Received: 25 January 2026 | Revised: 19 March 2026 | Accepted: 27 March 2026

https://doi.org/10.56946/jzs.v4i1.868
Volume 4 (1): 18-25

Journal of Zoology and Systematics

Research Article

Molecular Identification and Heavy Metal Resistance Profiles of *Escherichia coli* from Silver Carp in the Contaminated Waters of the River Kabul, Khyber Pakhtunkhwa

Abdur Rahman, Anees Ur Rehman, Mujahid Rahim, Shehzad Zareen, Noor ul Akbar, Muhammad Shehzad*

Department of Zoology, Kohat University of Science and Technology, Kohat, Khyber Pakhtunkhwa, Pakistan.
*Correspondence: (Muhammad Shehzad), muhammadshehzad962@gmail.com

Abstract

Heavy metal pollution in freshwater ecosystems presents considerable ecological and public health risks, particularly when toxic metals interact with pathogenic and metal-resistant bacteria in edible fish species. The present study investigated heavy-metal-resistant *Escherichia coli* isolated from silver carp obtained from the River Kabul in Pakistan. Thirty silver carp specimens were collected, and bacteria were isolated from their intestines and gills. Bacterial identification was performed using Gram staining and biochemical tests (Catalase, Oxidase, Motility, SIM, and Citrate). The Minimum Inhibitory Concentration (MIC) assay was used to assess *E. coli* resistance against $ZnSO_4$, $NiCl_2$, $CuSO_4$, and $HgCl_2$. Molecular identification was performed using the *16S rRNA* gene sequencing and phylogenetic analysis. *E. coli* was isolated from 23 of 30 fish (76%), yielding four phenotypically confirmed isolates, which were further subjected to MIC testing. All isolates were Gram-negative rods with biochemical characteristics consistent with *E. coli* (100% concordance). MIC assays showed high resistance to $ZnSO_4$, $NiCl_2$, and $CuSO_4$, while all tested isolates remained sensitive to $HgCl_2$. The statistical analysis revealed significant differences in resistance among the tested metals ($p < 0.05$). The phylogenetic analysis showed that the heavy metal-resistant isolates formed a tight clustering pattern with previously reported *E. coli* strains from Pakistan, suggesting possible regional adaptation in freshwater ecosystems contaminated with heavy metals. These results show that heavy metal contamination and heavy metal-resistant *E. coli* are both present in an important edible fish species.

Keywords: Heavy Metal, Metal-tolerance Bacteria, Nowshera, River Kabul, Silver Carp

1. Introduction

Rivers are open ecological systems, providing fertile scope for freshwater ecosystems and serving as a primary source of irrigation, soil enrichment, food supply, transportation, and drinking water [1, 2]. In addition, rivers serve as sinks for sewage from urban areas, industrial effluents, and municipal waste [3]. Thus, river water is often contaminated with organic pollutants and heavy metals from both anthropogenic and natural origins [4]. The contamination of water with heavy metals mainly results from human activities involving industrial processes, mining, soil erosion, agricultural runoff, waste from electronic products, industrial plants, and sewage discharge [2, 5, 6]. Some of the toxic heavy metals that affect the aquatic ecosystem badly include zinc (Zn), copper (Cu), chromium (Cr), arsenic (As), cadmium (Cd), nickel (Ni), mercury (Hg), and cobalt (Co) [7, 8]. This kind of pollution presents a serious challenge to aquatic life, including fish, other aquatic plants, molluscs, other invertebrates, and microorganisms, primarily bacteria [8, 9]. Fish are among the most sensitive species to heavy metal pollution; they may bioaccumulate in their tissues, exert toxic effects on fish, and pose health risks to humans via consumption of contaminated fish [10].

E. coli is a Gram-negative bacterium of the Enterobacteriaceae family [11]. It generally inhabits the intestinal tract of fish, but if it escapes the gut, it can cause pathogenesis, including an enterotoxigenic infection [12] Its pathogenicity arises from the presence of a large number of strains, each bearing different virulence factors [13, 14]. In addition, *E. coli* harbours genes that confer resistance to heavy metals, antibiotics, and disinfectants, enabling the bacterium to survive in water containing toxic metals [15]. These metal resistance traits are linked to antibiotic resistance, further complicating the control of antimicrobial resistance in both aquatic and terrestrial environments [16].

The coexistence of resistant bacteria with heavy metals poses a serious environmental and public health hazard and is

correlated with fish quality, water safety, and microbiological quality [17]. Such contamination of rivers is also expected in the Kabul River, which is polluted by industrial and domestic waste. Previously, the extent of heavy metal contamination and its biological effects in this river system were reported. For instance, Siraj et al. [18] assessed bioaccumulation of heavy metals in common carp (*Cyprinus carpio*) in the River Kabul and reported high metal concentrations, with resultant genotoxic effects [18]. Similarly, Siraj et al. [19] studied the freshwater catfish *Wallago attu* and identified patterns of heavy metal accumulation across various organs. Recently, Ali et al. [20] compared microbial diversity along the Swat and Kabul Rivers and concluded that excessive levels of heavy metals had adverse impacts on microbial morphology, particularly in the River Kabul, where anthropogenic pollution posed a significant threat. Despite substantial findings from earlier work, the characterization of heavy metal-resistant bacteria, especially fish-associated and particularly metal-resistant *E. coli*, in the River Kabul remains understudied.

This study aimed to identify and characterize heavy metal-resistant *Escherichia coli* strains in silver carp from the River Kabul, Nowshera, Khyber Pakhtunkhwa. Silver carp, a freshwater fish native to Asia, is recognised as one of the most commercially valuable species and is widely farmed for its economic significance [21]. The species is also notable for its high-quality proteins, essential minerals, polyunsaturated fatty acids, and fat-soluble vitamins, as reported by Jawdhari et al. [22] and Rezaei and Shahbazi [23]. Given its nutritional and economic importance, the presence of metal-resistant, potentially pathogenic bacteria in this fish poses direct risks to food safety and public health. The specific objectives were to (i) isolate and phenotypically identify *E. coli* from silver carp, (ii) determine the MICs of $ZnSO_4$, $NiCl_2$, $CuSO_4$, and $HgCl_2$ to evaluate metal resistance, and (iii) confirm isolate identity and explore the phylogenetic relationships of the isolates using *16S rRNA* sequencing. The findings show that metal-resistant *E. coli* strains are present in silver carp eaten by local communities, highlighting a potential foodborne exposure route not previously documented for this watershed. Additionally, the phylogenetic clustering of these isolates with regional Pakistani strains suggests local adaptation to human-made pollution, enhancing our understanding of bacterial evolution in contaminated freshwater systems.

2. Materials and Methods

2.1. Study area

The River Kabul, within the district of Nowshera, Khyber Pakhtunkhwa, was selected for this research activity (Fig. 1a). The River Kabul originates in Afghanistan. It enters Pakistan at Shalman, Khyber Agency [24]. In Khyber Pakhtunkhwa, the river runs through densely populated urban areas and agricultural fields and is highly contaminated with heavy metals [25]. Different industries and domestic sewage discharge untreated effluents into the river, increasing the concentrations of Zn, Pb, Cd, Cu, and Cr in the river water [25]. Further downstream, pollution from ghee industries and other industrial units increases heavy metal contamination in the river at Nowshera's Kund Park and Kashti Pul. Approximately 80 industries reportedly discharge untreated wastewater into the river, either directly or indirectly [26, 27]. A total of 54 fish species have been identified in the River Kabul and its tributaries, of which about 35 are common species [28]. Silver carp is included among these economically important fish species.

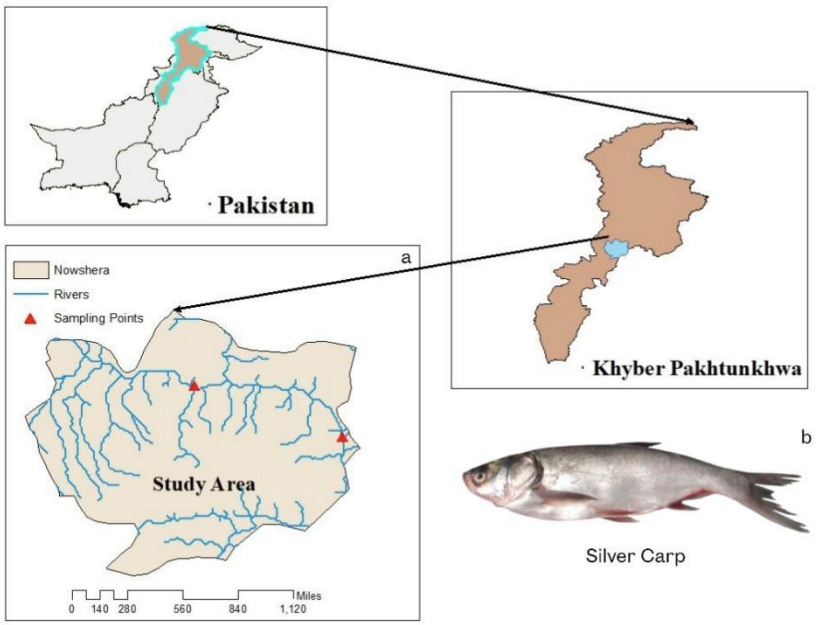

Figure 1. (a) Map of the study area representing the sample collection points, b) Photograph of the Silver carp captured from the River Kabul.

2.2. Fish sampling

Silver carp (Figure 1b) were collected from two sites in the River Kabul: Kund Park (33.92601°N, 72.2344°E) and Kashti Pul (34.0069°N, 71.9873°E) in the district of Nowshera. These locations were chosen because they are known to have high contamination levels, especially from heavy metals and household sewage. A total of 30 silver carp fish samples were collected with the help of local fishermen using hook-and-line and cast nets. After capture, the fish were identified to species using the key by Mirza and Sandhu [29], placed in iceboxes, and taken to the Fishery Lab, Department of Zoology, KUST, Kohat, for further analysis.

2.3. Isolation and Phenotypic Identification of *Escherichia coli*

In the lab, each fish was rinsed with distilled water to remove surface contamination, then dissected using sterile scissors and forceps. Samples from the intestine and opercular region were collected under sterile conditions. Samples were streaked onto MacConkey agar and Lysogeny Broth (LB) agar plates to isolate *E. coli*, then incubated at 37 °C for 24 hours [30]. Pink, lactose-fermenting colonies with typical *E. coli* features from MacConkey agar were transferred to fresh LB agar to get pure isolates. The Gram stain test helped separate bacteria into Gram-negative and Gram-positive groups. Smears from pure colonies were heat-fixed, stained with crystal violet and Gram's iodine, washed with 95% ethanol, and counterstained with safranin. Slides were checked under oil immersion to confirm Gram-negative rod-shaped bacteria [20]. The following biochemical tests were used to identify presumptive *E. coli* isolates following standard microbiological protocols: Catalase test, Oxidase test, Motility test, Sulphide Indole Motility (SIM) test, and Citrate utilisation test [31-33]

2.4. Heavy metal resistance assay (MIC)

To determine MICs, four *E. coli* isolates (S1–S4) were selected for detailed analysis based on the following criteria: (i) distinct colony morphology on MacConkey agar (variation in size, texture, and pigmentation intensity suggesting phenotypic diversity), (ii) sampling site diversity (two isolates from Kund Park and two from Kashti Pul to represent spatial variation), and (iii) source tissue variation (intestinal vs. gill-associated isolates to capture niche-specific populations). This selection strategy aimed to capture potential phenotypic and ecological diversity among the 23 *E. coli*-positive fish samples. While this subset represents only 17% of the total positive samples, resource constraints (costs of molecular sequencing and extensive MIC screening) limited comprehensive analysis of all isolates. The selection of four isolates is consistent with similar exploratory studies on metal resistance in fish-associated bacteria [34, 35]. MIC was measured to study the resistance exhibited by *E. coli* isolates to four metal salts: $ZnSO_4$, $NiCl_2$, $CuSO_4$, and $HgCl_2$, using concentrations from 1000 to 1900 µg/mL. Each MIC determination was performed in technical triplicate (three independent wells per concentration) using biological duplicates (two independent bacterial cultures per isolate), following standard microplate dilution methods for metal resistance assessment. We made serial dilutions of each metal salt in sterile LB broth using 96-well microplates. Each well received the bacterial suspension, and some wells without bacteria served as negative controls. The plates were incubated at 37 °C for 24 hours. The MIC was the lowest concentration at which no visible bacterial growth appeared. We recorded MIC values (in mg/L or mM, depending on the stock solution) for each metal to compare them statistically [36, 37].

2.5. Molecular characterisation of *E. coli*

Genomic DNA was extracted from pure *Escherichia coli* cultures using a commercial bacterial DNA extraction kit (prepared by BJ Micro Lab Pvt. Ltd., Rawalpindi, Pakistan) according to the manufacturer's instructions. The *16S rRNA* from each isolate was amplified with universal bacterial primers commonly used for taxonomic identification (e.g., Faniyan et al. [38]: Forward primer 5′-CCTAYGGGRBGCASCAG-3′ and Reverse primer 5′-GGACTACNNGGGTATCTAAT-3′. These primers are widely recognized for their efficacy in amplifying a highly conserved region of the 16S rRNA gene, making them suitable for accurate taxonomic identification and phylogenetic analysis of *E. coli*. PCR products were kept at 4 °C until further processing. The primers were chosen from previously published universal *16S rRNA* primer sets, rather than being explicitly designed for this study. Amplified products that showed a single, clear band of the expected size were purified and sent to a commercial sequencing facility (Macrogen Inc., Seoul, South Korea) for Sanger sequencing.

2.6 Phylogenetic analysis

We checked and edited raw 16S rRNA sequences in BioEdit (version 5.0.9) to remove low-quality bases and resolve ambiguities. High-quality consensus sequences were then submitted to the National Center for Biotechnology Information (NCBI) to obtain accession numbers. The accession numbers for the four isolates sequenced in this study are listed in Table 1. We used BLAST to search for similar sequences and identify the closest matches in the NCBI database. Representative sequences from related *E. coli* strains were downloaded and aligned with our study isolates using ClustalW in MEGA 11 (version 11.0.13). We built a phylogenetic tree using the Maximum Likelihood method with 100 bootstrap replications to assess branch support.

Table 1. List of accession numbers.

Sr. no	Accession Number
S1	PQ896686.1
S2	PQ896687.1
S3	PQ896688.1
S4	PQ896689.1

Although 1000 bootstrap replicates are often recommended for definitive phylogenetic studies [39, 40], 100 replicates offer an adequate preliminary assessment of clade support in

exploratory studies with limited sequence divergence, especially for 16S rRNA gene sequences within a single bacterial species, where topology tends to be stable [41, 42]. The tree was exported in Newick format and visualized with ChiPlot (tvBOT; 43) to display and annotate the phylogenetic relationships.

2.6. Statistical analysis

To compare MIC values among metals, a one-way ANOVA with metal type as the fixed factor was used. Prior to conducting ANOVA, we assessed the assumptions of normality and homogeneity of variance. The analyses and plots were done in R (v4.4.1) [44] with RStudio (v. 2024.04.2-764), using ggplot2 for visualization [45]. The study area map was created in ArcGIS v10.5.

3. Results and Discussion

We examined 30 silver carp and found *Escherichia coli* in 23 (76%). We identified four isolates, labelled S1-S4. All four were Gram-negative, rod-shaped bacteria, confirmed by Gram staining. Biochemical tests, including Catalase, Oxidase, Motility, SIM, and Citrate, matched the typical *E. coli* profile (Table 2). The isolation rate shows that *E. coli* exposed to heavy metals is found in a significant number of edible fish from polluted parts of the River Kabul. Other studies have also found *E. coli* in both farmed and wild freshwater fish, with the intestine and gills serving as key sites for these bacteria in aquatic food webs [46, 47]. The Gram-negative, rod-shaped appearance and biochemical traits of our isolates (positive indole and SIM, negative oxidase and citrate) match the standard descriptions of *E. coli* in microbiology references [32, 33, 48].

Table 2. Biochemical test results of *E. coli.*

Test	Result (*n* = *4*)
Gram staining	Gram-negative rods
Oxidase	–
Catalase	+
Motility	+
Indole	+
H_2S	–
Citrate	–

We chose four *E. coli* isolates (S1, S2, S3, and S4) to test for heavy metal resistance using minimum inhibitory concentration (MIC) assays with $ZnSO_4$, $NiCl_3$, $CuSO_4$, and $HgCl_2$. All isolates showed strong resistance to $ZnSO_4$, $NiCl_2$, and $CuSO_4$ at all tested concentrations, but were sensitive to $HgCl_2$, as demonstrated by lower MIC values (Figure 2 & 3). There was a statistically significant difference in MIC values among the heavy metals ($P<0.05$). These results show that the isolates are resistant to zinc, nickel, and copper, but still sensitive to mercury. This pattern of metal-specific resistance matches earlier studies on *E. coli* and other bacteria from metal-affected aquatic environments, which found higher tolerance to zinc, nickel, and copper, but lower tolerance to mercury [16, 34, 35]. Afzal et al. [48] also reported resistance of *E. coli* from industrially polluted waters in Faisalabad to $ZnSO_4$, $NiCl_2$, and $CuSO_4$, as observed with the River Kabul isolates [48]. The observation of significant ($P < 0.05$) variations in MICs of different metals against the same set of bacterial isolates suggests differential resistance mechanisms among the tested metals, with isolates showing higher tolerance to zinc, nickel, and copper compared to mercury.

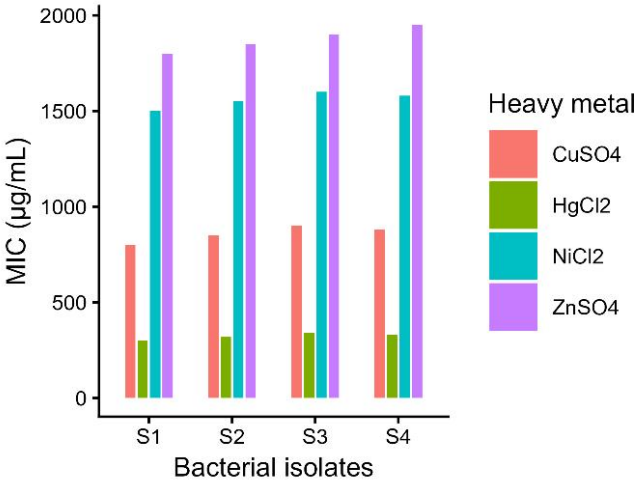

Figure 2. Heavy metal resistance profiles of *Escherichia coli* isolates S1 to S4 were measured using Minimum Inhibitory Concentration (MIC) assays. Each bar shows the MIC value for each metal and isolate.

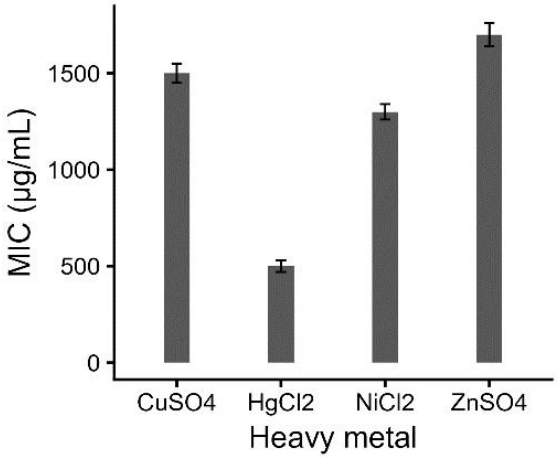

Figure 3. MIC values for *E coli* isolates S1, S2, S3, and S4 were measured. The figure compares MIC distributions across isolates and shows metal-specific resistance patterns. A one-way ANOVA showed that MIC values were significantly different among the metals ($P < 0.05$).

Given the documented history of heavy metal contamination in the River Kabul from industrial effluents and domestic sewage [24, 25], the observed resistance patterns may reflect selective pressure from long-term environmental exposure to these specific metals. However, without concurrent measurement of actual metal concentrations in our fish samples, water, or sediment from our sampling sites, we cannot definitively establish a quantitative link between environmental metal levels and the observed resistance phenotypes. The resistance patterns could alternatively reflect

exposure history at other locations (if fish migrate) or horizontal gene transfer from other bacterial populations. *E. coli* shows several strategies for heavy metal resistance, such as efflux pumps, enzymatic transformation, sequestration, and bioaccumulation [49].

The fact that all our isolates are environmental, with high MIC values for $ZnSO_4$, $NiCl_2$, and $CuSO_4$, suggests that these mechanisms might have developed after long-term exposure to industrial and household waste released into the River Kabul [5, 20]. Resistance genes against heavy metals also show linkage with antibiotic resistance genes on mobile genetic elements, such as plasmids and integrons, potentially leading to multidrug resistance in polluted water [15, 50]. In Pakistan, Saima et al. [51] reported multi-drug-resistant bacteria in wastewater streams, indicating that resistant strains and genes have already started moving between human-made waste and natural water. The finding of heavy-metal-resistant *E. coli* in fish from the River Kabul is part of this broader trend of coselection and the spread of resistance in the environment.

Further, we analysed the four heavy metal-resistant isolates (S1-S4) at the molecular level. PCR and 16S rRNA sequencing confirmed that all were *Escherichia coli*. We used 16S rRNA sequences to construct a phylogenetic tree and compared our isolates with *E. coli* strains from other regions (Figure 4). The results showed our isolates were very similar to strains from Lahore and Khyber Pakhtunkhwa, Pakistan. They grouped with strains KJ154050.1, KJ154054.1, and KJ154052.1 (Lahore) and PQ373160.1, PQ373117.1, PQ896688.1, and PQ896669.1 (Khyber Pakhtunkhwa), but were different from strains in Japan, China, South Korea, and the USA. This suggests a strong genetic link to Pakistani *E. coli* and points to regional adaptation or a shared environment among heavy metal-resistant *E. coli* in polluted freshwater.

These findings suggest that polluted rivers, fish farms, and wastewater streams in Pakistan may be connected, forming a network in which heavy metal-resistant and potentially multidrug-resistant *E. coli* strains can spread and evolve. Finding *E. coli* in the intestine and opercular region of silver carp has important implications for ecology and public health. The presence of metal-resistant bacteria in fish consumed by people raises the potential for bioaccumulation and/or the transmission of resistance traits to human pathogens through the food chain [46, 52]. The risk is exceptionally high when fish from polluted rivers are consumed without proper monitoring, processing, or cooking. On the other hand, stable indigenous bacteria that survive in such a metal-contaminated environment might be useful for cleaning wastewater by removing metals [53]. However, because *E. coli* can be pathogenic and vectors of resistance genes, any application in biotechnology would have to be very careful in strain selection and genetic details to avoid creating public health problems.

A critical limitation of this study is the absence of direct heavy metal concentration measurements in water and fish tissues from our sampling sites and time period. Although previous studies have documented heavy metal contamination in the River Kabul [6, 18, 19, 25, 26], our study lacks site-specific and temporally matched environmental chemistry data.

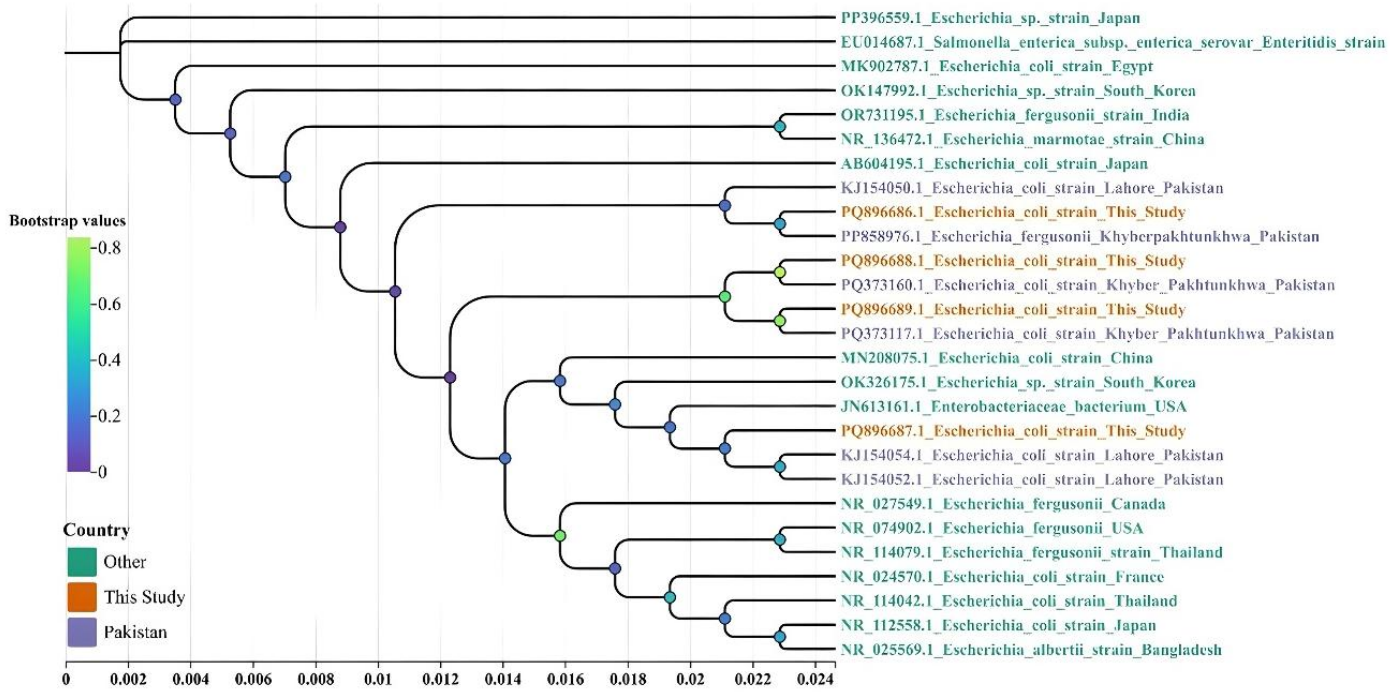

Figure 4. Maximum Likelihood phylogenetic tree is based on partial 16S rRNA gene sequences from heavy metal–resistant *E. coli* isolates found in silver carp, along with reference E. coli strains from GenBank.

This creates a disconnect between the observed bacterial resistance phenotypes and direct evidence of environmental selective pressure, limiting our ability to establish cause-and-effect relationships. Additionally, the selection of only four isolates of *E. coli* for MIC testing and molecular characterization. This small sample size may not fully represent the diversity of heavy metal resistance phenotypes or genetic lineages present in the River Kabul ecosystem. The observed resistance patterns, while informative, should be interpreted as preliminary characterization rather than definitive population-level traits.

4. Conclusion

The present study revealed the presence of heavy metal-resistant *Escherichia coli* in silver carp from the River Kabul. The bacterial isolates demonstrated high MIC values for zinc, nickel, and copper (resistance up to 1900 µg/mL), but remained sensitive to mercury at lower concentrations. Genetic analysis showed that these *E. coli* strains are closely related to those found in Pakistan, suggesting adaptation within local freshwater environments. The detection of heavy metal-resistant *E. coli* in silver carp, a commercially important and widely consumed fish species, highlights potential food safety and public health concerns for communities relying on the River Kabul for nutrition and income. However, comprehensive public health risk assessment requires additional data currently unavailable in this study, including: (i) actual heavy metal concentrations in consumed fish tissues and their comparison to food safety standards (FAO/WHO limits), (ii) antibiotic resistance profiles of these isolates to assess multidrug resistance potential, (iii) presence of virulence genes (e.g., Shiga toxin, enterotoxins) to determine pathogenic potential, (iv) survival of these bacteria through typical food preparation and cooking practices, and (v) prevalence studies across different seasons and fish species. These knowledge gaps should be addressed in future research to provide evidence-based risk assessments and inform food safety guidelines for fish consumption from the River Kabul.

Author contributions

Abdur Rahman: Investigation, Formal analysis, Methodology, Validation. Anees ur Rehman & Mujahid Rahim: Validation, Methodology. Noor ul Akbar: Writing, review, and editing. Shehzad Zareen: Conceptualization, Supervision. Muhammad Shehzad: Writing-original draft, Formal analysis, Validation. All authors have read and approved the final manuscript for publication.

Ethical approval

Ethical approval was obtained from the KUST Ethical Committee, KUST, Kohat (Dated: March, 2024).

Conflicts of Interest

The authors report no conflicts of interest.

Acknowledgment

The authors gratefully acknowledge the staff members of the Nowshera district fisheries for their assistance in collecting samples from the designated locations.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

REFERENCES

1. Gopal, B. Freshwater ecosystems: Ecological characteristics and ecosystem services. In: Ecosystems and integrated water resources management in South Asia, 2020, pp. 13–48. Routledge India.
2. Mishra, R. K. Fresh water availability and its global challenge. British Journal of Multidisciplinary and Advanced Studies, 2023. 4(3), 1–78. https://doi.org/10.37745/bjmas.2022.0208.
3. Ilyas, M., Ahmad, W., Khan, H., Yousaf, S., Yasir, M., & Khan, A. Environmental and health impacts of industrial wastewater effluents in Pakistan: a review. Reviews on Environmental Health, 2019. 34(2), 171–186. https://doi.org/10.1515/reveh-2018-0078.
4. Li, X., Liu, T., Zhang, Y., Cai, J., He, M., Li, M., et al. Growth of BiOBr/ZIF-67 nanocomposites on carbon fiber cloth as filter-membrane-shaped photocatalyst for degrading pollutants in flowing wastewater. Advanced Fiber Materials, 2022. 4(6), 1620–1631. https://doi.org/10.1007/s42765-022-00189-w.
5. Khan, B., Ullah, H., Khan, S., Aamir, M., Khan, A., & Khan, W. Sources and contamination of heavy metals in sediments of Kabul River: The role of organic matter in metals retention and accumulation. Soil and Sediment Contamination: An International Journal, 2016. 25(8), 891–904. https://doi.org/10.1080/15320383.2016.1224226.
6. Singh, V., Singh, J., & Mishra, V. Sorption kinetics of an eco-friendly and sustainable Cr(VI) ion scavenger in a batch reactor. Journal of Environmental Chemical Engineering, 2021. 9(2), Article 105125. https://doi.org/10.1016/j.jece.2021.105125.
7. Raychaudhuri, S. S., Pramanick, P., Talukder, P., & Basak, A. Polyamines, metallothioneins, and phytochelatins—Natural defense of plants to mitigate heavy metals. In: Studies in Natural Products Chemistry, 2021. 69, 227–261. https://doi.org/10.1016/B978-0-12-819487-4.00006-9.
8. Singh, A., Sharma, A., Verma, R. K., Chopade, R. L., Pandit, P. P., Nagar, V., et al. Heavy metal contamination of water and their toxic effect on living organisms. In: The Toxicity of Environmental Pollutants. IntechOpen, 2022. https://doi.org/10.5772/intechopen.105075.
9. Singh, V., Singh, N., Rai, S. N., Kumar, A., Singh, A. K., Singh, M. P., et al. Heavy metal contamination in the aquatic ecosystem: toxicity and its remediation using eco-friendly approaches. Toxics, 2023. 11(2), Article 147. https://doi.org/10.3390/toxics11020147.
10. Garai, P., Banerjee, P., Mondal, P., & Saha, N. C. Effect of heavy metals on fishes: Toxicity and bioaccumulation. Journal of Clinical Toxicology, 2021. Supplement S18, Article 001.
11. Gozde, E., & Emek, D. Escherichia coli and food safety. In: London, UK, 2019.
12. Novoslavskij, A., Terentjeva, M., Eizenberga, I., Valciņa, O., Bartkevičs, V., & Bērziņš, A. Major foodborne pathogens in fish and fish products: a review. Annals of Microbiology, 2016. 66(1), 1–15.

13. Braz, V. S., Melchior, K., & Moreira, C. G. Escherichia coli as a multifaceted pathogenic and versatile bacterium. Frontiers in Cellular and Infection Microbiology, 2020. 10, Article 548492. https://doi.org/10.3389/fcimb.2020.548492.
14. Pakbin, B., Brück, W. M., & Rossen, J. W. A. Virulence factors of enteric pathogenic Escherichia coli: A review. International Journal of Molecular Sciences, 2021. 22(18), Article 9922. https://doi.org/10.3390/ijms22189922.
15. Yang, S., Deng, W., Liu, S., Yu, X., Mustafa, G. R., Chen, S., et al. Presence of heavy metal resistance genes in Escherichia coli and Salmonella isolates and analysis of resistance gene structure in E. coli E308. Journal of Global Antimicrobial Resistance, 2020. 21, 420–426. https://doi.org/10.1016/j.jgar.2020.01.009.
16. Terzi, E., & Civelek, F. Cu, Cd, As and Hg resistance levels in Escherichia coli isolated from Mediterranean mussel and sea snail in the Southeastern Black Sea. Marine Science and Technology Bulletin, 2021. 10(1), 36–41. https://doi.org/10.33714/masteb.776902.
17. Nguyen, C. C., Hugie, C. N., Kile, M. L., & Navab-Daneshmand, T. Association between heavy metals and antibiotic-resistant human pathogens in environmental reservoirs: A review. Frontiers of Environmental Science & Engineering, 2019. 13(3), Article 46.
18. Siraj, M., Khisroon, M., Khan, A., Zaidi, F., Ullah, A., & Rahman, G. Bio-monitoring of tissue accumulation and genotoxic effect of heavy metals in Cyprinus carpio from River Kabul, Khyber Pakhtunkhwa, Pakistan. Bulletin of Environmental Contamination and Toxicology, 2018. 100, 344–349. https://doi.org/10.1007/s00128-017-2265-5.
19. Siraj, M., Khisroon, M., & Khan, A. Bioaccumulation of heavy metals in different organs of Wallago attu from River Kabul, Khyber Pakhtunkhwa, Pakistan. Biological Trace Element Research, 2016. 172, 242–250. https://doi.org/10.1007/s12011-015-0572-4.
20. Ali, B., Khan, A., Ali, S. S., Khan, H., Alam, M., Ali, A., Alrefaei, A. F., Almutairi, M. H., & Kim, K. I. Heavy Metals and Microbial Diversity: A Comparative Analysis of Rivers Swat and Kabul. Water, 2023. 15(18), Article 3297. https://doi.org/10.3390/w15183297.
21. Ahmed, I., Sheikh, Z. A., Wani, G. B., & Shah, B. A. Sex variation in hematological and serum biochemical parameters of cultured Chinese silver carp, Hypophthalmichthys molitrix. Comparative Clinical Pathology, 2019. 28(6), 1761–1767. https://doi.org/10.1007/s00580-019-03017-7.
22. Jawdhari, A., Mihăilescu, D. F., Fendrihan, S., Jujea, V., Stoilov-Linu, V., & Negrea, B.-M. Silver carp (Hypophthalmichthys molitrix) presence in Danube Delta and Romania—a review with data on natural reproduction. Life, 2022. 12(10), Article 1582. DOI: 10.3390/life12101582.
23. Rezaei, F., & Shahbazi, Y. Shelf-life extension and quality attributes of sauced silver carp fillet: A comparison among direct addition, edible coating and biodegradable film. LWT – Food Science and Technology, 2018. 87, 122–133. https://doi.org/10.1016/j.lwt.2017.08.068.
24. Salman, A., Kahlon, T. U. M., & Din, M. Pakistan–Afghanistan transboundary water governance. ISSRA Papers, 2018. 10(2).
25. Khan, K., Younas, M., Sharif, H. M. A., Wang, C., Yaseen, M., Cao, X., et al. Heavy metals contamination, potential pathways and risks along the Indus Drainage System of Pakistan. Science of the Total Environment, 2022. 809, Article 151994. https://doi.org/10.1016/j.scitotenv.2021.151994.
26. Khan, S., Kamal, M., Noor, S., & Afzal, S. M. Assessment of heavy metals and its treatment through phytoremediation in groundwater along River Kabul in district Charsadda. Frontiers in Environmental Science, 2024. 12, Article 1392892. https://doi.org/10.3389/fenvs.2024.1392892.
27. Ahmed, T., Nisar, U. B., Khan, S., Abid, M., Zahir, M., Murtaza, R., & Sagin, J. Effects of water qualities of Kabul River on health, agriculture and aquatic life under changing climate. Desalination and Water Treatment, 2022. 252, 319–331. https://doi.org/10.5004/dwt.2022.28259.
28. Rafique, M. Fish fauna of the Himalayas in Pakistan with comments on the origin and dispersal of its high Asian elements. Pakistan Journal of Zoology, 2001. 33(4).
29. Mirza, M., & Sandhu, A. Fishes of the Punjab Pakistan. Urdu Bazar, Lahore, 2007.
30. Zahoor, A., & Rehman, A. Isolation of Cr (VI) reducing bacteria from industrial effluents and their potential use in bioremediation of chromium-containing wastewater. Journal of Environmental Sciences, 2009. 21(6), 814–820. https://doi.org/10.1016/S1001-0742(08)62346-3.
31. Blazevic, D. J. Improved motility-indole medium. Applied Microbiology, 1968. 16(4), 668–668. https://doi.org/10.1128/am.16.4.668-.1968.
32. de Ambrosini, V. M., Martín, M., Merín, M., Batt, C., & Tortorello, M. Classification of the bacteria: traditional. Elsevier, London, United Kingdom, 2014.
33. Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. Diagnostic microbiology. Mosby, St. Louis, 2007.
34. Azam, M., Jan, A. T., Kumar, A., Siddiqui, K., Mondal, A. H., & Haq, Q. M. R. Study of pandrug and heavy metal resistance among E. coli from anthropogenically influenced Delhi stretch of river Yamuna. Brazilian Journal of Microbiology, 2018. 49(3), 471–480. https://doi.org/10.1016/j.bjm.2017.11.001.
35. Bhardwaj, R., Gupta, A., & Garg, J. K. Impact of heavy metals on inhibitory concentration of Escherichia coli—a case study of river Yamuna system, Delhi, India. Environmental Monitoring and Assessment, 2018. 190(11), Article 674. https://doi.org/10.1007/s10661-018-7061-0.
36. Lee, S. W., Najiah, M., & Wendy, W. Antibiogram and heavy metal resistance pattern of Aeromonas spp. isolated from Asian seabass (Lates calcarifer) hatchery. Annales Universitatis Mariae Curie-Skłodowska, Sectio DD, Medicina Veterinaria, 2009. 64(2), 9–13.
37. Allen, D. A., Austin, B., & Colwell, R. R. Antibiotic resistance patterns of metal-tolerant bacteria isolated from an estuary. Antimicrobial Agents and Chemotherapy, 1977. 12(4), 545–547. https://doi.org/10.1128/AAC.12.4.545.
38. Faniyan, O., Akpe, V., & Cock, I. E. Analyzing bacterial species from different environments using direct 16S rRNA gene sequencing methods. Pharmacognosy Communications, 2023. 13(1), 24–33. https://doi.org/10.5530/pc.2023.1.5.
39. Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39(4), 783–791. https://doi.org/10.2307/2408678
40. Hillis, D. M., & Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, 42(2), 182–192. https://doi.org/10.1093/sysbio/42.2.182
41. Patel, J. B. (2001). 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. Molecular Diagnosis, 6(4), 313-321. https://doi.org/10.1054/modi.2001.29158
42. Clarridge, J. E. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clinical Microbiology Reviews, 17(4), 840-862. https://doi.org/10.1128/CMR.17.4.840-862.2004

43. Xie, J., Chen, Y., Cai, G., Cai, R., Hu, Z., & Wang, H. Tree Visualization By One Table (tvBOT): a web application for visualizing, modifying and annotating phylogenetic trees. Nucleic Acids Research, 2023. 51(W1), W587–W592. https://doi.org/10.1093/nar/gkad359.
44. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2022.
45. Wickham, H. Modelling for visualisation. In: ggplot2: Elegant Graphics for Data Analysis, 2016, pp. 221–240. Cham: Springer International Publishing.
46. Kareem, S., Husain, R. H., & Rasheed, R. O. (2022). Bioaccumulation of heavy metals in common carp fish (*Cyprinus carpio*) and its relationship with the protein content. Iraqi Journal of Veterinary Sciences, 36(Supplement I), 173-178. https://doi.org/10.33899/ijvs.2022.135834.2531 .
47. Magray, M. S. U. D., Kumar, A., Rawat, A. K., & Srivastava, S. Identification of Escherichia coli through analysis of 16S rRNA and 16S–23S rRNA internal transcribed spacer region sequences. Bioinformation, 2011. 6(10), Article 370. https://doi.org/10.6026/97320630006370.
48. Afzal, A. M., Rasool, M. H., Waseem, M., & Aslam, B. Assessment of heavy metal tolerance and biosorptive potential of Klebsiella variicola isolated from industrial effluents. AMB Express, 2017. 7(1), Article 184. https://doi.org/10.1186/s13568-017-0482-2.
49. Silver, S., & Phung, L. T. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. Applied and Environmental Microbiology, 2005. 71(2), 599–608. https://doi.org/10.1128/AEM.71.2.599-608.2005.
50. Seiler, C., & Berendonk, T. U. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Frontiers in Microbiology, 2012. 3, Article 399. https://doi.org/10.3389/fmicb.2012.00399.
51. Saima, S., Fiaz, M., Manzoor, M., Zafar, R., Ahmed, I., Nawaz, U., & Arshad, M. Molecular investigation of antibiotic resistant bacterial strains isolated from wastewater streams in Pakistan. 3 Biotech, 2020. 10, 1–11. https://doi.org/10.1007/s13205-020-02366-3.
52. Sheng, L., & Wang, L. The microbial safety of fish and fish products: Recent advances in understanding its significance, contamination sources, and control strategies. Comprehensive Reviews in Food Science and Food Safety, 2021. 20(1), 738–786. https://doi.org/10.1111/1541-4337.12671.
53. Valls, M., & De Lorenzo, V. Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. FEMS Microbiology Reviews, 2002. 26(4), 327–338. https://doi.org/10.1111/j.1574-6976.2002.tb00618.x.

How to cite this article: Rahman, A., Rehman, A. U., Rahim, M., Zareen, S., Akbar, N., & Shehzad, M. (2026). Molecular Identification and Heavy Metal Resistance Profiles of Escherichia coli from Silver Carp in the Contaminated Waters of the River Kabul, Khyber Pakhtunkhwa. Journal of Zoology and Systematics, 4(1), 18–25.