2050 (Oneill, 2016). Therefore, it is crucial to better understand the environmental hotspots for the spread of AMR, such as livestock and aquaculture systems. Aquaculture farms are particularly concerning, as they can act as genetic reactors and hotspots for AMR

genes due to opportunities for genetic transfer and recombination.

This survey showed the high frequencies of resistance to florfenicol, erythromycin, flumequine, oxytetracycline, ciprofloxacin, chloramphenicol, trimethoprim sulphamethoxazole, tetracycline, and trimethoprim. Resistance levels to amoxicillin, cefotaxim, nitrofurantoin, and ceftazidime were low. These strains were identified as multi-drug resistant (MDR) as they were resistant to three or more antibiotics belonging to different antimicrobial classes. These findings suggest that commercial fishes could act as carriers of antibiotic-resistant *E. coli* obtained from contaminated water, posing a health risk to consumers of fish.

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Conflict of interest

The authors declare that they have no confict of interest.

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فنوتیپ ها و ژن های مقاومت آنتی بیوتیکی جدایه های *اشریشیاکلی* به دست آمده از قزل آلای رنگین کمان (*Oncorhynchus mykiss*) عرضه شده در فروشگاه های خرده فروشی در کرمان، ایران

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چکیده

آنتی‌بیوتیک‌ها به طور گسترده‌ای برای درمان بیماری‌های عفونی در انسان و حیوانات استفاده می‌شود. با این حال، استفاده بی رویه از آنتی‌بیوتیک‌ها به ظهور باکتری‌های مقاوم به آنتی‌بیوتیک کمک می‌کند و درمان عفونت‌ها را دشوارتر می‌کند. در این مطالعه سی و شش ماهی قزل آلای رنگین کمان را از خرده فروشی‌های مختلف شهر کرمان واقع در جنوب شرق ایران به صورت تصادفی جمع‌آوری کردیم. از هر ماهی با استفاده از سواب نمونه‌های پوستی تهیه شد و با استفاده از روش‌های کشت میکروبی، انتشار دیسک و PCR، جدایه‌های *اشریشیاکلی* (*E. coli*) از نظر فنوتیپ‌های مقاومت ضد میکروبی (AMR) و همچنین ژن‌های مرتبط مورد شناسایی و غربالگری قرار گرفتند. یافته‌های ما نشان داد که شیوع مقاومت فنوتیپی در برابر آنتی‌بیوتیک‌های مورد آزمایش بالا بود شامل اریترومایسین (۸۸/۵۷ درصد)، فلورفنیکول (۷۷/۱۴ درصد)، اکسی‌تتراسایکلین (۷۴/۲۸ درصد)، تری‌متوپریم–سولفامتوکسازول (۷۱/۴۲ درصد)، تری متوپریم (۶۵/۷۱ درصد)، کلرآمفنیکل (۶۲/۸۵ درصد)، فلومکوین (۶۰ درصد) سیپروفلوکساسین (۵۴/۲۸ درصد) و تتراسایکلین (۵۴/۲۸ درصد) که بالاترین میزان مقاومت را داشتند. علاوه بر این، ۸/۵۷ درصد از جدایه‌های *اشریشیاکلی*، سویه‌های تولید کننده ESBL بودند و ۷۴/۲۸ درصد از جدایه‌ها به چند دارو به طور همزمان مقاوم (MDR) بودند. بالاترین فراوانی ژن‌های مقاومت آنتی‌بیوتیکی برای bla_{TEM} ۵/۷۱ درصد، برای *qnrA* ۱۴/۲۸ درصد، برای *sul1* ۱۷/۱۴ درصد و برای *sul2* ۲۰ درصد بود. *اشریشیاکلی* یک باکتری مزوفیل است و به طور طبیعی در ماهی وجود ندارد. ماهی‌ها عمدتاً دارای باکتری‌های سرما دوست در میکرو فلور خود هستند. منشأ *اشریشیاکلی* روی پوست ماهی، آب آلوده به مدفوع انسان و حیوان است، بنابراین مقاومت آنتی‌بیوتیکی این باکتری با آبزی پروری ارتباط غیر مستقیم دارد. مطالعه ما نشان داد که *اشریشیاکلی* جدا شده از پوست ماهی قزل آلای رنگین کمان دارای سطح بالایی از مقاومت آنتی‌بیوتیکی است که ممکن است خطری برای سلامت عمومی باشد. بنابراین، کنترل استفاده از آنتی‌بیوتیک‌ها در پرورش ماهی برای کاهش فشار انتخاب برای ظهور و گسترش باکتری‌های مقاوم به آنتی‌بیوتیک بسیار مهم است.

کلمات کلیدی: *اشریشیاکلی*، مقاومت آنتی‌بیوتیکی، ماهی، کرمان

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