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Prevalence, Virulence Gene Profiles, and Multidrug Resistance of Shiga Toxin-Producing Escherichia coli in Farmed Carp (Labeo rohita) from Punjab, Pakistan: Implications for Food Safety and One Health

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Prevalence, Virulence Gene Profiles, and Multidrug Resistance of Shiga Toxin-Producing *Escherichia coli* in Farmed Carp (*Labeo rohita*) from Punjab, Pakistan: Implications for Food Safety and One Health

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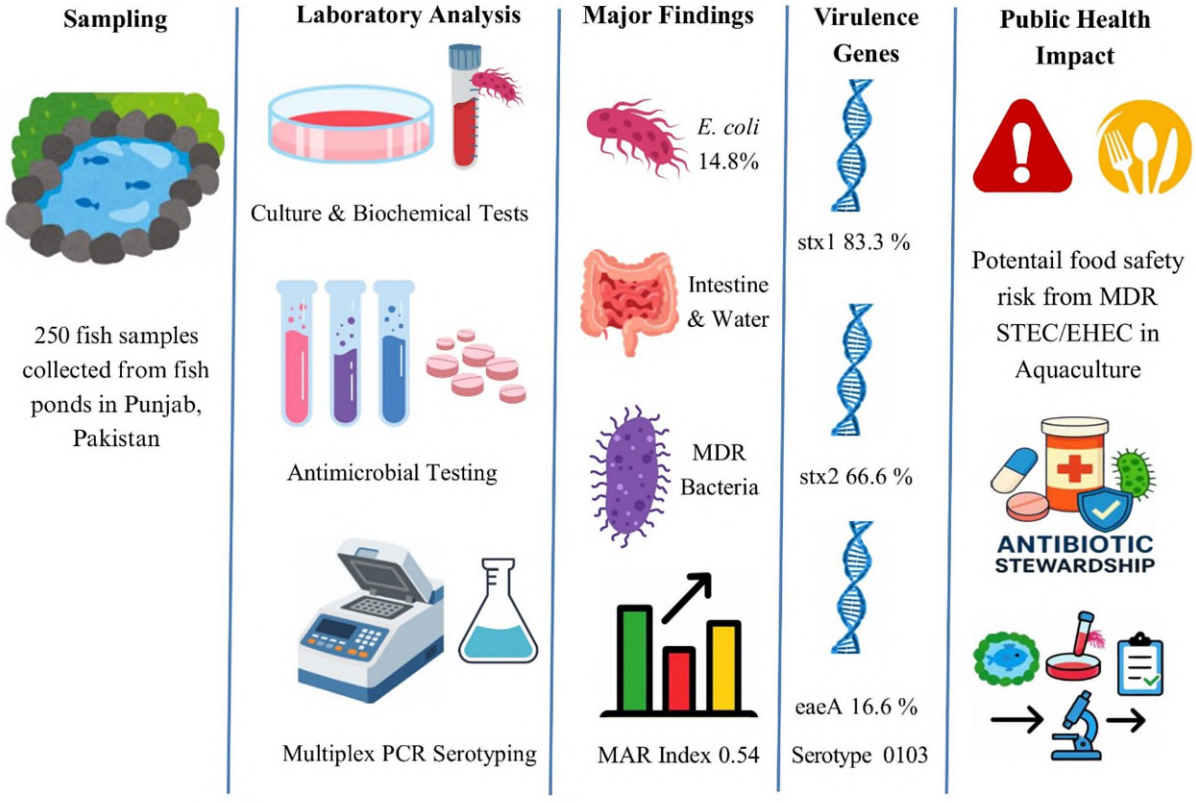
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GRAPHICAL ABSTRACT



ABSTRACT

Pathogenic multidrug-resistant (MDR) *Escherichia coli* (*E. coli*) may be harbored in farm-raised carp, and can be a serious but not fully recognised public health problem in Pakistan. The aim of this study was to determine the prevalence, antimicrobial resistance, virulence genes and serotypes of *E. coli* from the farm-raised carp of Punjab, Pakistan. Three fish ponds were sampled and a total of 250 samples (gills, muscle, body surface, intestine, and water). Culture, biochemical tests, serology, antimicrobial susceptibility testing, and multiplex PCR of stx1, stx2, and eaeA genes were used to identify isolates. The presence of *E. coli* in samples was observed in 14.8% of the samples with the highest prevalence in intestine (18.0%) and water (18.0%). The universal resistance to cephalexin and high resistance to rifampin and penicillin G were seen with a mean MAR index of 0.54. Tobramycin and amikacin were still totally effective. The isolates contained virulence genes stx1 (83.3%), stx2 (66.6%) and eaeA (16.6%). All three virulence genes were found in serotype O103. Thus, it is clear from these findings that antimicrobial stewardship and regular STEC monitoring is a dire need in the aquaculture industry of Pakistan.

Keywords: Shiga toxin-producing *Escherichia coli* (STEC); virulence genes; antimicrobial resistance; MAR index; carp aquaculture; Pakistan; One Health; food safety

1. INTRODUCTION

Aquaculture has become the fastest-growing food producing sector worldwide and major contributor to global aquatic food supply. According to FAO, global aquaculture production reached 130.9 million tonnes in 2022, while cultured aquatic animals contributed 94.4 million tonnes, representing 51% of total aquatic animal production [1]. Globally, per capita consumption of aquatic animals increased from 9.1 kg in 1961 to 20.7 kg in 2022 and 89% of aquatic animal production was directly consumed by human [2]. Aquatic foods are prime source of high-quality proteins, essential fatty acids, vitamins, and minerals contributing 15% of global animal protein intake and 3.2 billion people directly obtained 20% animal-based protein intake from aquatic animals [3, 4]. Future projections further emphasize the growing role of aquaculture, as it is expected to account for more than 85% of additional fisheries and aquaculture output and to supply 61% of fish and other aquatic products available for human consumption by 2034 (OECD-FAO Agricultural Outlook 2024-2033, 2024; FAO 2025).

Carp species of the family *Cyprinidae* including *Labeo rohita* (Rohu), *Catla catla* (Catla), *Cyprinus carpio* (common carp) and *Hypophthalmichthys molitrix* (silver carp) constitute the most extensively farmed fish groups in global aquaculture [7]. FAO-based data shows that carps, barbels and other cyprinids were top cultured fish specie groups in 2022, producing about 32.16 million tonnes while total shared 24.6% in global aquaculture production [1, 8]. FAO SOFIA 2024 reported that carp group contributed about 18% in total aquatic animal

production [9]. In Pakistan, aquaculture is based on freshwater culture and carp group is extensively cultured in Punjab, Sindh and Khyber Pakhtunkhwa. Pakistan has 1,120 km long coastline and 3.1 million hectares inland waters, which is major contributor of South Asian Aquaculture [10, 11]. Pakistan has strong potential for carp aquaculture which contributes in food security, rural livelihoods and freshwater aquaculture development for the sustainable production [12].

Despite this production potential, Pakistani aquaculture faces persistent bacterial disease challenges [13]. *Escherichia coli*, a member of the family *Enterobacteriaceae*, is a versatile commensal of vertebrate gastrointestinal tracts whose pathogenic strains particularly Shiga toxin-producing *E. coli* (STEC) are globally recognised agents of severe food-borne illness [14]. STEC strains carrying *stx1* and/or *stx2* genes encoding Shiga toxins, and the *eaeA* gene encoding the adhesin intimin, are associated with haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) a life-threatening complication characterised by microangiopathic haemolytic anaemia, thrombocytopaenia, and acute renal failure [15, 16]. Globally, STEC O157 and non-O157 serogroups (notably the 'Big Six': O26, O45, O103, O111, O121, O145) account for over 39,787 documented cases across 1,343 outbreaks since 1982, with the EU reporting 8,565 STEC cases in 2022 alone a 25% increase from 2021 [16, 17].

The unsupervised use of broad-spectrum antibiotics in aquaculture both as growth promoters and prophylactics has accelerated the emergence of antimicrobial-resistant (AMR) *E. coli* in farmed fish environments (Mohseni et al. 2025b). Antimicrobial resistance (AMR) bacteria are now well documented throughout Asian aquaculture systems (El Badawy et al., 2025). Meta-analysis and systematic review have revealed the excessive utilization of antibiotics in aquaculture and shows high resistance to penicillins and related β-lactams exceeded 60% in foodborne pathogens from fish [20].

Alarmingly, global antimicrobial consumption in aquaculture is projected to increase by 33% between 2017 and 2030 if current practices continue [21]. Aquaculture environments serve as hotspots for horizontal gene transfer (HGT) of resistance genes via mobile genetic elements (MGEs), enabling dissemination to human-pathogenic bacteria [22, 23]. A 2023 systematic review on ESBL-producing bacteria in retail seafood found a median contamination prevalence of 19.4%, significantly higher in Asian than European products [24].

Despite the recognised significance of carp aquaculture in Pakistan and growing the global concern regarding antimicrobial resistance (AMR), there remains a lack of baseline data on the virulence determinants and antimicrobial resistance profiles of pathogenic *Escherichia coli* isolated from farmed fish in Pakistan. The present study therefore aims to determine *E. coli* prevalence across anatomical compartments of farm-raised carp,

calculate multiple antibiotic resistance (MAR) indices, molecularly detect STEC virulence genes (*stx1*, *stx2*, *eaeA*) by multiplex PCR, and serotype confirmed isolates to inform public health risk.

2. MATERIALS AND METHODS

2.1 Ethical Statement

The present study was conducted in accordance with the guidelines of Society for Prevention of the Cruelty to Animals (SPCA), Pakistan. Ethical approval was granted by the Bioethical Committee of the Faculty of Fisheries and Wildlife, University of Veterinary and Animal Sciences Lahore, Pakistan (Approval No. DR/ 358-5, 12-02-2024). Fish were anesthetized by immersion in buffered tricaine methanesulfonate (MS-222, 0.10 g/L) until Stage III anesthesia was achieved. Following sampling, fish were euthanized by an overdose of MS-222 (300 mg/L) until cessation of opercular movements for ≥10 minutes. Death was confirmed by absence of heartbeat and loss of spinal reflexes, in accordance with AVMA Guidelines for the Euthanasia of Animals (2020).

2.2 Study Area and Sample Collection

The study was conducted across three selected fish-pond sites in Punjab, Pakistan during (**Fig. 1**): Dera Ghazi Khan, Multan and Kasur. These sites represent intensive freshwater carp aquaculture zones producing an estimated combined output of ~5,065 tons annually. Five fish were sampled per site per month over four months using gill nets, giving a total of 60 fish. Fish were handled, anesthetized and euthanized as described in Section 2.1. Samples were transported within 4 hours to the Fisheries & Aquaculture Laboratory, UVAS Ravi Campus Pattoki, in insulated cool boxes on ice. Species identity was confirmed using standard taxonomic keys (Jayaram et al., 2024). Total length (cm) and body weight (g) were recorded for each fish.

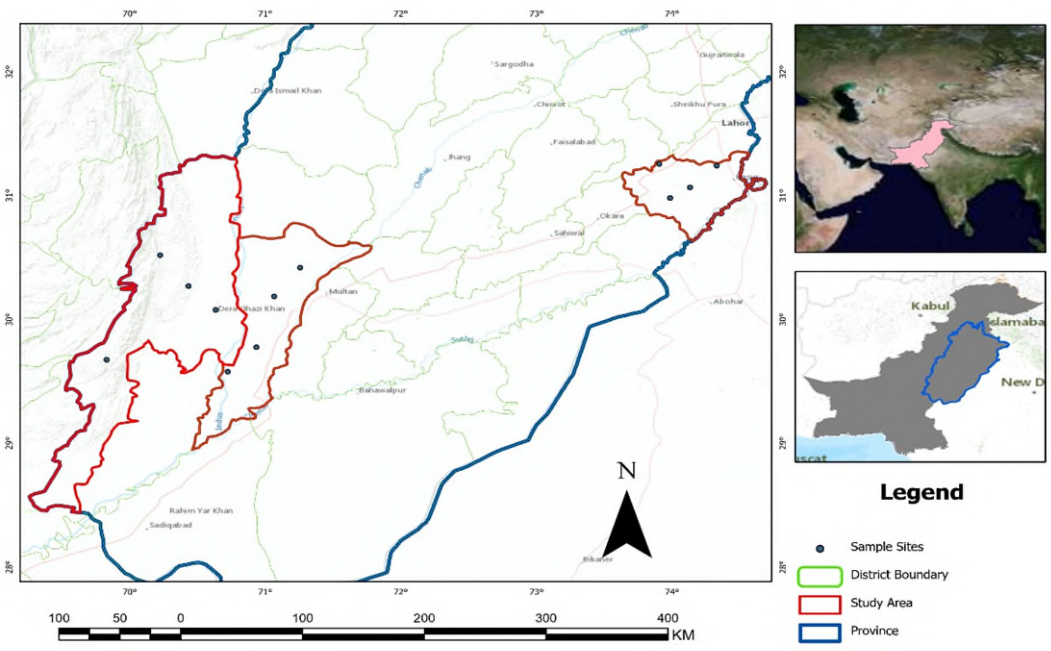


Fig. 1 Geographic distribution of fish-farm sampling sites in Punjab, Pakistan, across Dera Ghazi Khan, Multan, and Kasur. Sampling locations (blue dots) are shown within study areas (red), with district (green) and provincial (blue) boundaries and major rivers for reference; insets indicate the study region within Pakistan.

(A) 

(B) 

Fig. 2 Sample were captured by gill net and transported on ice to the UVAS laboratory. Morphometric measurements recorded.

2.3 Bacteriological Isolation and Biochemical Identification

Swabs were collected aseptically from gills, body surface, muscle, and intestine (following surface disinfection with 70% ethanol and red-hot scalpel). Water samples were collected from each pond. All swabs were inoculated into nutrient broth and incubated at 37°C for 24 h. Subcultures were made onto MacConkey agar and Eosin Methylene Blue (EMB) agar (37°C, 24 h). Colonies with a characteristic metallic green sheen on EMB were subjected to Gram staining and a full IMViC biochemical panel (indole, methyl red, Voges-Proskauer, citrate) for presumptive *E. coli* identification [25]. Triple sugar iron (TSI) agar, urease, and oxidase tests were performed for confirmatory identification.

2.4 Antimicrobial Susceptibility Testing and MAR Index

Antimicrobial susceptibility was determined by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar [26]. Agents tested: Penicillin G (10 µg), Tobramycin (10 µg), Rifampin (5 µg), Ofloxacin (5 µg), Chloramphenicol (30 µg), Cephalexin (30 µg), Cefotaxime (30 µg), Cefoxitin (30 µg), and Cephalothin (30 µg). *E. coli* ATCC 25922 served as quality control. Isolates with intermediate resistance zones were classified as resistant (conservative approach). The Multiple Antibiotic Resistance (MAR) index was calculated for each sample type as: MAR index = a / bc, where 'a' is the cumulative number of resistances across all isolates from that sample type, 'b' is the number of isolates, and 'c' is the number of antibiotics tested. MAR index >0.2 indicates high-risk antibiotic exposure environments [25].

2.5 Serological Identification

Confirmed *E. coli* isolates were serotyped using rapid diagnostic *E. coli* antisera sets targeting somatic O-antigens (DENKA SEIKEN Co., Japan), per manufacturer's instructions [27]. Isolates were screened against O26, O44, O103, O119, O125, O128, O145 and O172 antisera.

2.6 DNA Extraction and Multiplex PCR

DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany) per manufacturer's protocol with an additional heat-lysis step (100°C, 20 min). Multiplex PCR was performed in 25 µL reactions: 10 µL Emerald Amp GT PCR Master Mix (Takara, Japan), 3 µL nuclease-free water, 1 µL each forward and reverse primer (20 pmol each), and 6 µL template DNA. Primer sequences for *stx1* (614 bp), *stx2* (779 bp), and *eaeA* (890 bp) are listed in Table 1. PCR conditions: initial denaturation 94°C 5 min; 35 cycles of 94°C 30 s, 60°C 30 s, 72°C 1 min; final extension 72°C 10 min. Products were resolved on 1.5% agarose gel (100 V, 45 min, ethidium bromide staining) and visualised under UV. Gel images were analysed using AlphaImager software.

Table 1 Oligonucleotide primer sequences used for multiplex PCR detection of *stx1*, *stx2*, and *eaeA* virulence genes in *E. coli* isolates.

Primer	Sequence (5′→3′)	Product (bp)	Reference
stx1 (F)	5′ ACACTGGATGATCTCAGTGG 3′	614	[28]
stx1 (R)	5′ CTGAATCCCCCTCCATTATG 3′		
stx2 (F)	5′ CCATGACAACGGACAGCAGTT 3′	779	[28]
stx2 (R)	5′ CCTGTCAACTGAGCAGCACTTTG 3′		
eaeA (F)	5′ GTGGCGAATACTGGCGAGACT 3′	890	[29]
eaeA (R)	5′ CCCCATTCTTTTTCACCGTCG 3′		

2.7 Statistical Analysis

All data were expressed as frequency counts and percentages. Chi-square (χ^2) tests were used to assess statistical significance of differences in *E. coli* prevalence across sample types; a p-value <0.05 was considered statistically significant. MAR indices were calculated using the Krumperman formula. Analyses were performed using IBM SPSS Statistics v25.0 (IBM Corp., Armonk, NY, USA). Data were entered and managed in Microsoft Excel 2019.

3. RESULTS

3.1 Morphological and Biochemical Characterisation

E. coli colonies on EMB agar appeared as dark-centred colonies with a characteristic metallic green sheen, confirming acid production from lactose with strong gas production. Gram staining revealed Gram-negative, pink-staining, short rod-shaped organisms occurring singly or in pairs. The IMViC profile of all presumptive isolates was: indole-positive, methyl red-positive, Voges-Proskauer-negative, and citrate-negative fully consistent with *E. coli* (++−−). TSI reactions showed an acid butt with gas production and acid slant, urease was negative, and oxidase was negative, all consistent with the *E. coli* phenotype.

3.2 Prevalence of *E. coli* by Sample Type and MAR Index

E. coli was recovered from 37 of 250 samples (14.8% overall prevalence). Intestine and water samples yielded the highest positivity (18.0% each), followed by gills and body surface (16.0% each), while muscle was lowest (6.0%). Chi-square analysis showed statistically significant differences in prevalence across sample types (χ^2 = 9.38, df = 4, p = 0.009). MAR indices for all sample types exceeded 0.2, with intestinal samples yielding the highest MAR index (0.61), confirming all sample categories derived from high-risk antibiotic exposure environments. Full data are presented in Table 2.

Table 2. Prevalence of *Escherichia coli* and MAR index in farm-raised carp fishes from Punjab, Pakistan.

Sample Type	No. Examined	No. Positive	Prevalence (%)	MAR Index*
Gills	50	8	16.0	0.52
Muscle	50	3	6.0	0.43
Body Surface	50	8	16.0	0.55
Intestine	50	9	18.0	0.61
Water	50	9	18.0	0.58
Total / Mean	**250**	**37**	**14.8**	**0.54**

Note: MAR = multiple antibiotic resistance. *MAR index* = *a*/(*bc*), where *a* = total number of antibiotic resistances recorded, *b* = number of isolates tested, and *c* = total number of antibiotics evaluated. A MAR index > 0.2 indicates exposure to a high-risk environment with frequent antibiotic use.

3.3 Antimicrobial Resistance Profile

All 44 *E. coli* isolates were tested against nine antimicrobial agents. Cephalexin showed universal resistance (100%), followed by rifampin (95.4%) and penicillin G (90.9%). Cephalothin, Cefoxitin and Chloramphenicol

showed moderate resistance (86.4%, 70.5% and 29.5% respectively). Ofloxacin showed low resistance (13.6%). Tobramycin and amikacin retained complete susceptibility (0% resistance). The overall MDR rate (resistance to ≥3 antibiotic classes) was 54.0% of all isolates. Data are presented in Table 3 and Figure 4.

Table 3 Antimicrobial resistance profile of *Escherichia coli* isolates (n = 44) recovered from farm-raised *Labeo rohita* in Punjab, Pakistan.

Antimicrobial Agent	Class	R (n/44)	R (%)	Interpretation
Cephalexin	Cephalosporin	44/44	**100.0**	High MDR risk
Rifampin	Rifamycin	42/44	**95.4**	High MDR risk
Penicillin G	β-Lactam (penicillin)	40/44	**90.9**	High MDR risk
Cephalothin	Cephalosporin	38/44	**86.4**	Moderate
Cefoxitin	Cephamycin	31/44	**70.5**	Moderate
Chloramphenicol	Phenicol	13/44	**29.5**	Moderate
Ofloxacin	Fluoroquinolone	6/44	**13.6**	Low
Tobramycin	Aminoglycoside	0/44	**0.0**	Fully susceptible
Amikacin	Aminoglycoside	0/44	**0.0**	Fully susceptible

Note: Red indicates high resistance (≥90% of isolates); yellow indicates moderate resistance (20–89%); and green indicates complete susceptibility (0% resistance). Values are expressed as the percentage of resistant isolates for each antimicrobial agent tested.

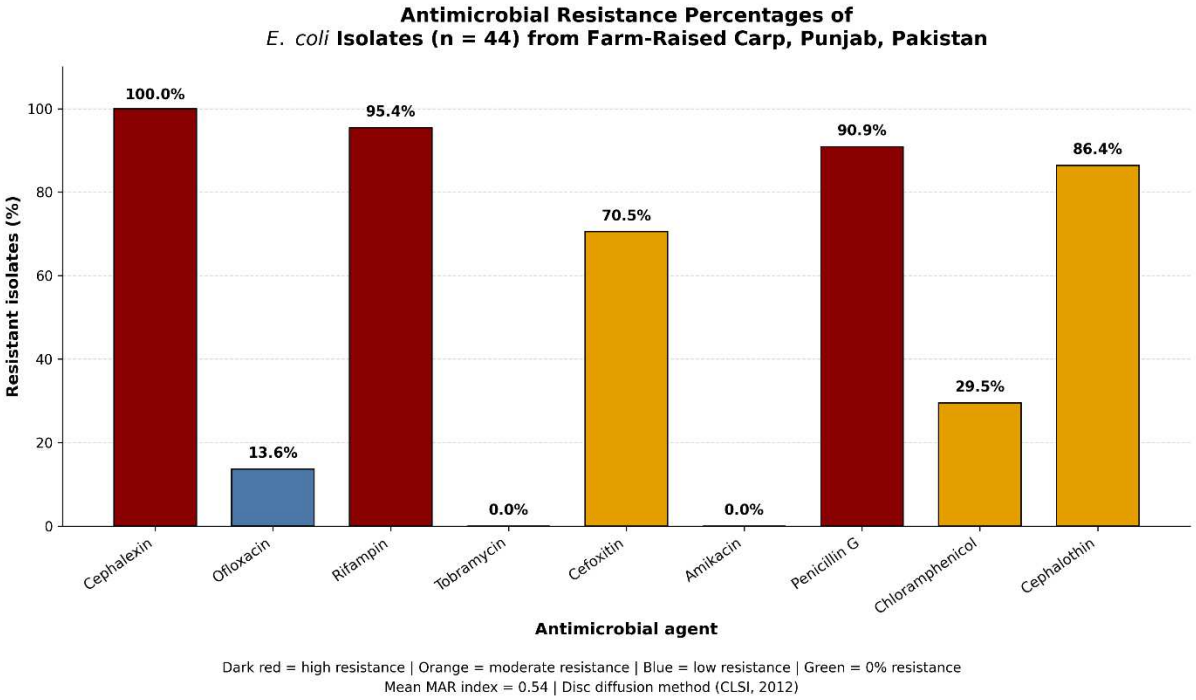


Fig. 3 Antimicrobial resistance profile of *E. coli* isolates (n = 44) from farm-raised carp in Punjab, Pakistan. Dark red, orange, blue and green categories represent high resistance (≥90%), moderate resistance (20–89%), low resistance (<20%), and complete susceptibility (0% resistance), respectively. Tobramycin and amikacin showed no resistant isolates. Mean MAR index = 0.54; antimicrobial susceptibility was determined by the disc diffusion method according to CLSI guidelines.

3.4 Serotyping

Five distinct O-serogroups were identified: O44, O103, O119, O125, and O172. O125 was the most prevalent (20.4%), followed by O119 (18.1%), O103 (16.3%), O172 (14.3%), and O44 (12.2%)

3.5 PCR Detection of Virulence Genes

Multiplex PCR targeting *stx1*, *stx2*, and *eaeA* was performed on all 44 confirmed *E. coli* isolates. *stx1* (614 bp) was detected in 83.3% of isolates (Figure 5); *stx2* (779 bp) in 66.6% (Figure 6); and *eaeA* (890 bp) in 16.6% (Figure 7). Serotype O103 harboured all three virulence genes simultaneously meeting molecular EHEC criteria (Table 4). *stx1* and *stx2* co-occurred in serotypes O119 and O172, while O44 and O125 carried *stx1* only (STEC phenotype). Virulence gene prevalence across serotypes is summarised in Table 4 and Figure 8.

Table 4. Distribution of virulence genes and assigned pathotypes among *E. coli* serogroups isolated from farm-raised carp in Punjab, Pakistan. + = gene detected; − = gene not detected. Serogroup O103 (highlighted) harboured all three genes (EHEC phenotype).

Serogroup	stx1	stx2	eaeA	Pathotype
O44	+	−	−	STEC (stx1 only)
O103	+	+	+	**EHEC (all three genes)**
O119	+	+	−	STEC (stx1 + stx2)
O125	+	−	−	STEC (stx1 only)
O172	+	+	−	STEC (stx1 + stx2)

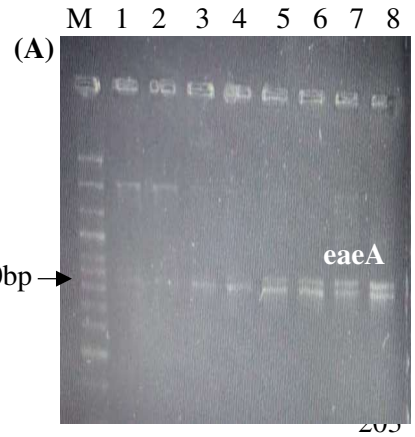


Fig. 4 Agarose gel electrophoresis showing PCR amplification of the eaeA intimin gene (890 bp) from *E. coli* isolates. Lane M: 100 bp DNA ladder; Lanes 1–6: positive isolates (890 bp band); Lanes 7–8: negative controls.

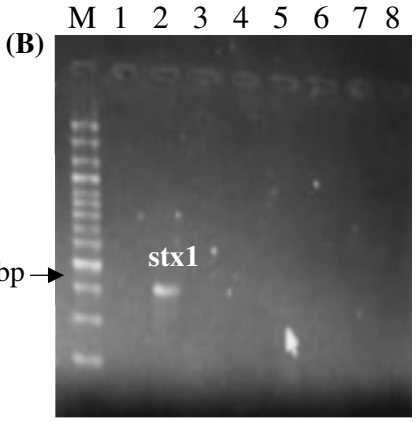


Fig. 5 Agarose gel electrophoresis showing PCR amplification of the stx1 Shiga toxin 1 gene (614 bp) from *E. coli* isolates. Lane M: 100 bp DNA ladder; Lanes 1–7: positive isolates (614 bp band); Lane 8: negative control.

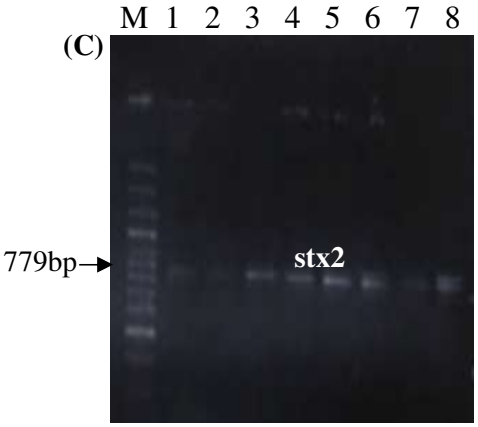


Fig. 6 Agarose gel electrophoresis showing PCR amplification of the stx2 Shiga toxin 2 gene (779 bp) from *E. coli* isolates. Lane M: 100 bp DNA ladder; Lanes 1–5: positive isolates (779 bp band); Lanes 6–8: negative controls.

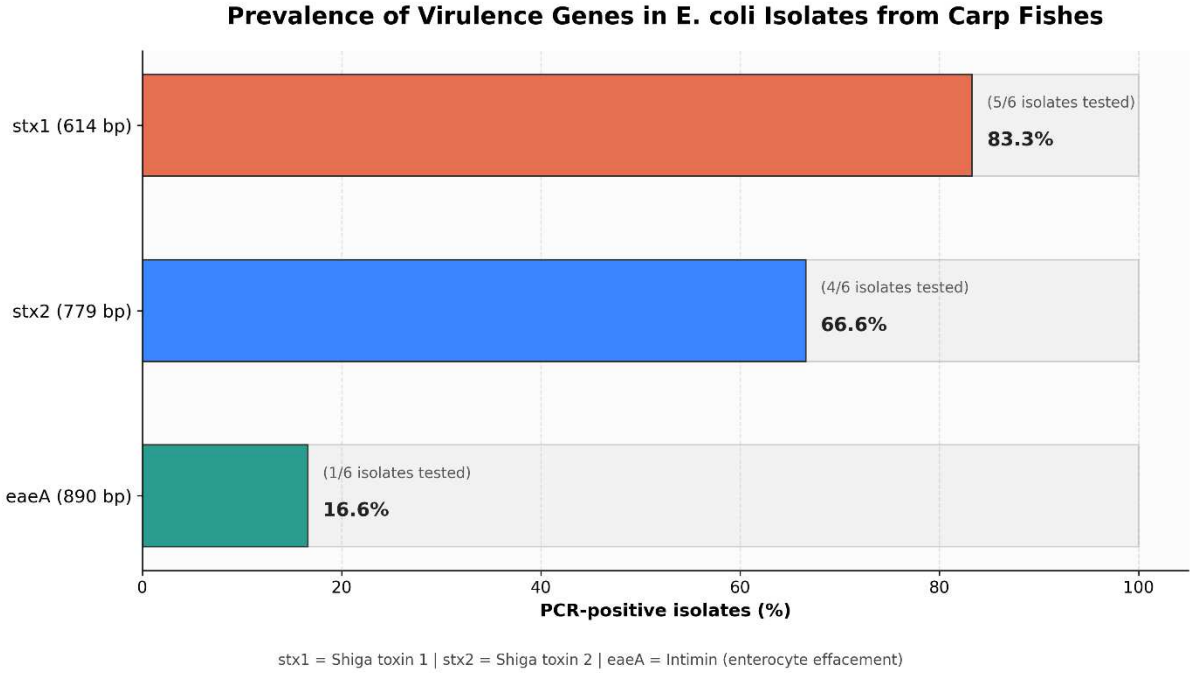


Fig. 7 Horizontal bar chart showing prevalence of virulence genes (stx1 83.3%, stx2 66.6%, eaeA 16.6%) in *E. coli* isolates from farm-raised carp, Punjab, Pakistan. Multiplex PCR; Emerald Amp GT Master Mix (Takara, Japan).

4. DISCUSSION

4.1 Prevalence of *E. coli*: Context and Seasonal Considerations

The overall *E. coli* prevalence of 14.8% recorded in farm-raised carp from Punjab, Pakistan falls within the range documented in comparable aquaculture systems internationally: Dissasa et al. [30] reported 22.8% from Ethiopian rift-valley Lakes; Yohans et al. [31] detected 23.5 % in raw fish from Dhaka city of Bangladesh; similar findings were findings were observed by Yohans et al. [31] A more recent study from Northwest Ethiopia by Ayalew et al. [32] demonstrated that *E. coli* contamination rates in market fish were strongly modulated by hygiene practices and season, corroborating the view that pond management not geography alone drives contamination burden. The relatively conservative prevalence in our study likely reflects a winter sampling window (November–February). Recent evidence from Egyptian aquaculture by El Badawy et al. [33] in *Scientific Reports* confirmed that both *E. coli* pathotype prevalence and AMR gene expression are significantly higher in summer months compared to winter suggesting our data represent a conservative lower estimate of the true seasonal burden in Punjab carp farms.

Tissue-specific prevalence showed intestine and pond water as highest-yielding sources (18.0% each), consistent with *E. coli*'s enteric ecology and its persistence in aquatic environments. The statistically significant difference across sample types ($\chi^2 = 9.38$, $p = 0.009$) confirms that site of collection matters clinically, with muscle being least contaminated (6.0%). This is consistent with Liao et al. [34], who found muscle tissue acts as a partial barrier to bacterial penetration in non-septicaemic fish. The high-water positivity (18.0%) and a MAR index of 0.58 from water samples points to persistent environmental faecal contamination likely from organic manure fertilisation, a common practice in small-scale Pakistani carp farming. Schar et al. [21] confirmed in their 749-survey meta-analysis that pond water is a critical hotspot for antibiotic-resistant bacteria dissemination in Asian aquaculture.

4.2 Antimicrobial Resistance: Patterns, Mechanisms, and ESBL Concerns

All MAR index values exceeded 0.2 across sample types (mean 0.54), indicating that all sampled farm environments qualify as high-risk antibiotic exposure settings per the criteria of Krumperman [35]. Universal resistance to cephalexin (100%) and near-universal resistance to rifampin (95.4%) and penicillin G (90.9%) represent among the highest AMR rates reported for aquaculture-derived *E. coli* in South Asia. These findings align with the global systematic review by Schar et al. [21], which identified penicillin resistance (>60%) as the

most prevalent AMR phenotype in Asian aquaculture foodborne pathogens. A comprehensive global review of AMR in *E. coli* from fish and aquaculture by Rana et al. [36] confirmed that β-lactams, tetracyclines, quinolones, and sulfonamides dominate resistance profiles globally, with ESBL-producing strains carrying *blaTEM*, *blaCTX-M*, and *blaSHV* genes now representing an urgent food safety concern.

The universal cephalexin resistance (100%) detected here is of particular concern as it phenotypically suggests ESBL production or AmpC beta-lactamase activity [22, 27]. A recent systematic review by Uhland et al. [37] on ESBL-producing bacteria in retail seafood found a global median ESBL contamination rate of 19.4% in fish products significantly higher in Asia with CTX-M (93.6%), TEM (6.7%), and SHV (5.0%) as predominant gene types. Similar results observed reported from Indian retail market fish detected 48.88% ESBL-producing *E. coli*, with CTX-M-15 (47%) as the dominant variant by Sajeev et al. [27] . In the present study, although ESBL genotyping was not performed, the phenotypic cephalosporin resistance pattern strongly warrants future molecular confirmation using blaCTX-M, blaTEM, and blaSHV PCR assays a key recommendation for follow-up research.

Near-universal rifampin resistance (95.4%) is an unusual finding in an aquaculture context since rifampin is not a licensed veterinary aquaculture antibiotic. This might be due to co-selection pressure by other resistance mechanisms, to acquisition of rpoB mutations by horizontal gene transfer or to indirect exposure of the environment from clinical discharge of humans into pond catchment areas, documented in Pakistani river systems [38]. In a similar finding, Lin et al. [39] found that the prevalence of *E. coli* resistance to rifampin in epizootic ulcerative syndrome fish in China was 5.46%, which may imply that the prevalence of *E. coli* resistance to rifampin in the aquaculture environment in South Asian countries might be systematically underestimated. This is a significant development from a One Health perspective because rifampin is an anti-tuberculosis cornerstone drug and any gene pool that gives rise to cross-resistance to rifampin also poses a risk of transferring to *Mycobacterium tuberculosis* and therefore requires urgent epidemiological vigilance.

Complete susceptibility to tobramycin and amikacin (0% resistance) means 0% of the isolates were resistant to these two aminoglycosides, making them effective and therefore they can be used for empirical treatment of severe infections with STEC, and are rarely used in the fish farming industry in Pakistan. This finding is consistent with the results obtained by Patterson et al. [40]. The MDR of cultured fish was found to be 86.4 % (resistance to ≥3 drug classes) which is comparable to the MDR *E. coli* in cultured fish of Bangladesh reported by Islam et al. (2023) 86.4% of cultured fish were MDR. This study found high MAR index value with 0.54 which is much higher than each threshold value 0.2 and similar to the range (0.5–0.88) mentioned by high AMR aquaculture

environment in Southeast Asia [41]. There is an urgency to address the multi-drug resistance of the protein source food and strictly monitored the utilization of multi-drugs in aquaculture sector.

4.3 Virulence Gene Prevalence and STEC Pathotype Significance

The detection of *stx1* (83.3%), *stx2* (66.6%), and *eaeA* (16.6%) confirms the widespread circulation of clinically dangerous STEC pathotypes in Pakistani carp aquaculture. These prevalence rates are notably higher than those reported by Begadiya et al. [42] from Indian freshwater fish (*stx1* 9.3%, *stx2* 16.3%) and by Mohseni et al. [43] from Iranian fish and shrimp (triple-gene combination in only 10%). In contrast, a recent multi-seafood survey by Al Qabili et al. [44] in India detected *stx1*, *stx2*, and *eae* in 1,518 out of 3,742 STEC isolates from fish and coastal waters, indicating that the burden in intensively farmed environments may be substantially higher than in wild fish.

The high prevalence of *stx2*-positive strains (66.6%) is the most clinically significant finding. Shiga toxin 2 is 10–100-fold more potent than Stx1 in damaging renal tubular endothelial cells, and its presence is the primary molecular risk factor for HUS progression particularly in children under five and the elderly [15]. Similar findings reported by King et al. [45] confirmed that *stx2a* the most clinically severe Shiga toxin subtype was detected in 18% of confirmed non-O157 STEC clinical cases in England (2016–2023), where O103:H2 was the sixth most common non-O157 serogroup [46]. Future subtyping of *stx2* in our isolates using the validated PCR is recommended, as stx2a-positive strains carry a substantially higher public health burden than stx2c or stx2d subtypes.

The co-detection of *stx1*, *stx2*, and *eaeA* in serotype O103 (Table 4) represents the most alarming finding of this study. O103 is classified by the USDA as one of the 'Big Six' non-O157 STEC serogroups strains capable of causing severe haemorrhagic colitis and HUS in humans Rana et al., 2025). Soycan et al. [23] detected O103 as the most prevalent STEC serogroup (32.6%) across diverse food matrices in Turkey, including fish samples, and identified O103 as frequently co-carrying *stx* and *eae* genes. The intimin encoded by *eaeA* enables intimate bacterial adhesion to intestinal epithelial cells via the Locus of Enterocyte Effacement (LEE) pathogenicity island, amplifying toxin delivery and colonisation efficiency the molecular basis of the EHEC phenotype [36]. Strains simultaneously positive for *stx2* and *eaeA* are most strongly associated with HUS, making the O103 isolates identified here among the most pathogenic *E. coli* strains recoverable from food fish globally.

4.4 Serotype Diversity and Epidemiological Implications

Five O-serogroups were identified O44, O103, O119, O125, O172 with O125 (20.4%) and O119 (18.1%) predominating. This diversity corroborates findings by Soliman. [48] from Egyptian carp aquaculture, where

O119 and O125 were similarly predominant. O125 is a well-characterised enteropathogenic serogroup historically associated with infantile diarrhoea in developing countries [43]. Its high prevalence in pond water and fish intestines in the present study is consistent with anthropogenic faecal contamination of pond catchments likely from inadequately treated human sewage, a documented pathway in Pakistani river basins (Iqbal et al., 2017). The concurrent detection of multiple serogroups simultaneously carrying virulence genes and MDR phenotypes constitutes a dual risk: direct STEC food-borne disease from consumption of undercooked fish, and indirect dissemination of resistance genes to human gut microbiota via the food chain [49, 50]. A global comparison of our findings with published studies is presented in Table 5.

Table 5. Comparative summary of *E. coli* prevalence, virulence gene detection, and key antimicrobial resistance findings in farm-raised and market fish across published studies. NR = not reported. Present study values are highlighted.

Study (Country)	Fish/Source	*E. coli* Prev. (%)	Virulence Genes	Key AMR Finding
Present study (Pakistan)	Carp *(Labeo rohita)*	14.8	stx1, stx2, eaeA	Cephalexin 100%; Penicillin 90.9%
Begadiya et al. (2018, India)	Freshwater fish	NR	stx1 9.3%, stx2 16.3%	Ampicillin 67%; Streptomycin 32%
Yohans et al. (2022, Ethiopia)	Market fish	NR	STEC detected	Ampicillin resistant
Behshood et al. (2022, Iran)	Fish & shrimp	17.5	stx1+eaeA 20%	Multi-drug resistant
Sajeev et al. (2023, India)	Retail market fish	39.8	ESBL-producing	CTX-M-15 dominant
Liao et al. (2021, China)	Rainbow trout	76.5	STEC, EPEC, EHEC	blaTEM 14.2%; MDR
El Badawy et al. (2025, Egypt)	Nile tilapia	NR	stx1, stx2, eaeA, bfpA	β-lactamase + carbapenemase

4.5 Public Health and One Health Perspective

The concurrent detection of MDR phenotypes (MAR mean 0.54) and STEC virulence genes in farm-raised Punjab carp represents a convergent public health hazard demanding urgent One Health intervention. The WHO has identified AMR as one of the ten greatest global health threats, with an estimated 1.27 million deaths directly attributable to AMR bacteria in 2019 with LMICs including Pakistan disproportionately affected (Naghavi et al., 2024). Consuming raw or undercooked contaminated carp can directly transmit STEC to humans, with a 5–10% risk of HUS progression carrying up to 5% mortality even with intensive medical care [36]. Children under five a major consumer demographic for family-prepared freshwater fish in rural Punjab face the highest risk of life-threatening HUS sequelae.

Aquaculture ponds act as established hotspots for HGT of resistance genes carried on MGEs (integrons, conjugative plasmids, transposons) between fish pathogens, environmental bacteria, and human-pathogenic *E. coli* (Cho et al., 2023). ESBL-producing *E. coli* in aquatic environments can persist for months in pond sediments and re-enter human exposure pathways through water, vegetable irrigation, or farm worker contact. Caputo et al. (2023) identified aquaculture as a globally critical AMR dissemination source, with global antimicrobial consumption in aquaculture projected to rise 33% by 2030 under current trends. WorldFish [51], which leads One Health AMR surveillance in Pakistan, Bangladesh, Nepal, and Sri Lanka, has explicitly flagged the lack of standardised AMR frameworks and limited laboratory capacity in Pakistan as the primary barriers to evidence-based aquaculture AMR management.

4.6 Study Limitations

The present study has several limitations that should be acknowledged. First, sampling was restricted to the winter season (November–February), which may underestimate peak warm-season *E. coli* burdens. Second, ESBL genotyping (blaCTX-M, blaTEM, blaSHV) and stx subtyping were not performed key analyses recommended in follow-up research. Third, the sample size (250 samples from 60 fish) and three-site design limits statistical power for sub-group analyses. Fourth, fish from only one dominant species (*Labeo rohita*) were sampled, limiting generalisability to other carp species (*Catla catla*, *Cyprinus carpio*, *Hypophthalmichthys molitrix*). Fifth, minimum inhibitory concentrations (MICs) were not determined, limiting mechanistic resistance interpretation. Future studies should incorporate whole-genome sequencing (WGS) for complete resistance gene characterisation, stx subtyping, and seasonal sampling.

5. CONCLUSION

This study provides the first comprehensive baseline molecular evidence that farm-raised carp fishes in Punjab, Pakistan harbour pathogenic, multi-drug-resistant *E. coli* strains carrying critical STEC virulence determinants.

The concurrent detection of *stx1* (83.3%), *stx2* (66.6%), and *eaeA* (16.6%) particularly the EHEC phenotype in serotype O103 signals a tangible and immediate public health risk in a region where carp is a dietary staple. A mean MAR index of 0.54 confirms that all sampled farm environments operate as high-risk antibiotic exposure zones. The findings make a compelling case for: (i) urgent implementation of evidence-based antimicrobial stewardship programmes in Pakistani aquaculture; (ii) mandatory pre-market STEC/EHEC screening for pond-raised carp; (iii) *stx* subtyping and ESBL genotyping in follow-up surveillance; (iv) whole-genome sequencing of EHEC O103 isolates to characterise resistance gene content and clonal relatedness to clinical strains; and (v) a national One Health AMR action plan for the fisheries and aquaculture sector, aligned with WHO and FAO global frameworks.

DECLARATIONS

Ethics Approval: This study was conducted in accordance with the institutional ethical guidelines for animal research at the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. Ethical approval was granted by the relevant institutional committee under Approval No. DR/358-5, dated 12 February 2024. Permission to collect fish and pond-water specimens was obtained from the respective fish pond owners before sampling. Collection of specimens complied with institutional ethical approval and local requirements for sampling from farmed aquaculture ponds. No protected or endangered species were involved.

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

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Consent for Publication

Not applicable

Author Contributions: TH: sample collection, laboratory work, data analysis, writing original draft. FR: study design, supervision, manuscript review and editing. SM: laboratory work, data analysis and writing original draft. AS: Formal analysis, Visualization and editing. SS: Writing review and editing. AT: Data curation, formal analysis, review and editing. SS review manuscript, data analysis and editing. All authors have read and agreed to the published version of the manuscript.

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REFERENCE

1. FAO. The State of World Fisheries and Aquaculture 2024. Blue Transformation in action. The State of World Fisheries and Aquaculture 2024. 2024.

2. Meinam M, Deepti M, Ngasotter S. Emerging aquaculture technologies for food and nutritional security. In: Food security, nutrition and sustainability through aquaculture technologies. 2025.

3. Calisi A, Gualandris D, Gamalero E, Dondero F, Semeraro T, Verri T. Eco-Sustainability in Aquaculture: Questions and Perspectives. Environments. 2026;13:208. https://doi.org/10.3390/environments13040208.

4. Ohia CMD. Aquaculture Technologies and Practices: Balancing Innovation, Environment and Economy for Sustainability. In: Food Security, Nutrition and Sustainability Through Aquaculture Technologies. Cham: Springer Nature Switzerland; 2025. p. 417–24. https://doi.org/10.1007/978-3-031-75830-0_23.

5. FAO O. Agricultural Outlook 2025-2034. 2025.

6. OECD-FAO Agricultural Outlook 2024-2033. 2024. https://doi.org/10.4060/cd0991en.

7. Song Y, Zhang W. Balancing Growth and Sustainability in China's Carp Aquaculture: Practices, Policies, and Sustainability Pathways. Sustainability (Switzerland). 2025;17. https://doi.org/10.3390/su17125593.

8. Taylor B, Ofori KF, Parsaeimehr A, Akdemir Evrendilek G, Attarwala T, Ozbay G. Exploring the Complexities of Seafood: From Benefits to Contaminants. Foods. 2025;14. https://doi.org/10.3390/foods14091461.

9. Gillani R. Trade Development Authority of Pakistan (TDAP). Pakistan Mango. 2023;1.

10. Shahzad SM. DISCOURSE ANALYSIS AND FUTURE PERSPECTIVE OF FISH INDUSTRY OF PAKISTAN. Pak J Sci. 2023;74. https://doi.org/10.57041/pjs.v74i4.761.

11. Mohsin M, Yin H, Mehak A. Sustainable solutions: exploring risks and strategies in Pakistan's seafood trade for marine conservation. Front Mar Sci. 2024;11. https://doi.org/10.3389/fmars.2024.1420755.

12. Mahmood MA, Xin D, Saleem CM, Zhu C, Xiaona X, Jiang Y, et al. Population Dynamic Parameters of Rohu, Labeo rohita (Hamilton, 1822) in the Indus River, Sindh, Pakistan. Pak J Zool. 2025;57. https://doi.org/10.17582/journal.pjz/20231229182844.

13. Irshath AA, Rajan AP, Vimal S, Prabhakaran VS, Ganesan R. Bacterial Pathogenesis in Various Fish Diseases: Recent Advances and Specific Challenges in Vaccine Development. Vaccines. 2023;11. https://doi.org/10.3390/vaccines11020470.

14. Salinas C, Rodriguez F, Muñoz-Barrera A, Lorenzo Salazar JM, González-Montelongo R, Flores C, et al. Genetic variability of Shiga toxin-producing Escherichia coli strains isolated from Paraguayan cattle . Microbiol Spectr. 2025;13. https://doi.org/10.1128/spectrum.00596-25.

15. Matussek A, Mernelius S, Chromek M, Zhang J, Frykman A, Hansson S, et al. Genome-wide association study of hemolytic uremic syndrome causing Shiga toxin-producing Escherichia coli from Sweden, 1994–2018. European Journal of Clinical Microbiology and Infectious Diseases. 2023;42. https://doi.org/10.1007/s10096-023-04600-1.

16. Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, et al. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. The Lancet. 2024;404. https://doi.org/10.1016/S0140-6736(24)01867-1.

17. Alhadlaq MA, Aljurayyad OI, Almansour A, Al-Akeel SI, Alzahrani KO, Alsalman SA, et al. Overview of pathogenic Escherichia coli, with a focus on Shiga toxin-producing serotypes, global outbreaks (1982–2024) and food safety criteria. Gut Pathogens. 2024;16. https://doi.org/10.1186/s13099-024-00641-9.

18. Mohseni P, Ghanbarpour R, Jajarmi M, Bagheri M. Molecular and phenotypic characteristics of isolated Escherichia coli from the skin, gills, and intestine of rainbow trout in retail stores of Kerman, Iran. Iran J Vet Res. 2025;26. https://doi.org/10.22099/IJVR.2025.49736.7331.

19. Schar D, Klein EY, Laxminarayan R, Gilbert M, Van Boeckel TP. Global trends in antimicrobial use in aquaculture. Sci Rep. 2020;10. https://doi.org/10.1038/s41598-020-78849-3.

20. Debnath PP, Chokmangmeepisarn P, Papadopoulou A, Coyle NM, Baker-Austin C, van Aerle R, et al. Diversity and antimicrobial resistance among bacterial isolates from finfish aquaculture in Thailand. BMC Vet Res. 2025;21. https://doi.org/10.1186/s12917-025-04942-2.

21. Schar D, Zhao C, Wang Y, Larsson DGJ, Gilbert M, Van Boeckel TP. Twenty-year trends in antimicrobial resistance from aquaculture and fisheries in Asia. Nat Commun. 2021;12. https://doi.org/10.1038/s41467-021-25655-8.

22. Cho S, Jackson CR, Frye JG. Freshwater environment as a reservoir of extended-spectrum β-lactamase-producing Enterobacteriaceae. Journal of Applied Microbiology. 2023;134. https://doi.org/10.1093/jambio/lxad034.

23. Soycan A, Er DK, Dündar D. Prevalence and serotype distribution of Shiga toxin-producing Escherichia coli (STEC) in raw animal and plant food samples from Kocaeli province, Türkiye. Food Control. 2025;176. https://doi.org/10.1016/j.foodcont.2025.111382.

435 24. Pearce R, Conrady B, Guardabassi L. Prevalence and Types of Extended-Spectrum β-Lactamase-Producing
436 Bacteria in Retail Seafood. Foods. 2023;12. https://doi.org/10.3390/foods12163033.
437 25. Mohammed R, Nader SM, Hamza DA, Sabry MA. Public health concern of antimicrobial resistance and
438 virulence determinants in E. coli isolates from oysters in Egypt. Sci Rep. 2024;14.
439 https://doi.org/10.1038/s41598-024-77519-y.
440 26. Samad MA, Karim MdR, Mahmud MA, Sagor MS, Hossain MS, Uddin ASMA, et al. Molecular
441 characterization of multi-drug resistance Escherichia coli O157:H7 from calf feces and cow milk in Bangladesh.
442 Sci Rep. 2026;16:9940. https://doi.org/10.1038/s41598-026-36237-3.
443 27. Sajeev S, Hamza M, Rajan V, Vijayan A, Sivaraman GK, Shome BR, et al. Resistance profiles and
444 genotyping of extended-spectrum beta-lactamase (ESBL) -producing and non-ESBL-producing E. coli and
445 Klebsiella from retail market fishes. Infection, Genetics and Evolution. 2023;112.
446 https://doi.org/10.1016/j.meegid.2023.105446.
447 28. Dhanashree B, Shrikar Mallya P. Detection of shiga-toxigenic Escherichia coli (STEC) in diarrhoeagenic
448 stool & meat samples in Mangalore, India. Indian Journal of Medical Research. 2008;128.
449 29. Mazaheri S, Ahrabi SS, Aslani MM. Shiga toxin-producing Escherichia coli isolated from lettuce samples in
450 Tehran, Iran. Jundishapur J Microbiol. 2014;7. https://doi.org/10.5812/jjm.12346.
451 30. Dissasa G, Lemma B, Mamo H. Isolation and identification of major bacteria from three Ethiopian rift
452 valley lakes live and processed fish, and water samples: implications in sanitary system of fish products. BMC
453 Vet Res. 2022;18. https://doi.org/10.1186/s12917-022-03508-w.
454 31. Yohans H, Mitiku BA, Tassew H. Levels of Escherichia coli as Bio-Indicator of Contamination of Fish
455 Food and Antibiotic Resistance Pattern Along the Value Chain in Northwest Ethiopia. Veterinary Medicine:
456 Research and Reports. 2022;Volume 13. https://doi.org/10.2147/vmrr.s373738.
457 32. Assefa A, Regassa F, Ayana D, Amenu K, Abunna F. Prevalence and antibiotic susceptibility pattern of
458 Escherichia coli O157:H7 isolated from harvested fish at Lake Hayq and Tekeze dam, Northern Ethiopia.
459 Heliyon. 2019;5. https://doi.org/10.1016/j.heliyon.2019.e02996.
460 33. El Badawy A, Hamza D, Ahmed Z, Sabry MA. Seasonal and environmental drivers of antibiotic resistance
461 and virulence in Escherichia coli from aquaculture and their public health implications. Sci Rep. 2025;15.
462 https://doi.org/10.1038/s41598-025-98498-8.

34. Liao CY, Balasubramanian B, Peng JJ, Tao SR, Liu WC, Ma Y. Antimicrobial Resistance of Escherichia coli From Aquaculture Farms and Their Environment in Zhanjiang, China. Front Vet Sci. 2021;8. https://doi.org/10.3389/fvets.2021.806653.

35. Krumperman PH. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol. 1983;46. https://doi.org/10.1128/aem.46.1.165-170.1983.

36. Rana ML, Ullah MA, Hoque MN, Hassan J, Siddique MP, Rahman MT. Preliminary survey of biofilm forming, antibiotic resistant Escherichia coli in fishes from land based aquaculture systems and open water bodies in Bangladesh. Sci Rep. 2025;15. https://doi.org/10.1038/s41598-024-80536-6.

37. Uhland FC, Li XZ, Mulvey MR, Reid-Smith R, Sherk LM, Ziraldo H, et al. Extended Spectrum β-Lactamase-Producing Enterobacterales of Shrimp and Salmon Available for Purchase by Consumers in Canada—A Risk Profile Using the Codex Framework. Antibiotics. 2023;12. https://doi.org/10.3390/antibiotics12091412.

38. Iqbal MS, Ahmad MN, Hofstra N. The relationship between hydro-climatic variables and e. Coli concentrations in surface and drinking water of the kabul river basin in pakistan. AIMS Environ Sci. 2017;4. https://doi.org/10.3934/environsci.2017.5.690.

39. Lin Z, Deng Y, Su W, Wang Q, Chen H, Sun Y, et al. Prevalence and distribution of antibiotic resistance in the water environment of sea bass (Lateolabrax maculatus) breeding area in spring in South China. Front Mar Sci. 2023;10. https://doi.org/10.3389/fmars.2023.1139641.

40. Patterson J. Prevalence of Antibiotic Resistant Listeria monocytogenes in Sea Foods of Tuticorin Coast, Southeastern India. European Journal of Applied Sciences. 2016;8.

41. Suyamud B, Chen Y, Quyen DTT, Dong Z, Zhao C, Hu J. Antimicrobial resistance in aquaculture: Occurrence and strategies in Southeast Asia. Science of the Total Environment. 2024;907. https://doi.org/10.1016/j.scitotenv.2023.167942.

42. Begadiya HB, Nayak JB, Brahmbhatt MN, Chaudhary JH, Solanki KS. Detection of Virulence Gene and Antimicrobial Resistance Pattern of Escherichia coli Isolated from Fresh Water Fish in and around Anand City, Gujarat, India. Int J Curr Microbiol Appl Sci. 2018;7. https://doi.org/10.20546/ijcmas.2018.705.391.

43. Mohseni P, Ghanbarpour R, Jajarmi M, Bagheri M. Molecular and phenotypic characteristics of isolated Escherichia coli from the skin, gills, and intestine of rainbow trout in retail stores of Kerman, Iran. Iran J Vet Res. 2025;26. https://doi.org/10.22099/IJVR.2025.49736.7331.

493 44. Al Qabili DMA, Aboueisha AKM, Ibrahim GA, Youssef AI, El-Mahallawy HS. Virulence and
494 antimicrobial-resistance of shiga toxin-producing E. coli (STEC) Isolated from edible shellfish and its public
495 health significance. Arch Microbiol. 2022;204. https://doi.org/10.1007/s00203-022-03114-2.
496 45. King G, Jenkins C, Hayden I, Rodwell E V., Quinn O, Godbole G, et al. Epidemiology of Shiga toxin-
497 producing Escherichia coli other than serotype O157:H7 in England, 2016-2023. J Med Microbiol. 2025;74.
498 https://doi.org/10.1099/jmm.0.001947.
499 46. Millanao AR, Barrientos-Schaffeld C, Siegel-Tike CD, Tomova A, Ivanova L, Godfrey HP, et al.
500 Antimicrobial resistance in Chile and the one health paradigm: Dealing with threats to human and veterinary
501 health resulting from antimicrobial use in Salmon aquaculture and the clinic. Revista Chilena de Infectologia.
502 2018;35. https://doi.org/10.4067/s0716-10182018000300299.
503 47. Nguyen Y, Sperandio V. Enterohemorrhagic E. coli (EHEC) pathogenesis. Frontiers in cellular and infection
504 microbiology. 2012;2. https://doi.org/10.3389/fcimb.2012.00090.
505 48. Soliman F, Khalil MK;, Saad RH;, El-Gamal TT* ;, Gebril MHL. Isolation and Identification of E. coli from
506 Cultured Freshwater. 2010.
507 49. Souza HDF de, Ferreira PFA, Patricio TC da C, Santiago GS, Coelho I da S, Souza MMS de, et al. Is green
508 metallic sheen in emb agar accurate to assist the Escherichia coli identification? Revista Pró-UniverSUS.
509 2025;15. https://doi.org/10.21727/rpu.v15i4.4520.
510 50. Dewi RR, Hassan L, Daud HM, Matori MF, Zakaria Z, Ahmad NI, et al. On-Farm Practices Associated with
511 Multi-Drug-Resistant Escherichia coli and Vibrio parahaemolyticus Derived from Cultured Fish.
512 Microorganisms. 2022;10. https://doi.org/10.3390/microorganisms10081520.
513 51. HQ W. WorldFish 2025. Annual Report. 2025.
514