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RESEARCH ARTICLE

Antibiotic Resistance and its Gene Profile in *Escherichia coli* Isolated from Diseased Farm-Raised Carps in Punjab, Pakistan

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ABSTRACT

Antibiotic resistance has become alarming to public health. The present study aimed to investigate the multi-drug resistance (MDR) of *Escherichia coli* (*E. coli*) in farm-raised fish. A total of 216 diseased fish samples from different fish farms were screened for *E. coli*. Molecular confirmation was done with Polymerase chain reaction (PCR) using *usp*A gene. All the positive *E. coli* isolates were subjected to eight different antibiotics. Antimicrobial sensitivity was performed via the Kirby Bauer disk diffusion method. Antibiotic resistance genes; bla_{SHV} bla_{TEM}, bla_{CTX-M}, *tet(A)*, *tet(B)*, *erm (A)*, *erm (B)*, *erm (C)*, *vga (A)*, *str(A)*, *str(B)*, *aadA1* and *aac (3)-I* were examined. A total of 79 (36.57%) *E. coli* was confirmed through PCR. Antibacterial susceptibility test showed 86.1% of isolates were highly resistant towards oxytetracycline and 22.7% of isolates were sensitive against azithromycin, respectively. Results for all antimicrobial agents were highly significant $p<0.01$, except streptomycin. Phenotypic multi-drug resistant (MDR) was documented against 8 different antibiotics. About 7.59% of isolates stood resistant against eight different antibiotics. The higher prevalence for bla_{CTX-M} was 86.07%, *tetA* 91.13%, and *ermB* 69.62% while for *strA* 75.94% was recorded. While bla_{SHV}, *ermA*, and *vga(A)* genes were not detected. All isolates were recorded for genotypic MDR. About 10.12% of isolates were sustained for ten different antibiotic resistance genes. The results for antibiotic susceptibility and resistance genes pattern in the present study have provided a valuable check and balance in term of unhygienic practices and the misuse of antimicrobial agents.

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INTRODUCTION

Fish is an important component of the human diet and has proven beneficial for health. The consumption rate has increased globally, almost doubling during the last four decades (Caires *et al*., 2017). Despite the nutritional benefits, it causes illness in humans due to unhygienic runoffs from animals and anthropogenic sources, which contaminate fish and fishery products with different microorganisms (Herrera *et al*., 2006). Fish are considered the reservoirs for zoonotic microorganisms, which causes infection via food or direct contact (Onmaz *et al*., 2015). To improve the survival rate of fish enormous strategies are commonly employed such as water quality management, alteration in stocking density, formulating good quality feed, and use of antimicrobial agents with no side effects to avoid pathogens (Watts *et al*., 2017).

Underdeveloped and developing countries where there is a lack of regulatory legislation, mostly use different supplements and antimicrobial agents as primary line treatment to control the disaster of pathogenic bacteria in fish farming and fishery products and play an indispensable function in contemporary medicine (Davies *et al*., 2013).

World Organization for Animal Health (OIE) has documented that 160 countries are still using

antimicrobial agents for better growth in animals (OIE, 2021). Common antimicrobial agents: macrolides, tetracyclines, penicillins, quinolones, aminoglycosides and sulphonamides are used in aquaculture (WHO, 2017). Aquaculture systems have been determined as genetic reactors for antimicrobial resistance genes (AMR) (Obimakinde *et al*., 2017).

E. coli is synergetic, commonly found in healthy humans and animal's guts, on the other hand, it is considered an opportunistic pathogen and determined water pollutant, and disease causative agent both in humans and animals (Jang *et al*., 2017).

Assortment of sulphonamide, tetracycline, quinolone, aminoglycoside, and β-lactamase antibiotic resistance genes have been identified in cage culture sediments (Ture *et al*., 2018). *E. coli* from fish farms and river sediments in Zhanjiang, China, was carried resistance both phenotypic and genotypic against different antibiotics; β-lactams, aminoglycosides, macrolide, and tetracycline (Liao *et al*., 2021).

Based on previous studies, the bacteria circulating in fish farms, aquaculture systems and aquatic environments have shown resistance to an extensive range of antimicrobial agents. Therefore, we hypothesized that *E. coli*, isolated from farmed fish are resistant to various antibiotics. This study aims to illustrate the antimicrobial resistance of *E. coli*, in farm raised carp in Punjab, Pakistan.

MATERIALS AND METHODS

Experimental species, collection and transportation of fish samples: From August 2021 to January 2022, a total of 216 fish samples were collected from 12 fish farms (2 fish farm of each district) of different districts of Punjab *viz*; Muzaffargarh, Gujranwala, Bahawalnagar, Multan, Toba Tek Singh, and Kasur. Approximately 36 samples of carps; *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix* and *Cyprinus carpio* were screened for *E. coli*. Sample size was decided on the basis of number of individuals harvested at the time of sampling and samples were collected at ratio of 20:1. Fish organs *i.e*. gut, gills, kidney, fins, and skin were used for *E. coli* isolation. All organs were kept in an individual falcon tube (50ml) that contained 40ml of transport media (buffered peptone water) and transported in the icebox to the Department of Fisheries and Aquaculture, the University of Veterinary and Animal Sciences Lahore, Ravi campus Pattoki for further processing. The samples were incubated at 41.5°C for 6h to increase the recovery rate of stressed cells (ISO, 2017).

Isolation and biochemical characterization of *E. coli:* The organs gut, gills, kidneys, fins and skin were homogenized using a homogenizer (SCILOGEX D500, USA). From each homogenized tissue approximately, 100µl of the sample were cultured on MacConkey, and Methylene Blue (EMB) (Oxoid, UK), which were incubated at 37°C for 24 h. Colonies were recognized by their color, and shape and further identified through the biochemical test (catalase, oxidase, indole and urease test) and on a molecular basis following the set procedures (Fattahi *et al*., 2013; ISO, 2017).

DNA extraction and molecular confirmation of *E. coli*: Genomic DNA of a total of 79 *E. coli* isolated from farmed fish was extracted by boiling method (Monstein *et al*., 2007). Species-specific *Usp*A gene in Table 1 was used for molecular confirmation of *E. coli* isolates by *E. coli* (ATCC 25922) as a positive control. Amplification was performed in a thermal cycler (Bio-Rad, USA, Model 1861096) with 25 µl reaction volume. The PCR amplification conditions as demonstrated in Table 1.

Antibiotic susceptibility: An antimicrobial susceptibility test was performed through the disk diffusion method. All isolates were tested against commonly used antimicrobials agent; ceftriaxone (CRO) 30µg, amoxicillin (AML) 25µg, azithromycin (AZM) 15µg, erythromycin (E) 15µg, oxytetracycline (OTC) 30µg, doxycycline (DO) 30µg, gentamicin (CN) 10µg and streptomycin (S) 10µg. The disc diffusion method consists of Inoculum preparation (0.5 Mc-Farland Standard), preparation of bacteria lawn on Muller Hinton (MHA) agar (Oxoid, UK). Diverse antibiotic discs were positioned on Muller Hinton (MH) agar plate and incubated for 24 hours at 37°C. The inhibition zone was calculated according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2020). Furthermore, multidrug resistance (MDR) was measured as resistance to eight antimicrobial agents belonging to four different classes (two antibiotics from each class) (Baran *et al*., 2020).

Molecular detection of antibiotic resistance genes: All the *E. coli* isolates were subjected to bla_{SHV}, bla_{TEM}, and bla_{CTX-M}, *tet(A), tet(B), erm (A), erm (B), erm (C), vga (A), str(A), str(B), aadA1* and *aac (3)-I* as shown Table 1, 25µl reaction volume was prepared; the amplification program was carried out in a thermal cycler (Bio-Rad, USA, Model 1861096). The PCR amplification conditions are in Table 1. (Ng *et al*., 2001; Monstein *et al*., 2007; Van *et al*., 2008; Piotrowska *et al*., 2017).

Gel Electrophoresis: A total of 5µl of the PCR product was assayed through 1% agarose stained with 7µl ethidium bromide and visualized through a Gel Documentation System (Bio-Rad, USA).

Sequences and phylogenetic analysis: Sequencing of detected genes *Usp*A, bla_{TEM}, bla_{CTX-M}, *tet(A), tet(B), erm (B), erm (C), str(A), str(B), aadA1* and *aac (3)-I* was carried out through Sanger sequencing (BGI Hong Kong Co. Ltd., China). For accession numbers sequenced data were submitted to NCBI GenBank, the sequences were aligned with the sequences from NCBI using Bio-Edit and ClustalW, and the Neighbor-Joining tree of *Usp*A gene was constructed using MEGA 11 BLAST software (Tamura *et al*., 2013; Rajput *et al*., 2014).

Statistical analysis: Descriptive statistics (Frequency Tables) was used to interpret the percentage of the antibiotic resistance and resistance genes; further more chi-square test were performed to compare the antibiotic resistance at *p*-value (< 0.05) using IBM SPSS Statistic version 25 software (Kibret and Abera, 2011).

Table 1: Primers for molecular detection of *E. coli* and antibiotic resistance genes

Genes	Sequence	Amplicon (bp)	Annealing	Time	Cycles	Reference
*Usp*A	F: CCGATACGCTGCCAATCAGT R: ACGCAGACCGTAGGCCAGAT	884	58°C	1mins	30	Rajput *et al.* (2014)
*bla*SHV	F : ATGCGTTATATTCGCCTGTG R: TGCTTTGTTATTCGGGCCAA	747	58°C	30s	30	Monstein *et al.* (2007)
CTX-M	F : ATGTGCAGYACCAGTAARGTKATGGC R:TGGGTRAARTARGTSACCAGAAYCAGCGG	593	52°C	30s	35	
TEM	F: TCGCCGCATACACTATTCTCAGAATGA R: ACGCTCACCGGCTCCAGATTTAT	445	52°C	30s	35	
tet(A)	F: GCTACATCCTGC TTGCCTTC R: CATAGATCGCCGTGAAGAGG	210	54°C	1mins	35	Ng *et al.* (2001)
tet(B)	F: TTGGTTAGGGGCAAGTTTTG R: GTAATGGGCCAATAACACCG	659	52°C	1mins	35	
erm(A)	F: GAAAAACCCTAAAGACACGCAAAA R: AGTGACATTTGCATGCTTCAAAG	658	50°C	30s	35	Piotrowska *et al.* (2017)
erm(B)	F: AAAAATATAAAATATTCTCA R: TAGACAATACTTGCTCATAAGTAAC	694	45°C	30s	35	
erm(C)	F: TATTAAATAATTTATAGCTATTGAAAA R: TGAACATGATAATATCTTTGAAAT	644	45°C	30s	35	
vga(A)	F: GTAGGCCGTAATGGAGCTGG R: CGTCTACTCTTAGCCATGCC	841	52°C	30s	35	
str(A)	F: GAGAGCGTGACCGCCTCATT R: TCTGCTTCATCTGGCGCTGC	862	49°C	90s	35	
str(B)	F: GCTCGGTCGTGAGAACAATC R: AGAATGCGTCCGCCATCTGT	859	45°C	90s	35	
aadA1	F: TATCCAGCTAAGCGCGAACT R: ATTTGCCGACTACCTTGGTC	284	52°C	90s	35	Van *et al.* (2008)
aac(3)-I	F: TTACGCAGCAGCAACGATGT R: GTTGGCCTCATGCTTGAGGA	402	54°C	90s	35	Piotrowska *et al.* (2017)

RESULTS

Biochemical and molecular confirmation and phylogenetic analysis of *E. coli:* A total of 216 farmed fish samples were collected to carry out the biochemical and molecular characterization of *E. coli* and its antibiotic resistance profile. About 100/216 (46.29%) *E. coli* isolates were confirmed via biochemical tests. PCR was performed for *Usp*A gene with a positive control group and a total of 79/216 (36.57%) isolates were observed positive for *Usp*A gene. PCR products of *Usp*A gene in *E. coli* were sequenced and nucleotide sequence was BLAST and aligned with NCBI GenBank data to get the accession number *Usp*A (ON745313) and compared the nucleotide similarity potential of *E. coli* strains with other exiting in the database, which showed 100% of similarity. Further, the sequenced data were analyzed with phylogeny (Neighbor-Joining tree) characterization as depicted in Fig. 1.

Antimicrobial susceptibility: A total of 79 *E. coli* isolates confirmed via PCR were examined against ceftriaxone, amoxicillin, azithromycin, erythromycin, oxytetracycline, doxycycline, gentamicin, and streptomycin. Isolates from farmed fish in Fig. 2 showed a higher resistance towards oxytetracycline 86.1%, followed by 81.0% amoxicillin, 77.2% to ceftriaxone and doxycycline, on other hand sensitive against erythromycin and azithromycin has been recorded 18.9% and 22.7%, respectively. Cross-tabulation of different antimicrobial agents used against the isolates showed highly significant ($p<0.01$) and only streptomycin were non- significant ($p>0.05$) in Table 2.

Detection of antibiotic resistance genes: All isolates were screened for ESBLs genes *bla*SHV, *CTX-M*, and *TEM*. Amplification of *CTX-M* and *TEM* gene in Fig. 3 were documented as 86.07% and 78.48%, while 0% *bla*SHV gene was observed for all isolates. Further, the isolates were screened for tetracycline efflux (*tetA* and *tetB*) genes.

Table 2: Cross-tabulation of Antibiotics against *E. coli* isolated from farm raised fish

Antibiotics	Code/Concentration	Resistant isolates%	P-value
Ceftriaxone	CRO (30µg)	61(77.2)	P<0.01
Amoxicillin	AML (25µg)	64(81.0)	P<0.01
Azithromycin	AZM (15µg)	52(65.8)	P<0.01
Erythromycin	E (15µg)	47(59.5)	P<0.01
Oxytetracycline	OTC (30µg)	68(86.1)	P<0.01
Doxycycline	DO (30µg)	61(77.2)	P<0.05
Gentamicin	CN (10µg)	57(72.2)	P<0.05
Streptomycin	S (10µg)	54(68.4)	P=0.009

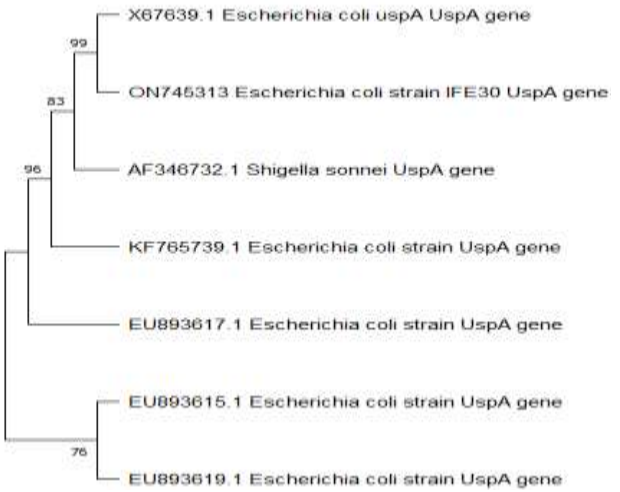

Fig. 1: The Neighbor-Joining tree for *UspA* gene. Evolutionary analyses were conducted in MEGA 11.

The higher frequency of *tetA* and *tetB* in Fig. 3 has examined, which was 91.13% and 82.27%, respectively. The detection and frequency rate of the erythromycin resistance genes were also recorded. The isolates carried a higher rate for *ermB* 69.62% and *ermC* 64.55% depicted in Fig. 3, on the other hand, 0% detection of *ermA* and *vga(A)* genes were recorded. The prevalence of the aminoglycoside resistance genes was confirmed in *E. coli*

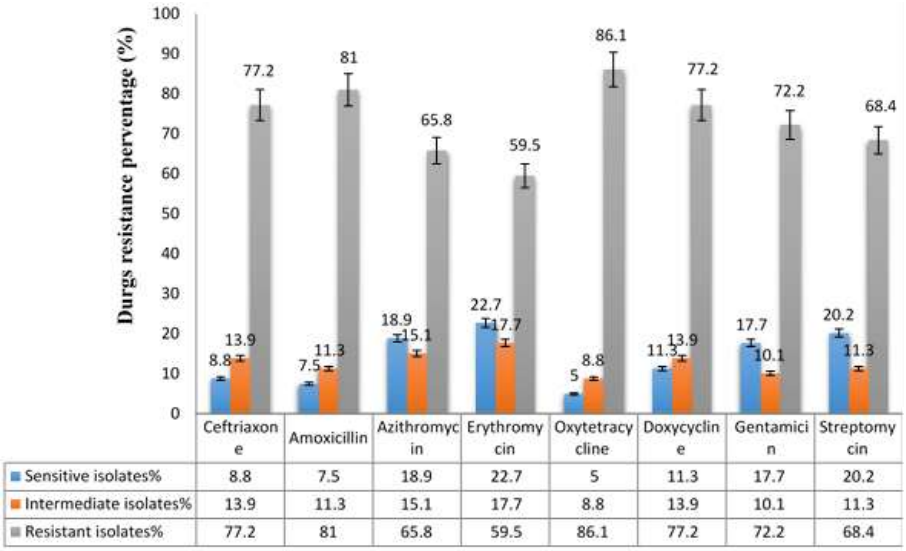

Fig. 2: Results of antibiotic susceptibility test for 8 different antibiotics for *E. coli* from diseased farm raised fish.

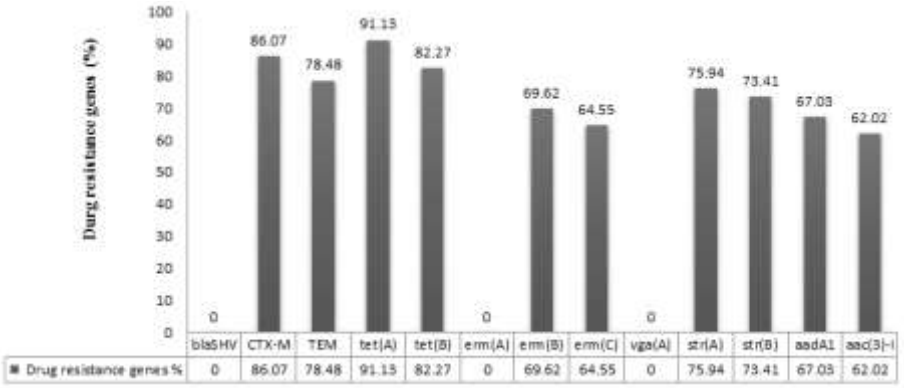

Fig. 3: Results of antibiotic resistance genes for *E. coli* from diseased farm raised fish.

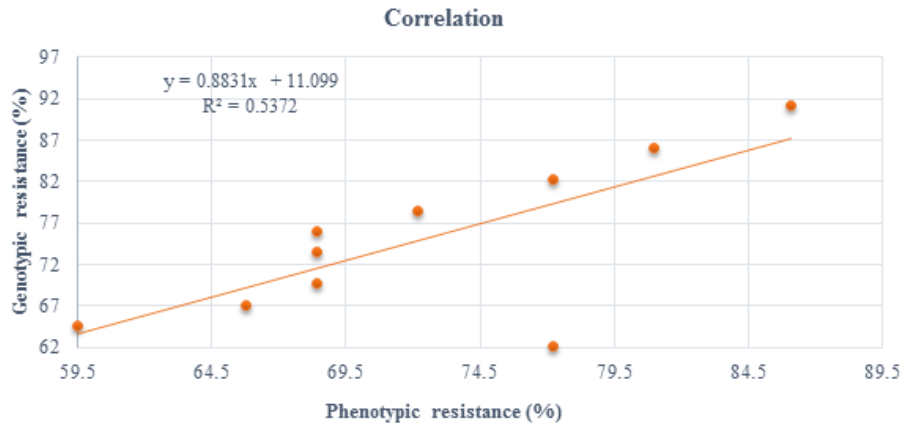

Fig. 4: Correlation between phenotypic and genotypic antibiotic resistance among E. coli isolates from diseased fish.

isolates in Fig. 3. The determinants of the streptomycin resistance genes were predominantly detected. Among 79 *E. coli* isolates carried *strA* 75.94%, *strB* 73.41%, *aadA1* 67.03%, and *aac(3)-I* 62.02%. Further, the highly positive correlation was observed in phenotypic and genotypic resistance presented in Fig 4.

Multiple drug resistance (MDR) and resistance genes prototypes in *E. coli*: About 1.26% of isolates were resistant to a single antibiotic, and 11.39% of isolates were tested resistant to two different antimicrobial agents. About 5.06% of isolates were resistant to three different antibacterial agents and 12.65% of isolates were recorded as resistant to the four diverse antibiotics. 17.78% of isolates have shown resistance to five different antibiotics and 24.05% of isolates demonstrated resistance against six different antibacterial agents. On the other hand, 20.25% of isolates showed resistance to seven antibiotics and 7.59% of isolates carried resistance against eight different antibiotics. A total of 79 isolates showed 34 diverse MDR patterns in Table 3.

Further, Extended-spectrum beta-lactamases, tetracycline, erythromycin, and aminoglycoside resistance genes were screened in all isolates. The four different antibiotic resistance genes carried by 2.53% of isolates and 13.92% of bacterial strains conceded five different antibiotic resistance genes. Six different genes were seen in 11.39% of isolates and 13.92% of isolates had held seven different resistance genes. About 29.11% of isolates carried the higher rates of eight different antibiotic resistance genes.

Table 3: Phenotypic MDR profile for *E. coli* isolated from farm raised fish.

Antibiotics Combinations	Number of Isolates (n=79)	Number of Resistant Antibiotics
OTC	1	1
AZM, DO	5	2
AZM, OTC	1	2
CRO, OTC	2	2
E, OTC	1	2
AZM, E, OTC	1	3
CRO, OTC, DO	1	3
CRO, AML, OTC	1	3
E, OTC, DO	1	3
AZM, OTC, DO, S	1	4
CRO, AML, OTC, S	1	4
E, DO, CN, S	6	4
AML, E, OTC, S	2	4
CRO, AML, OTC, CN, S	3	5
AZM, E, DO, CN, S	1	5
AZM, E, DO, CN, S	1	5
AML, E, DO, CN, S	2	5
CRO, AML, AZM, OTC, CN	3	5
AML, E, OTC, DO, CN	2	5
CRO, AML, E, DO, CN	1	5
CRO, AML, E, OTC, S	1	5
AML, AZM, E, DO, CN, S	1	6
CRO, AML, E, DO, CN, S	1	6
CRO, AML, AZM, OTC, CN, S	3	6
CRO, AML, AZM, OTC, DO, CN	1	6
CRO, AML, AZM, E, OTC, CN	1	6
CRO, AML, E, OTC, CN, S	1	6
CRO, AML, E, OTC, DO, CN	5	6
CRO, AML, E, OTC, DO, S	1	6
CRO, AML, AZM, OTC, DO, S	5	6
CRO, AML, E, OTC, DO, CN,S	1	7
CRO, AML, AZM, OTC, DO, CN, S	10	7
CRO, AML, AZM, E, OTC, CN, S	2	7
CRO, AML, AZM, E, OTC, DO, S	3	7
CRO, AML, AZM, E, OTC, DO, CN, S	6	8
Total	79	167

Nine different resistance genes stood by 18.98% of isolates and 10.12% of isolates have sustained ten different antibiotic resistance genes in Table 4.

Sequences analysis and accession numbers: Resistant isolates were sequenced to study the partial nucleotide sequences of *CTX-M*, *TEM*, *tetA*, *tetB*, *ermB*, *ermC*, *strA* *strB*, *aadA1*, and *aac(3)-I* genes, the sequenced genes were aligned with published sequences in NCBI database. Sequences of the present study showed 99-100% similarity with published data. The partial coding sequence (CDS) of sequenced genes was submitted to GenBank, NCBI with the accession numbers *CTX-M* (OP156931), *TEM* (OP227115), *tet*A (OP227133), *tet*B (OP227118), *erm*B (OP227121), *erm*C (OP227124), *str*A (OP227127), *str*B (OP227130), *aac(3)-I* (ON968444), and *aadA1* (OP184806).

DISCUSSION

The present study results demonstrated in form figures were compared to the local data published by Shah *et al*. (2012) and Ahmad *et al*. (2022) who worked on isolated bacteria from farm water, sediments, diseased and market fish. In their published data they reported the resistances profile of tetracycline 43.3%, streptomycin 36.2%, amoxicillin 71.6%, erythromycin 75.6%, and ESBLs 16% against the isolated bacteria these results were almost in line for the confirmation of the present study.

Table 4: Resistance genes profile for *E. coli* isolated from farm raised fish.

Resistance genes	No. of isolates (n=79)	No. of Resistance Genes
tetB, ermB, StrA, aadA1	1	4
TEM, tetA, StrB, aadA1	1	4
CTX-M, TEM, tetA, StrA, aadA1	2	5
TEM, tetB, ermB, StrA, aac(3)-I	1	5
TEM, tetB, ermB, StrA, aadA1	1	5
CTX-M, tetB, ermB, StrA, aac(3)-I	1	5
tetA, tetB, ermB, StrA, aac(3)-I	1	5
CTX-M, TEM, ermC, StrB, aac(3)-I	1	5
CTX-M, TEM, tetA, ermC, StrB	1	5
CTX-M, TEM, tetA, ermB, StrB	1	5
CTX-M, tetA, ermB, StrB, aac(3)-I	1	5
tetA, tetB, ermB, StrA, aadA1	1	5
TEM, tetB, ermB, StrA, StrB, aac(3)-I	1	6
CTX-M, TEM, tetA, ermB, StrB, aac(3)-I	3	6
CTX-M, TEM, tetA, tetB, ermB, aadA1	1	6
CTX-M, tetA, tetB, ermB, StrB, aadA1	1	6
CTX-M, tetA, tetB, ermB, StrA, aadA1	3	6
CTX-M, tetA, tetB, ermB, StrA, StrB, aadA1	2	7
CTX-M, TEM, tetA, tetB, ermB, StrA, StrB	1	7
CTX-M, tetA, tetB, ermB, ermC, StrA, aadA1	1	7
CTX-M, TEM, tetA, ermB, ermC, StrB, aac(3)-I	2	7
TEM, tetA, tetB, ermB, StrA, StrB, aadA1	1	7
TEM, tetA, tetB, ermC, StrA, StrB, aadA1	2	7
CTX-M, TEM, tetA, tetB, ermB, ermC, aac(3)-I	1	7
CTX-M, tetA, tetB, ermB, StrA, aadA1, aac(3)-I	1	7
CTX-M, TEM, tetA, tetB, ermB, StrA, StrB, aac(3)-I	2	8
CTX-M, TEM, tetA, tetB, ermB, ermC, StrA, aadA1	1	8
CTX-M, TEM, tetA, ermB, ermC, StrA, StrB, aac(3)-I	2	8
CTX-M, TEM, tetA, tetB, ermC, StrA, StrB, aadA1	6	8
CTX-M, TEM, tetB, ermB, ermC, StrA, StrB, aadA1	1	8
CTX-M, TEM, tetA, tetB, ermB, ermC, StrB, aac(3)-I	4	8
CTX-M, TEM, tetA, tetB, ermB, ermC, aadA1, aac(3)-I	1	8
CTX-M, tetA, tetB, ermB, ermC, StrA, aadA1, aac(3)-I	2	8
CTX-M, TEM, tetA, tetB, ermC, StrA, StrB, aac(3)-I	1	8
CTX-M, tetA, tetB, ermB, ermC, StrA, StrB, aac(3)-I	1	8
CTX-M, TEM, tetA, tetB, ermB, StrA, aadA1, aac(3)-I	1	8
CTX-M, TEM, tetA, tetB, ermC, StrB, aadA1, aac(3)-I	1	8
CTX-M, TEM, tetA, tetB, ermC, StrA, StrB, aadA1, aac(3)-I	9	9
CTX-M, TEM, tetA, tetB, ermB, ermC, StrA, StrB, aadA1	2	9
CTX-M, tetA, tetB, ermB, ermC, StrA, StrB, aadA1, aac(3)-I	1	9
TEM, tetA, tetB, ermB, ermC, StrA, StrB, aadA1, aac(3)-I	1	9
CTX-M, TEM, tetA, tetB, ermB, ermC, StrA, StrB, aac(3)-I	1	9
CTX-M, TEM, tetA, tetB, ermB, ermC, StrA, aadA1, aac(3)-I	1	9
CTX-M, TEM, tetA, tetB, ermB, ermC, StrA, StrB, aadA1, aac(3)-I	8	10
Total	79	304

In contrast, a study from Uganda, documented *E. coli* isolated from the gut of *Oreochromis niloticus* carried a higher resistance against ampicillin 72.7%, tetracycline 81.8%, and erythromycin 72.7% (Kikomeko, 2016). In Egypt, *E. coli* strains from deployed workers and healthy *O. niloticus*, reported higher sensitivity to erythromycin (AbdEl-Tawab *et al*., 2018). The results of the other published data for the antibiotic resistance pattern in *E. coli* isolated from cultured fish species; the documented results of the experiment showed the resistance rates as follows: gentamicin 88.3%, tetracycline 88.1%, and streptomycin 88.1% (Hon *et al*., 2016).

Furthermore, in the present study, we examined the $bla_{\text{CTX-M}}$, bla_{TEM}, *tetA*, *tetB*, *ermB* and *ermC*, *strA*, *strB*, *aadA1*, and *aac(3)-I* resistance genes in *E. coli* isolated farms fish. Results regarding these findings of the present study were compared with the previous study had a close

resemblance with those, postulated in studies conducted in Pakistan and Ireland, which was reported as *CTX-M* 40% and 94.25%, respectively in *E. coli* from market fish and aquatic environment (Hooban *et al*., 2021; Ahmad *et al*., 2022). The recent study by Liao *et al*. (2021) evaluated three different aquaculture farms in China to determine the positive *E. coli* isolates; the analysis has reported $^{bla}CTX{-}M$ 80% and ^{bla}TEM 60% isolated from the farm I water and sediments, in farm II $^{bla}CTX{-}M$ 70%, and ^{bla}TEM 60%, farm III $^{bla}CTX{-}M$ 80%, and ^{bla}TEM 80%, were reported in water isolates. In Egypt an experiment has been conducted by Hamza *et al*. (2020) documented $^{bla}CTX\text{-}M\text{-}15$ and ^{bla}TEM in *E. coli* from integrated fish farms. The cited studies concluded that *E. coli* isolates from different sources (farm, fish, and fishery products) carried the ESBL gene, to these findings justified the results of present study.

In the present study, further investigation regarding tetracycline, erythromycin and streptomycin resistance genes of the present study was compared with published data of the Shah *et al*. (2012) in which they reported the presence of *tetA* and *tetA(G)* in isolates from aquaculture farms. The same study in Sri Lanka reported the tetracycline resistance genes in an aquaculture environment; they postulated a higher frequency of *tetA* and *tetB* (Liyanage and Manage, 2019). The resulted documented for bacteria isolated from aquaculture sources in Australia reported 75% *tet* genes (Akinbowale *et al*., 2007). Resistance genes ^{bla}TEM 47.66%, *tetA* 45.33%, and 29.91% *strA* has been reported in *E. coli* from Laguna lake water and fish (Salvador-Membreve and Rivera, 2021), and Silva *et al*. (2019) detected *aadA* in *E. coli* isolates from fish. According to Singh *et al*. (2021) isolates from water carried 12% *strA* and *strB*. Erythromycin *aac(3)-II* and *ermB* has been documented by Liao *et al*. (2021) in *E. coli* isolated from an aquaculture farm in China.

All these reported studies supported the results of the present study. However, the cited reports realized the prevalence of antibiotic resistance and AMR genes in *E. coli* isolated from aquaculture farms and different water bodies. The higher and lower degrees of various AMR resistance genes might be due to unhygienic water sources, misuse of antibiotics, anthropogenic activities, and poor processing systems and feed which has been confirmed by all the cited results in line with the present study.

Conclusions: In conclusion, a high rate of antibiotic resistance and resistance genes in *E. coli* from farm raised fish in selected districts of Punjab indicates the unhygienic environment and misuse of antibiotics in the aquaculture sector. Therefore, it is recommended that to improve the present alarming situation of the aquaculture of Pakistan, which is in its infancy legislators should be realized to deceive some policies according to HACCP set standards. Moreover, the utilization of fertilizer, manure (excretes of animals), supplements, and other materials used at fish farms should pass through prescribed safety measures for hygienic production and healthy utilization.

Declaration of competing interest: The authors declare that they have no known financial interests or personal relationship that could have appeared to influence the work reported in this paper.

Authors contribution: MU, FR, NK, SA, and AAS conceptualized and designed the study. FR, SA and AAS assisted in conducting the experiments and analyzed the data. MU performed the experiments and interpreted the results. MU, FR, and SA wrote the manuscript. NK edited the figures, tables and references in the manuscript.

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