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RESEARCH ARTICLE OPEN ACCESS

Environmental Reservoirs of Antimicrobial Resistance: Phenotypic and Genotypic Characterization of *Escherichia coli* from Aquatic Sources in Northern India

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Abstract

***Escherichia coli* is a key indicator of faecal contamination and a reservoir of antimicrobial resistance genes (ARGs) in aquatic environments. Monitoring its environmental prevalence and resistance patterns in water sources is essential for public health and environmental safety. In this study, a total of 300 water samples were collected from diverse sources. *E. coli* were isolated from 32% samples, with the highest prevalence in river water (72.72%) and the lowest in tap water (10%). All isolates were positive for the *yaiO* gene. Complete (100%) resistance was observed against ampicillin-sulbactam and polymyxin-B, while high resistance was displayed by isolates against Tigecycline (94.6%), ciprofloxacin (94.4%), colistin (94.4%), and amikacin (94.4%). Comparatively, higher sensitivity was noted to azithromycin (46%) and meropenem (41.1%). PCR analysis detected *tetA*/*tetB* genes in 37.5% and *dfrA* in 20.83% of isolates, with significant variation in gene distribution across water sources. The study revealed a substantial prevalence of multidrug-resistant *E. coli* in surface and groundwater sources, posing a potential risk to human and animal health. The presence of ARGs highlights the aquatic environment as a reservoir for resistance determinants, underscoring the need for regular water quality surveillance, improved sanitation, and prudent antibiotic stewardship within a One Health framework.**

Keywords: *Escherichia coli*, Water Contamination, Antimicrobial Resistance, Multidrug-resistance, One Health

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INTRODUCTION

Antimicrobial resistance (AMR) constitutes a significant global health challenge, impacting human, animal, and environmental health. Among bacterial species, *Escherichia coli* plays a pivotal role due to its capacity to acquire and disseminate resistance to multiple antibiotic classes, including β-lactams, fluoroquinolones, aminoglycosides, and tetracyclines.[1] Although typically a commensal organism within the intestinal microbiota, pathogenic strains of *E. coli* are capable of causing urinary tract, gastrointestinal, and systemic infections.[2,3] Furthermore, *E. coli* serves as a sentinel indicator of fecal contamination in aquatic environments.

The waterborne transmission of *E. coli* poses a substantial public health risk, particularly in low-resource settings where inadequate sanitation and limited access to safe drinking water facilitate the spread of resistant bacteria. Contaminated water not only acts as a conduit for human exposure but also serves as a reservoir for resistance determinants that can be horizontally transferred to other bacterial pathogens.[4,5] Environmental contamination is further exacerbated by anthropogenic activities, including agricultural runoff, wastewater discharge, and inadequate sanitation infrastructure, all of which introduce antibiotics and resistant bacteria into surface and groundwater.[6,7] Such water systems function as ecological hotspots for the selection, maintenance, and dissemination of antimicrobial resistance.

The mechanisms by which *E. coli* acquires resistance are diverse and frequently involve horizontal gene transfer mediated by plasmids, integrons, and transposons.[8] These mobile genetic elements facilitate rapid adaptation and the spread of resistance genes across bacterial communities. The selective pressure exerted by widespread antibiotic use in healthcare and agriculture, coupled with the persistence of antibiotics in aquatic ecosystems, accelerates the emergence of resistant strains.[9,10] Consequently, monitoring *E. coli* in environmental waters provides critical insights into both fecal pollution and the broader dynamics of AMR transmission within the community.

Advancements in molecular techniques have enhanced the detection of resistance genes in environmental isolates, thereby improving the understanding of AMR dissemination and informing surveillance strategies.[11] Given the increasing role of aquatic ecosystems in the AMR burden, this study aimed to isolate and characterize *E. coli* from different water sources, determine their antimicrobial resistance profiles, and detect selected resistance genes. The findings are anticipated to support environmental AMR surveillance and guide water quality management and promote responsible stewardship efforts.

MATERIALS AND METHODS

Sampling of water

A total of 300 water samples were collected from different locations across Uttar Pradesh, India. Samples of 10-15 ml were collected in sterile centrifuge tubes and transported to the Veterinary Microbiology laboratory under cold-chain condition for microbiological analysis. The sampling locations were selected across different localities, representing seven types of water sources: tap water, streams, ponds, rivers, handpumps, dams and wells. These sites were identified after several preliminary visits to various communities. Details of samples collected are given in Table 1.

Standard bacterial culture

Standard strain of *E. coli* ATCC 25922 procured from HiMedia (India) was used as a reference strain for the present study.

Bacterial Culture

Pre-enrichment of water samples

Water samples were mixed with pre-enrichment broth, Buffered Peptone water (BPW, 2% w/v) (HiMedia, India), at a 1:10 dilution (1 ml of sample + 9 ml of BPW) to provide a suitable environment to help stressed or low-population bacteria recover and multiply before being transferred to a more selective medium. The mixture in tubes were incubated in a shaking incubator for 12-18 hrs at 37 °C for pre-enrichment.

Table 1. Details of water samples collected in the present study

No.	Source of water sampling	No. of samples collected
1.	Tap water	40
2.	Stream	42
3.	Pond	93
4.	River	22
5.	Handpump	32
6.	Dam	17
7.	Well	54
	Total	**300**

Isolation and characterization of *E. coli*

A loopful of each pre-enriched water sample was streaked onto MacConkey Lactose Agar (MLA, HiMedia, India) and incubated aerobically at 37 °C for 18-24 hrs to encourage the selective growth of Gram-negative bacteria like *E. coli*. MLA is a selective and differential medium which selectively inhibit the growth of Gram-positive bacteria and supports the growth of Gram-negative lactose fermenting bacteria only. Pink colonies on MLA were further sub-cultured on Eosin Methylene Blue (EMB) agar for the development of greenish-black colonies with a characteristic metallic sheen, typical feature of *E. coli.*[12] Morphological, cultural, and bio-chmemical tests were carried out for confirmation of *E. coli* isolates, such as Gram staining (shape, size, arrangement of colonies), catalase, oxidase, IMViC (Indole, Methyl red, Voges Proskauer, Citrate utilization test), Nitrate reduction, Urease, and sugar fermentation (glucose, lactose, sucrose, maltose) tests. Isolates showing Gram-negative rods, positive for catalase, indole, and methyl red, and negative for oxidase, Voges-Proskauer, citrate, and urease were identified as pure colonies of *E. coli* and preserved on nutrient agar slants at 4 °C for future analysis.

DNA extraction (snap chill method)

The snap-chilling method was used to extract bacterial DNA from *E. coli*.[13] Isolates were incubated in 5 ml Brain Heart Infusion (BHI) broth at 37 °C overnight with constant shaking. 1 ml of the broth culture was centrifuged at 8000 rpm for 10 min at 4 °C. The bacterial pallet was washed three times with sterile normal saline solution (0.85% NaCl) and resuspended in 300 µl nuclease-free sterile distilled water. The bacterial suspension was boiled for 5-10 mins in a boiling water bath followed by immediate chilling for 10 min and then lysate was centrifuged at 5000 rpm for 5 min. The supernatant containing crude DNA was collected and stored at -20 °C. The purity and concentration of DNA were checked using a Nanodrop and gel electrophoresis.

PCR confirmation of *E. coli*

All the isolates of *E. coli,* identified by both cultural and biochemical characterization, were confirmed by PCR targeting the *yaiO* gene, a highly conserved and species-specific molecular maker for *E. coli*[14] and the primer sequences used were F: TGATTTCCGTGCGTCTGAATG and R: ATGCTGCCGTAGCGTGTTTC. Agarose gel electrophoresis (1.5% agarose in 1X TAE buffer) was used to evaluate the amplified PCR products. It was run at 80 V/cm for 45 min and stained with ethidium bromide (0.5 µg/ml). PCR product of each sample (5-10 µl) was loaded in individual wells of gel alongside a positive control. The amplicon size was determined by a 100 bp DNA ladder as a molecular weight marker. The products were visualized under a UV transilluminator and documented using a gel documentation system (AlphaImager).

Antibiotic sensitivity test

All the pure culture isolates of *E.coli* were subjected to in vitro antibiotic sensitivity testing by disc diffusion method[15] against 22 commonly used antibiotics for *E. coli* (Gentamycin, Ampicillin sulbactam, Doxycycline, Azithromycin, Ofloxacin, Amikacin, Nitrofurantoin, Tetracycline, Trimethoprim, Sulphafurazole, Aztreonam, Co-trimoxazole, Fosfomycin, Enrofloxacin, Polymyxin B, Colistin, Tigecycline, Levofloxacin, Ciprofloxacin, Meropenem, Cefepime, Imipenem, and Amoxycillin clavulanic acid) following CLSI guidelines. *E. coli* ATCC 25922 culture served as quality control strain. Fresh colonies of *E. coli* were suspended in sterile normal saline (0.85% NaCl) and adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 1.5 × 10^8 CFU/mL). A sterile cotton swab was used to uniformly inoculate the suspension onto Mueller-

Table 2. PCR cycling conditions for detection of *tetA, tetB* and *dfrA gene* of *E. coli*

Target gene	Primer sequence	PCR product size	PCR conditions	PCR reaction volume (25 µl)
tetA	F: 5'-GGTTCACTCGAACGACGTCA-3' R: 5'-CTGTCCGACAAGTTGCATGA-3'	577 bp	1. First cycle (95 °C for 3 min), 2. Subsequent 35 cycles of Denaturation (94 °C for 1 min), Annealing (56 °C for 1 min), & Extension (72 °C for 1 min) 3. Final extension(72 °C for 10 min)	12.5 µl of PCR master mix (1X, Thermo fisher) 10 picomole/µl of each primers forward and reverse, 4.5 µl DNA template
tetB	F: 5'-CCTCAGCTTCTCAACGCGTG-3' R: 5' -GCACCTTGCTGATGACTCTT-3'	634 bp		
dfrA	F: 5'-GGAGTGCCAAAGGTGAACAGC-3' R: 5'-GAGGCGAAGTCTTGGGTAAAAAC-3'	367 bp	1. First cycle (95 °C for 3 min), 2. Subsequent 35 cycles of Denaturation (94 °C for 1 min), Annealing (45 °C for 1.5 min), & Extension (72 °C for 1 min) 3. Final extension (72 °C for 10 min)	12.5 µl of PCR master mix (1X, Thermo fisher) 10 picomole/µl of each primers forward and reverse, 4.5 µl DNA template

Hinton agar (MHA) plates, ensuring complete coverage of the agar surface. After drying the plates for 3-5 minutes at room temperature, antibiotic-impregnated disks (HiMedia, India) were placed on the agar surface using sterile forceps and incubated at 37 °C for 18-24 hrs. Results were recorded after 18-24 hrs incubation and interpreted as sensitive, intermediate, or resistant according to CLSI standards.[16]

Multiplex PCR for detection of *tetA* and *tetB* gene

All the *E. coli* isolates were screened by multiplex PCR assay for *tetA* and *tetB* genes[17] with suitable modifications. PCR was carried out in a 0.2 ml thin wall PCR tubes with final volume of 25 µl reaction mixture with PCR cycling conditions listed in Table 2. Agarose gel electrophoresis (1% agarose in 1X TAE buffer) was used to evaluate the amplified PCR products. The gel was run at 80 V/cm for 45 min and stained with ethidium bromide (0.5 µg/ml). PCR product of each sample (5-10 µl) was loaded in individual wells of gel alongside a positive control. The amplicon size was determined by a 1 kb DNA ladder as a molecular weight marker. The products were visualized under a UV transilluminator and documented using a gel documentation system (AlphaImager).

PCR for detection of *dfrA* gene

For the *dfrA* gene detection, conventional PCR was carried out in all *E. coli* isolates[17] with PCR cycling condition listed in Table 2. Rest of the procedure was same as used for *tetA* and *tetB* detection and the amplicon size was determined by a 100 bp DNA ladder as the molecular weight marker.

Statistical analysis

The data on the prevalence of *E. coli* across water sources, distribution of resistance genes (*tetA*/*tetB*, *dfrA*), and antibiotic susceptibility profiles were analyzed using the Chi-square (χ^2) test of independence. Fisher's exact test was applied where expected frequency were $\leq$5. Statistical analysis was performed using SPSS version 26 (IBM, USA). A P-value <0.05 was considered statistically significant.

RESULTS

Isolation and Identification of *E. coli*

Morphological characterization using Gram staining revealed Gram-negative rod-shaped bacteria in scattered arrangement (Figure 1). Cultural characterization of isolates revealed pink lactose-fermenting colonies on MLA and a greenish metallic sheen on EMB agar (Figures 2a, 2b). TSI (Triple Sugar Iron) agar slant inoculation showed fermentation of all the three sugars; glucose, lactose, and sucrose with the production of gas turning the media to yellow colour completely. Biochemical characterization revealed the isolates positive for catalase, Indole, Methyl red, and nitrate reduction, whereas they tested negative for oxidase, Voges-Proskauer, citrate utilization, and Urease (Figures 3a, 3b). These morphological and biochemical characteristics confirmed a total of 96 isolates as *E. coli* recovered from 300 water samples.

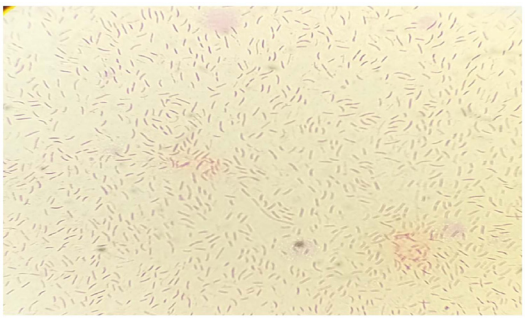


Figure 1. Gram staining of *E. coli* exhibiting pink coloured, rod shaped bacteria in scattered arrangement

Prevalence of *E. coli* in water samples

A total of 96 (32%) *E. coli* isolates were recovered from 300 water samples collected from different sources (Table 3). The prevalence varied across water sources: the highest was in river water (72.7%), followed by ponds (43.0%), and canals (35.7%), while the lowest was observed in hand pump (12.5%) and tap water (10%) samples. The results indicate higher contamination levels in surface water compared to treated or groundwater sources.

Molecular confirmation of *E. coli* by detection of *yaiO* gene

All the 96 isolates of *E. coli* identified through cultural and biochemical characterization were further confirmed as *E. coli* (96/96 = 100%) by PCR targeting *yaiO* gene, which is a species-specific marker gene for identification of *E. coli* (Figure 4).

Antibiotics sensitivity test

Antibiotic sensitivity testing of all the 96 *E. coli* isolates using Kirby-Bauer method against 22 commonly used antibiotics revealed varying resistance patterns (Table 4). The highest

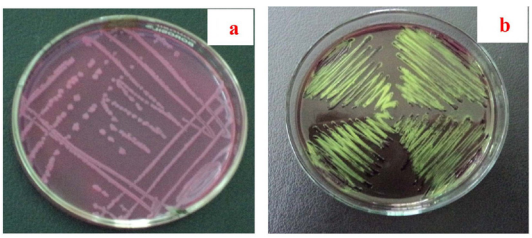


Figure 2. (a) *E. coli* exhibiting pink colored lactose fermenting colony on MLA; (b) *E. coli* exhibiting greenish metallic sheen colony on EMB agar

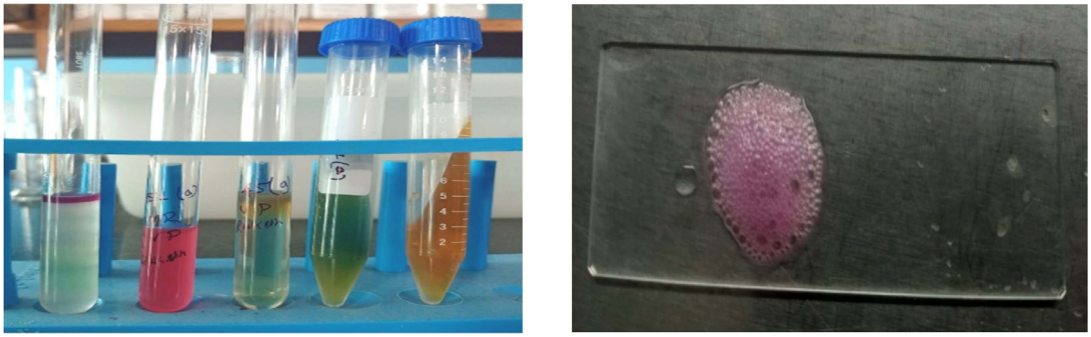
Table 3. Percentage prevalence of *E. coli* isolates in water samples collected from different sources

No.	Source of water sampling	No. of samples collected	No. of *E. coli* isolated	Prevalence (%)
1	Tap water	40	04	10.0
2	Stream	42	15	35.71
3	Pond	93	40	43.01
4	River	22	16	72.72
5	Hand pump	32	04	12.5
6	Dam	17	05	29.41
7	Well	54	12	22.22
	Total	300	96	32

sensitivity was exhibited to Azithromycin (46%), followed by Meropenem (41.1%), Co-trimoxazole (36.8%), Sulphafurazole (36.3%), Imipenem (30.4%), Gentamycin (28.57%), Ofloxacin (28.5%), Cefepime (21%), Levofloxacin (20%), Fosfomycin (17.3%), Aztreonam (11.1%), Enrofloxacin (5.5%), Tigecycline (5.4%) and Doxycycline (1.3%). On the other hand, complete resistance (100%) was exhibited against Ampicillin sulbactam and Polymixin-B, followed by Tigecycline (94.6%), Ciprofloxacin (94.4%), Colistin (94.4%), Enrofloxacin (94.4%), Amikacin (94.4%), Azteronam (88.8%), Amoxycillin clavulinic (80%), Nitrofurantoin (70.5%), Azithromycin (68.4%), Fosfomycin (60.8%), Co-trimoxazole (52.63%), Levofloxacin (50%), Trimethoprim (50%), Cefepime (47.8%), Ofloxacin (47.6%), Imipenem (43.4%), Gentamycin (42.8%), Sulphafurazole (36.36%), Meropenem (35.2%), and Doxycycline (33.3%) (Figure 5). These results display widespread multidrug-resistance (MDR), with more than 80% showing resistance to three or more antibiotics.

Multidrug-resistance profile of *E. coli* isolates from different sources

A total of 13 *E. coli* isolates exhibited diverse multidrug-resistance (MDR) profiles, with resistance ranging from 3-7 antimicrobial classes (Table 5). The majority of isolates showed resistance to ≥5 antibiotic classes, indicating a high burden of MDR. Resistance was most frequently observed against ampicillin–sulbactam, ciprofloxacin, amikacin, polymyxin B, colistin, and tigecycline, while comparatively fewer isolates exhibited resistance to tetracycline, co-trimoxazole, sulphafurazole, and nitrofurantoin. Isolates from river and pond sources demonstrated higher resistance complexity, with some exhibiting resistance to up to 7 antibiotic classes, highlighting substantial variability and environmental influence on resistance patterns.



(a) (b)

Figure 3. (a) IMViC test of *E. coli* with indole positive, MR positive, VP negative and Citrate utilization test negative results. TSI slant exhibiting all 3 sugars fermentation; (b) Catalase test positive result for *E. coli*

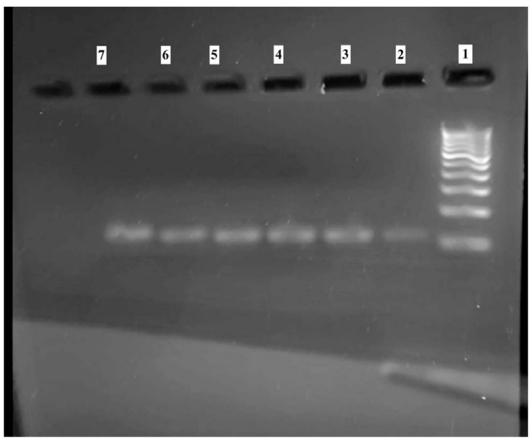


Figure 4. PCR amplification of *yaiO* gene
Lane 1: 100 bp DNA ladder
Lane 2-7: *yaiO* positive sample (amplicon size 115 bp)

Detection of antibiotic resistance genes

Detection of *tetA* and *tetB* genes

Multiplex PCR screening of 96 *E. coli* isolates, using *tetA* and *tetB* specific primers, revealed that 37.5% isolates (36/96) were positive for *tetA* and *tetB* genes (Table 6, Figure 6) and all of these isolates were phenotypically resistant

Table 4. Result of antibiotic sensitivity testing by disc diffusion method

No.	Name of antibiotics	Disc concen.	Sensitive	Intermediate	Resistance
1.	Ampicillin Sulbactam (A/S)	10/10 mcg	-	-	100%
2.	Polymyxin-B (PB)	300 units	-	-	100%
3.	Tigecycline (TGC)	15 mcg	5.4%	-	94.6%
4.	Amikacin (AK)	30 mcg	-	5.5%	94.4%
5.	Enrofloxacin (EX)	10 mcg	5.5%	-	94.4%
6.	Ciprofloxacin (CIP)	5 mcg	5.5%	-	94.4%
7.	Aztreonam (AT)	30 mcg	11.11%	-	88.8%
8.	Amoxycillin Clavulanic acid (AMC)	30 mcg	-	20%	80%
9.	Nitrofurantoin (NIT)	2 mcg	-	29.4%	70.5%
10.	Azithromycin (AZM)	15 mcg	46%	-	68.4%
11.	Fosfomycin (FO)	50 mcg	17.3%	21.7%	60.8%
12.	Co-trimoxazole (COT)	25 mcg	36.8%	10.5%	52.63%
13.	Tetracycline (TR)	30 mcg	16%	33.3%	50%
14.	Levofloxacin (LE)	5 mcg	20%	30%	50%
15.	Trimethoprim (TE)	5 mcg	-	50%	50%
16.	Cefepime (CPM)	50 mcg	21%	31.2%	47.8%
17.	Ofloxacin (OF)	5 mcg	28.5%	23.8%	47.6%
18.	Imipenem (IPM)	10 mcg	30.4%	26.2%	43.4%
19.	Gentamycin (GEN)	10 mcg	28.57%	28.57%	42.8%
20.	Sulphafurazole (SF)	300 mcg	36.3%	27.2%	36.36%
21.	Meropenem (MRP)	10 mcg	41.1%	23.7%	35.2%
22.	Doxycycline (DO)	30 mcg	1.3%	58.33%	33.3%

Table 5. Multidrug-resistance profiles of *E. coli* isolates from water sources

Isolate ID	Source	No. of Antibiotic Classes Resistant	Resistant Antibiotics
EC-01	Tap water	5	A/S, CIP, AK, TR, TGC, PB
EC-02	Stream	5	A/S, CIP, AK, TE, COT
EC-03	Stream	6	A/S, CIP, AK, PB, COL, TGC
EC-04	Stream	3	TR, COT, SF
EC-05	Pond	4	CIP, LE, OF, EX
EC-06	Pond	5	CIP, OF, LE, AK, TE
EC-07	Pond	5	CIP, OF, LE, EX
EC-08	River	6	A/S, PB, COL, CIP, AK, AMC
EC-09	River	7	A/S, CIP, AK, PB, COL, EX, TGC
EC-10	River	4	TE, COT, SF, NIT
EC-11	River	3	CIP, LE, OF
EC-12	Dam	5	A/S, COL, CIP, TR
EC-13	Well	5	A/S, AK, PB, COL, TGC

A/S: Ampicillin Sulbactam, CIP: Ciprofloxacin, AK: Amikacin, TR: Tetracycline, PB: Polymyxin-B, TE: Trimethoprim, COT: Co-trimoxazole, COL: Colistin, TGC: Tigecycline, SF: Sulphafurazole, LE: Levofloxacin, OF: Ofloxacin, EX: Enrofloxacin, AMC: Amoxycillin clavulanic acid, NIT: Nitrofurantoin

Table 6. Multiplex PCR result for detection of *tetA*, *tetB*, and *dfrA* genes in *E. coli*

Source of sample collection	No. of samples collected	No. of *E. coli* isolated	No. of isolates positive for *tetA* gene	No. of isolates positive for *tetB* gene	No. of isolates positive for *tetA* & *tetB* gene	No. of *E. coli* isolate positive for *dfrA* gene
Tap water	40	04	01	0	0	0
Canal	42	15	04	02	01	03
Pond	93	40	09	05	3	10
River	22	16	03	02	01	04
Hand pump	32	04	01	0	0	0
Dam	17	05	01	01	0	01
Well	54	12	04	03	02	02
Total	300	96	23	13	07	20

to tetracycline. These genes encode tetracycline efflux pumps, which confer resistance by actively pumping tetracyclines out of the bacterial cell.

Detection of *dfrA* gene

The *dfrA* gene encodes a modified dihydrofolate reductase, which is insensitive to trimethoprim inhibition, and thus confers resistance against trimethoprim, a co-trimoxazole component. Conventional PCR revealed that 20.83% (20/96) of the isolates were positive for the *dfrA* gene (Table 6, Figure 7)

DISCUSSION

The present study investigated the identification, prevalence, and antimicrobial resistance (AMR) profiles of *Escherichia coli* isolated from different water sources in Uttar Pradesh, India. The isolates displayed morphological characteristics typical to *E. coli* and culturally they produced pink lactose-fermenting colonies on MLA, with a characteristic metallic-green sheen on EMB agar which confirmed their identity as *E. coli*. Identity of the isolates was further validated by biochemical characterization, whereby all the isolates were positive for catalase, indole, methyl red, and nitrate reduction tests; and negative for oxidase, Voges-Proskauer, citrate utilization, and urease tests. The complete agreement between the cultural and biochemical results suggests that the conventional approach remains a reliable and cost-effective method for preliminary screening of *E. coli* from environmental samples.[18,19] Molecular confirmation targeting *yaiO* gene, a species specific molecular marker for *E. coli*, further validated all 96 isolates as *E. coli*. This gene is a reliable marker for species confirmation and complements traditional culture methods, reducing false negatives and improving diagnostic accuracy.[20] These findings are consistent with Anbazhagan et al.[21] and Molina et al.,[20] who demonstrated that *yaiO*-based PCR offers high specificity and sensitivity for *E. coli* identification. Integrating molecular methods strengthens environmental surveillance by identifying viable but non-culturable cells that conventional techniques often miss.[22]

The detection of *E. coli* in environmental water sources is a well-recognized indicator of fecal contamination and poses significant risks for the transmission of waterborne diseases. In the present study, *E. coli* was isolated from 32% (96/300) of water samples collected from diverse aquatic sources. The highest prevalence was recorded in river water (72.7%), followed by pond water (43.0%), and canal water (35.71%). On the other hand, comparatively lower prevalence was observed in hand pump (12.5%) and tap water (10%). The high prevalence in rivers and ponds may be attributed to direct discharge of untreated domestic sewage, animal waste runoff, and agricultural effluents.[23] Though at different rates, the prevalence of *E. coli* in different water sources in India is a consistent observation, such as 28.7% in Ayodhya,[24] 9.6% in Himachal Pradesh,[25] and 56.5% in Punjab.[26] Similarly, the prevalence rate of 25.2% was reported in Nepal[27] and 56% in Bangladesh[28] with whom India shares the river waters. In contrast, the relatively low prevalence in tap and hand-pump water reflects the effect of chlorination and filtration practices that limit bacterial survival. However, even the presence of

E. coli in these treated sources indicates possible post-treatment contamination or leakages in water pipelines. The groundwater contamination may also be by percolation or inadequate protection as well.

The antibiotic susceptibility testing of 96 *E. coli* isolates revealed a diverse and concerning resistance pattern, indicating widespread multidrug-resistance (MDR) among isolates obtained from water sources in Uttar Pradesh. Multidrug-resistance (MDR) has been defined as acquired non-susceptibility to at least one agent in three or more antimicrobial classes.[29] The results demonstrated complete resistance (100%) to ampicillin-sulbactam and polymyxin B, along with very high resistance (≥94%) to tigecycline, ciprofloxacin, colistin, enrofloxacin, and amikacin. Moderate resistance was observed to imipenem (43.4%), gentamicin (42.8%), and co-trimoxazole (52.6%), while azithromycin (46%), meropenem (41.1%), and co-trimoxazole (36.8%) were among the few antibiotics still exhibiting some efficacy. These findings signify the alarming presence of MDR *E. coli* in environmental waters, which could act as both reservoir and channel for resistance genes transferable to human and animal pathogens. The high resistance to β-lactams, polymyxins, and fluoroquinolones indicates extensive antibiotic selection pressure, likely arising from the misuse of these drugs in human medicine, livestock production, and agricultural applications.[30,31] The 100% resistance to ampicillin-sulbactam is consistent with reports by Avatsingh et al.[32] and Johnson et al.,[33] who documented complete loss of β-lactam efficacy in *E. coli* isolated from wastewater and river samples. Similarly, colistin resistance (94.4%) is particularly concerning since colistin is a last-resort antibiotic for carbapenem-resistant Enterobacteriaceae. The high colistin resistance in environmental isolates may be attributed to the dissemination of *mcr* (mobilized colistin resistance) genes.[34] In this study, the resistance to third-generation cephalosporins such as cefepime and

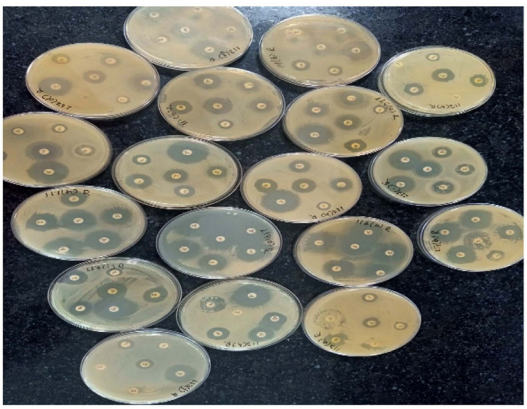


Figure 5. MHA plates showing different sensitivity and resistance pattern in ABST test result

Figure 6. Multiplex PCR of *tetA* and *tetB* gene detection
Lane 1: 1 kb DNA ladder (1st band-250 bp, 2nd band-500 bp and so on
Lane 3: *tetA* positive sample with 577 bp PCR product size
Lane 4: *tetA* (577 bp) and *tetB* (634 bp) positive sample

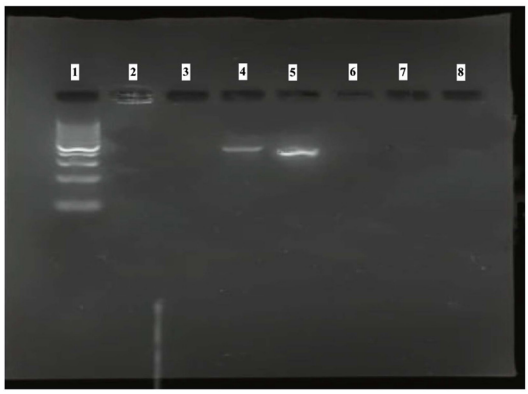


Figure 7. PCR amplification of *dfrA* gene
Lane 1: 100 bp DNA ladder
Lane 4-5: *dfrA* gene positive sample (amplicon size 367 bp)

to fluoroquinolones exceeded the levels previously reported from India and other low and middle-income countries,[25] suggesting dissemination of ESBL-producing strains and plasmid-mediated quinolone resistance mechanisms.[35]

The moderate resistance to carbapenems (imipenem 43.4%; meropenem 35.2%) suggests the potential emergence of carbapenemase-producing *E. coli* strains even in natural water systems. This incomplete resistance is consistent with earlier reports that carbapenems still retain efficacy against many ESBL-producing *E. coli.*[36] This trend mirrors findings by Akiba et al.,[37] who observed similar resistance levels among *E. coli* isolates from Indian rivers receiving untreated hospital effluents. However, the presence of carbapenem resistance in environmental isolates underscores the role of aquatic environments in the persistence and dissemination of highly resistant pathogens. Interestingly, despite overall resistance trends, azithromycin (46%) retained partial effectiveness. Azithromycin's residual sensitivity might be due to its relatively lower use in environmental contexts compared to β-lactams or fluoroquinolones. A critical finding in this study was that 83.3% of isolates were multidrug-resistant (MDR), which is equivalent to that reported from Panjab (86.5%),[26] but exceeding the average of 43.6% in low and middle-income countries.[38] The high MDR prevalence suggests strong environmental selection pressures driven by antibiotic misuse in humans, livestock, and aquaculture, as well as persistence of resistance genes in sediments and biofilms.[39-41] Mobile genetic elements such as plasmids, integrons, and transposons likely facilitated resistance gene dissemination among aquatic bacteria.[42]

Multiplex PCR analysis revealed that 37.5% of the *E. coli* isolates carried tetracycline resistance genes (*tetA* and *tetB*). These genes encode efflux pump proteins that actively expel tetracycline molecules from bacterial cells, preventing inhibition of protein synthesis. The presence of *tetA* and *tetB* genes in environmental *E. coli* isolates suggests that tetracycline residues are present in the aquatic environment, likely originating from livestock farms and aquaculture effluents where tetracyclines are extensively used as growth promoters and prophylactics. The presence of tetracycline resistance genes in environmental *E. coli* has been documented in other studies as well.[43-45] Similar trend in the detection rates of these resistance genes have been reported by Daghrir et al.[46] from wastewater and soil in North India (35%-45%) and Rathinavelu et al.[47] and Karkman et al.[48] from river and pond water (30%-40%). On the other hand, lower prevalence (7%) of these resistance genes has been reported in *E. coli* isolates from the River Yamuna[32] and far higher prevalence (70.0%) has been reported from drinking water in China.[49] The consistent reports of these findings across different studies reinforces the notion that *tet* genes are highly stable and easily transmissible in environmental microbiota. The findings reflect the environmental dissemination of ARGs, likely driven by selective pressure from antibiotic use in human, veterinary, and agricultural settings.[50,51] Similarly, the *dfrA* gene was detected in 20.83% of the isolates, conferring resistance to trimethoprim, a component of co-trimoxazole. This detection rate is consistent with earlier reports which revealed the prevalence rate of *dfrA* gene in the range of 18%-25% from environmental and clinical sources.[52-55] The gene encodes an altered dihydrofolate reductase enzyme that is insensitive to inhibition by trimethoprim, thereby maintaining folate synthesis and bacterial growth. In this study, relatively lower prevalence of *dfrA* compared to *tetA/B* can be ascribed to the fact that trimethoprim is clinically less used compared to tetracyclines. However, the presence of *dfrA* in waterborne isolates is epidemiologically significant because of the fact that this gene is frequently located on plasmids or class 1 integrons, which facilitate horizontal transfer among bacterial species. The detection of resistance genes at higher frequencies in isolates form river and pond water reinforces the fact that stagnant and open water bodies serve as hotspots for the persistence and propagation of resistant strains. Environmental persistence of antibiotic resistance genes has been shown to correlate strongly with nutrient load, biofilm formation, and organic pollution.[56] The considerable level of concurrence between phenotypic resistance and the presence of *tet* and *dfrA* genes in several isolates in this study indicates that genetic determinants are the primary contributors to the observed MDR phenotype. However, the presence of phenotypically resistant

isolates without resistant gene detection suggests the involvement of other mechanisms such as mutations in target sites, reduced membrane permeability, or efflux pump overexpression other than *tet* and *dfrA.*[57]

CONCLUSION

This study establishes the widespread distribution of *E. coli* along with an alarming rise of antimicrobial resistance among the environmental isolates from the aquatic ecosystems of Uttar Pradesh posing a serious public health and environmental safety concern. The presence of MDR *E. coli* harboring *tetA*, *tetB*, and *dfrA* genes and their persistence in the environment increases the risk of genetic exchange with pathogenic bacteria, potentially leading to recalcitrant infections under both medical and veterinary settings. Hence, this study underscores the need for regular surveillance of antimicrobial residues and resistance genes in aquatic systems, rational antibiotic stewardship in agriculture and clinical sectors, and wastewater treatment and sanitation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

JB conceptualized the study and collected resources. JB, RRM and NAM performed validation. RRA supervised the study. JB, RK, ESR, RRM and VK applied methodology. RK, ESR and VK performed investigation and data curation. NAM performed formal analysis and wrote the original draft. RRM and JB wrote, reviewed and revised the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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