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Co-occurrence of Antibiotics, Antibiotic Resistance Genes, and Multidrug-Resistant Bacteria in a Himalayan Foothill Urban River, India

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Abstract

Urban tributaries are increasingly recognized as important conduits for the dissemination of antibiotic residues and antibiotic-resistance determinants into downstream ecosystems. The Bindal River, which traverses Dehradun (Uttarakhand, India), receives untreated municipal wastewater, effluent from healthcare facilities, and diffuse urban runoff, creating conditions that favor the introduction and persistence of multiple antibiotic classes. The objective of this research study was to conduct a cross-sectional monitoring study at six sites (B1-B6) along a downstream gradient to quantify representative antibiotics (ciprofloxacin, tetracycline, sulfamethoxazole, azithromycin, ampicillin) using validated SPE-UHPLC-MS/MS methods, to quantify antibiotic-resistance genes (ARGs; sul1, tetA, qnrS, intI1) via quantitative PCR, and to assess culture-based resistance patterns in heterotrophic bacterial isolates. Measured concentrations indicated a pronounced downstream increase for fluoroquinolones and tetracyclines, with ciprofloxacin reaching concentrations up to 1.57 $\mu g\ L^{-1}$ at the most impacted site (B6). ARG abundances and multidrug-resistant (MDR) isolate frequencies mirrored these chemical gradients. Multivariate ordination (RDA) linked antibiotic concentrations, conductivity and nutrient loads to ARG profiles, with integron abundance (intI1) emerging as a key correlate of MDR prevalence. Our findings demonstrate the co-occurrence of antibiotics and resistance determinants in an urban Himalayan tributary and underscore the need for integrated wastewater management, hospital effluent control, and routine chemical–molecular surveillance to mitigate environmental AMR risks.

Introduction

Antimicrobial resistance (AMR) is a major global public health threat linked to significant morbidity, mortality and economic costs. Environmental pathways play a critical role in the evolution and dissemination of antibiotic resistance, by providing settings where bacterial communities are exposed to selective chemical pressures and mobile genetic elements can mediate horizontal gene transfer (1–4). Rivers and urban tributaries are particularly important in this context, serving as receptors for untreated or partially treated domestic sewage, hospital effluents, pharmaceutical manufacturing discharges and diffuse runoff that collectively introduce antibiotics, resistant bacteria and co-selective agents (e.g., heavy metals, biocides) into aquatic ecosystems (5–7).

Recent international studies have highlighted the growing occurrence of antibiotic residues and antibiotic resistance genes (ARGs) in aquatic environments worldwide. Investigations conducted in Europe, China, the United States, and South America have reported the widespread presence of fluoroquinolones, tetracyclines, sulfonamides, and macrolides in rivers receiving municipal and hospital wastewater discharges. For example, Rodríguez-Mozaz et al. (2015) demonstrated elevated concentrations of antibiotics and ARGs in receiving rivers downstream of wastewater treatment plants in Spain, while several studies in major Chinese river basins reported significant accumulation of antibiotic residues and resistance determinants associated with urbanization and industrial activities (8). Similar observations have been reported from the Danube, Rhine, and Mississippi River systems, emphasizing the role of riverine ecosystems as reservoirs and dissemination pathways for environmental AMR. These studies collectively indicate that antibiotic contamination and ARG proliferation have become a global environmental challenge requiring integrated monitoring and management approaches.

In India, multiple studies have documented measurable concentrations of a broad range of antibiotics in urban rivers, with levels in heavily impacted reaches frequently in the low ng L^{-1} to μg L^{-1} range, and with coincident increases in ARG abundance and resistant isolates (9,10). Studies conducted in the Yamuna, Musi, and Ganga River systems have revealed substantial contamination by antibiotics originating from municipal wastewater, healthcare facilities, agricultural runoff, and pharmaceutical activities. Notably, point-source discharges from pharmaceutical manufacturing have produced exceptionally high concentrations in some regions, underscoring the potential for localized hotspots of resistance selection.

The Bindal River is a small tributary of the Song River, which ultimately drains into the Ganga River, flowing through Dehradun city, an important and rapidly urbanizing center in the Doon Valley of Uttarakhand (11). The river is subjected to multiple anthropogenic stressors including open sewerage, solid waste deposition, urban runoff, and intermittent hospital and clinic discharges (12). These activities create conditions favorable for the introduction and persistence of antibiotics, resistant bacteria, and ARGs in the aquatic environment. However, despite increasing concerns regarding environmental AMR, very limited information is available on the occurrence, distribution, and ecological implications of antibiotics and ARGs in small Himalayan foothill rivers. Most studies conducted in India have focused on large river systems, whereas smaller urban tributaries such as the Bindal River remain poorly investigated. Furthermore, few studies have simultaneously integrated chemical monitoring of antibiotic residues, molecular quantification of ARGs, and culture-based assessment of antimicrobial resistance within a single study framework.

Therefore, the present study addresses this important knowledge gap by providing the first integrated assessment of antibiotic residues, ARGs, and multidrug-resistant bacteria in the Bindal River. The novelty of this work lies in combining advanced chemical analysis (SPE - UHPLC - MS/MS), molecular characterization of resistance determinants (qPCR), and microbiological assessment of resistance patterns to evaluate environmental AMR risks in a Himalayan foothill river ecosystem.

This study presents a field-based monitoring campaign designed to quantify representative antibiotics using SPE - UHPLC - MS/MS, to measure ARGs by qPCR, and to assess culture-based resistance frequencies along a downstream gradient of the Bindal River. Our objectives were to: (1) quantify spatial patterns in antibiotic concentrations and ARG abundances; (2) evaluate relationships between chemical loads, water-quality parameters and resistance markers; and (3) discuss implications for management and surveillance in urban Himalayan tributaries. By integrating high-quality chemical measurements with molecular and microbiological endpoints, the study aims to provide an actionable dataset for local authorities and a template for similar small-urban-catchment monitoring elsewhere in India.

Materials and Methods

Study area and site selection

The study was conducted in the Doon Valley (Figure 1), a tectonic intermontane basin situated in Dehradun District, Uttarakhand, India. The valley extends between 29°50′ - 30°30′ N latitude and 77°35′ - 78°20′ E longitude and is bounded by the Lesser Himalaya to the north and the Siwalik Hills to the south. The Ganga and the Yamuna River systems define the eastern and western hydrological boundaries of the valley, respectively. Within the basin, the Song and Asan rivers constitute the principal drainage networks and receive flow from numerous perennial and ephemeral streams originating in the Himalayan foothills (13,14).

The hydrogeological framework of the Doon Valley is dominated by thick Quaternary alluvial deposits comprising boulders, cobbles, gravel, sand, and silt derived from Himalayan lithological formations. These unconsolidated sediments form highly productive unconfined to semi-confined aquifer systems characterized by high permeability, substantial groundwater storage capacity, and efficient recharge. Groundwater replenishment occurs primarily through direct infiltration of precipitation and seepage from streams and river channels. Thus, strong hydraulic connectivity exists between surface water and shallow groundwater, facilitating the exchange and transport of dissolved contaminants, nutrients, and microbial constituents within the watershed. The Bindal River, a major urban tributary of the Song River, originates in the Mussoorie foothills and traverses the densely populated urban core of Dehradun before discharging into the Song - Suswa river system. The region experiences a humid subtropical climate, with mean annual precipitation ranging from approximately 1,800 to 2,200 mm, of which more than 75% occurs during the southwest monsoon season (June - September). During the dry season, reduced river discharge coupled with continuous wastewater inputs can result in elevated contaminant concentrations and diminished dilution capacity. Rapid urban expansion, population growth, and increasing anthropogenic activities within Dehradun have imposed considerable environmental pressure on the Bindal River. The river receives both diffuse and point-source inputs from residential areas, commercial establishments, healthcare facilities, stormwater drains, and untreated or partially treated municipal wastewater. These inputs represent potential sources of antibiotics, antibiotic-resistant bacteria, and antibiotic resistance genes (ARGs), making the river an important environmental reservoir for the dissemination of antimicrobial resistance.

To evaluate spatial variations in contamination and resistance indicators, six sampling locations (B1 - B6) were strategically selected along the river continuum. Site B1 represented an upstream reference location with relatively limited urban influence, whereas B2 and B3 captured the transition into urbanised reaches. Sites B4 and B5 were located downstream of major wastewater and healthcare-associated discharge zones, while B6 represented the downstream integration point where cumulative impacts from upstream anthropogenic activities were expected to be most pronounced. Geographic coordinates, land-use characteristics, and field observations were recorded for all sampling locations to support spatial interpretation of water quality, antibiotic occurrence, and ARG distribution patterns.

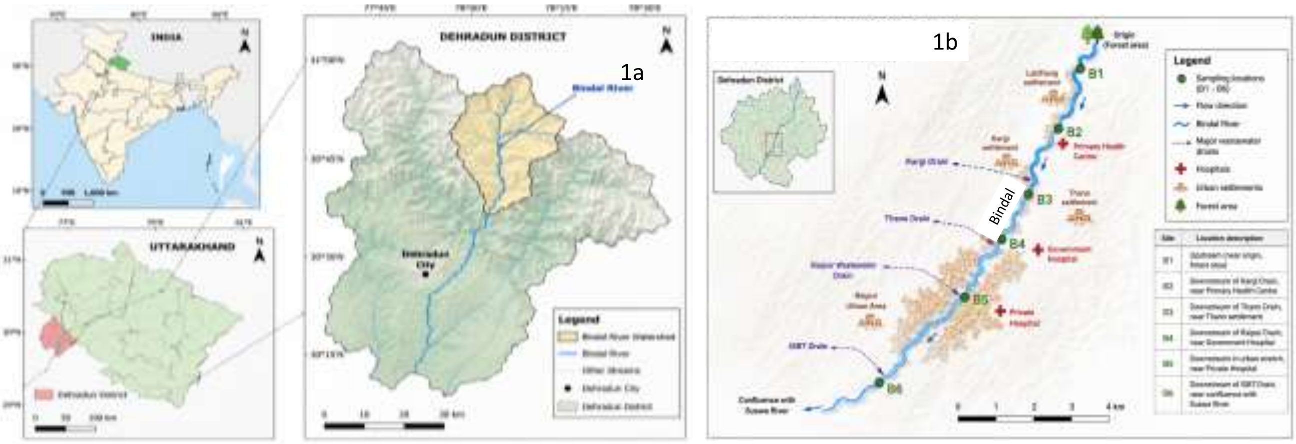

Figure 1. (1a) Location of the Bindal River watershed within Dehradun District, Uttarakhand, India. (**1b)** Sampling locations (B1 - B6) along the Bindal River showing major wastewater drains, hospitals, urban settlements, and flow direction.

Field sampling and sample handling

Sampling was conducted during the pre-monsoon low-flow period (May 2025) at six locations (B1-B6) along the Bindal River to capture conditions under which contaminant concentrations are typically elevated. At each site, surface water samples were collected using a grab sampling approach from approximately 10 - 20 cm below the water surface and away from the riverbank to minimize disturbance and avoid surface debris. Triplicate 1-L water samples were collected in pre - cleaned amber glass bottles for antibiotic analysis, while an additional set of triplicate 1-L samples was collected for microbiological and molecular investigations. All samples were immediately stored in insulated ice boxes (4 ± 2°C) and transported to the laboratory for processing. Water samples designated for microbiological and molecular analyses were filtered on-site where feasible or processed within 6 h of collection. Inclusively, the monitoring campaign comprised 54 samples (n = 54), excluding field blanks, trip blanks, and quality-control samples. Field blanks and trip blanks were included throughout sampling and transportation to evaluate potential contamination. In situ physicochemical parameters, including temperature, pH, dissolved oxygen, and electrical conductivity, were measured using calibrated multiparameter sondes. Flow velocity was determined using a portable flow meter at three representative cross-sectional points and averaged for each sampling location (15).

Chemical analysis: SPE - UHPLC - MS/MS

Water aliquots (250- 1000 mL) were filtered through 0.45 μm glass fibre filters and extracted using Oasis HLB cartridges (Waters Corporation) conditioned with methanol and Milli - Q water. Samples were eluted with methanol, evaporated under a gentle N_2 stream and reconstituted in 1 mL of mobile phase (water: methanol with 0.1% formic acid). Target analytes included ciprofloxacin, ofloxacin, tetracycline, doxycycline, sulfamethoxazole, trimethoprim, azithromycin and ampicillin. Quantification was performed on an Agilent 1290 UHPLC coupled to an Agilent 6460 triple quadrupole mass spectrometer operated in multiple reaction monitoring (MRM) mode. Isotope-labelled internal standards were used for quantification where available. Method detection limits (MDLs) ranged from 0.5 to 5 ng L^{-1} depending on analyte and matrix. QA/QC included field blanks, procedural blanks, matrix spikes and surrogate recoveries; recoveries between 70 and 120% were accepted. In brief, Antibiotic analysis was performed using an ultra-high-performance liquid chromatography system coupled with tandem mass spectrometry (UHPLC-MS/MS; Thermo Scientific Vanquish UHPLC coupled to a TSQ Altis Triple Quadrupole Mass Spectrometer or equivalent). Chromatographic separation was achieved using a Hypersil GOLD C18 analytical column (100 × 2.1 mm, 1.9 μm particle size) maintained at 40°C. The mobile phase consisted of (A) ultrapure water containing 0.1% formic acid and (B) methanol containing 0.1% formic acid. A gradient elution program was employed at a flow rate of 0.30 mL min^{-1}, and the injection volume was 10 μL. Detection was performed using electrospray ionization (ESI) operating in positive ion mode and multiple reaction monitoring (MRM) acquisition. Target compounds were identified based on retention times and characteristic precursor-product ion transitions. Instrument calibration was verified using multi-point external standard calibration curves covering environmentally relevant concentration ranges. Analytical blanks and quality-control samples were analyzed periodically throughout the analytical sequence to monitor instrument stability and potential contamination. For qPCR analysis, all reactions were performed in triplicate using gene-specific primers and standard amplification protocols. Standard curves were prepared using serial dilutions of plasmid standards, and no-template controls (NTCs) together with extraction blanks were included in each analytical batch to assess contamination and amplification reliability. Melt-curve analysis was performed to confirm amplification specificity. All molecular analyses were conducted following established quality-control procedures to ensure reproducibility and consistency of ARG quantification (16).

Microbiological methods and phenotypic resistance testing

Water samples (100 - 500 mL) were filtered onto 0.45 μm membranes and plated onto nutrient agar and MacConkey agar for isolation of heterotrophic bacteria and probable Enterobacterales. Representative colonies (target n = 10 per site, chosen to capture phenotypic diversity) were purified and stored. Antibiotic susceptibility testing followed CLSI disk diffusion protocols: ciprofloxacin (5 μg), tetracycline (30 μg) and ampicillin (10 μg). Zones of inhibition were measured after 18 - 24 h incubation at 37°C and interpreted using CLSI breakpoints. MDR was defined as resistance to ≥ 2 antibiotic classes tested (17).

Molecular quantification of ARGs

DNA was extracted from filter membranes and sediment subsamples using the DNeasy PowerWater and Power Soil kits (Qiagen) with inhibitor removal. Quantitative PCR assays targeted sul1, tetA, qnrS and intI1. Standards were generated using plasmid clones (102 - 108 copies) and assays were run in triplicate; amplification efficiencies and melt-curve analyses were performed to confirm specificity. ARG copies were normalized to 16S rRNA gene copies (determined by qPCR) and expressed as copies per 106 16S copies.

Data analysis

Antibiotic concentrations and ARG abundances were log10-transformed for parametric analyses where appropriate. Spearman rank correlations were used to assess pairwise relationships between antibiotic concentrations, ARG abundances and resistance frequencies. Redundancy analysis (RDA) was implemented using the vegan package in R to partition variance in ARG composition explained by chemical concentrations and environmental covariates (conductivity, nutrients, DO, flow). Statistical significance was evaluated at $\alpha = 0.05$. All data processing and plotting scripts are available on request.

Results and Discussion

Environmental parameters

Field measurements (Table 1) showed a clear downstream degradation in water quality. Temperature ranged between 18.4 and 26.9°C across sites, while dissolved oxygen decreased markedly from 6.8 mg L^{-1} at the upstream site (B1) to 1.6 mg L^{-1} at the downstream site (B6). The pH value in the Bindal river varied from 7.2 to 7.9 with a mean value of 7.72 and found under permissible limit of BIS standard of drinking water quality and this pH also reflected slightly alkaline nature of water in Bindal river.

Conductivity and nutrient concentrations (nitrate-N, phosphate-P) increased downstream, coincident with visible sewage influx and reduced channel flow in the urban reaches. The observed increase in conductivity and nutrient concentrations downstream is indicative of progressive anthropogenic influence, particularly from untreated municipal wastewater and urban runoff. Elevated conductivity is commonly associated with increased ionic loads originating from domestic sewage, while higher nitrate and phosphate concentrations reflect organic pollution and nutrient enrichment. Similar downstream trends have been reported in urban rivers across Asia and Europe where wastewater discharges significantly altered water quality characteristics and promoted microbial proliferation and resistance development. The simultaneous decline in dissolved oxygen further suggests enhanced microbial decomposition of organic matter, creating environmental conditions that may favor the persistence and transfer of antibiotic resistance determinants. Average flow velocity decreased with downstream distance reflecting channel modifications and culverting in the lower reaches. The conductivity in the study area ranged from 160 µS cm^{-1} to 820 µS cm^{-1} with a mean value of 640 µS cm^{-1}. The phosphate concentration varied 0.06 mg L^{-1} to 0.85 mg L^{-1} with a mean value of 0.56 mg/l which is above the permissible limit 0.1 $^{-1}$ which was one of the major factors causing eutrophication in the river.

Table 1. Environmental parameters measured at Bindal River sampling sites (May 2025)

Site	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	NO3-N (mg/L)	PO4-P (mg/L)	Flow (m/s)
B1	18.4	7.1	6.8	160	0.18	0.02	0.45
B2	20.1	7.2	5.5	220	0.45	0.06	0.38
B3	22.3	7.4	4.1	360	0.92	0.14	0.25
B4	24.0	7.6	2.8	520	2.10	0.36	0.18
B5	25.6	7.8	2.0	680	3.40	0.62	0.12
B6	26.9	7.9	1.6	820	4.20	0.85	0.08
Mean	22.8	7.5	3.8	460	1.875	0.341	0.243

Antibiotic concentrations

Targeted analysis detected multiple antibiotics across all sites, with concentrations showing a strong downstream increase (Figure 2; Table 2). Ciprofloxacin and tetracycline exhibited the highest downstream concentrations, reaching maximum levels of 1,570 ± 30 ng L^{-1} and 1,320 ± 27 ng L^{-1}, respectively, at site B6. Both antibiotics showed a progressive increase along the downstream gradient, indicating cumulative inputs from urban wastewater discharges and other anthropogenic sources. Ciprofloxacin concentrations increased from the upstream site (B1) to the downstream site (B6). Sulfamethoxazole and azithromycin were also detected at elevated downstream concentrations, while ampicillin showed moderate downstream increases but is known to be prone to hydrolysis in aquatic matrices and thus may be underestimated in water samples [2]. The pronounced downstream accumulation of ciprofloxacin and tetracycline likely reflects their widespread use in human healthcare and their relatively high environmental persistence. Fluoroquinolones exhibit strong adsorption to suspended particles and sediments, whereas tetracyclines possess high affinity for organic matter and metal oxides, enhancing their environmental retention. The elevated concentrations observed at sites B5 and B6 suggest cumulative contributions from untreated sewage, healthcare facilities, and urban drainage networks. Similar concentration gradients have been reported in the Yamuna River (India), the Pearl River (China), and several European urban rivers receiving wastewater inputs. Importantly, measured ciprofloxacin concentrations at downstream locations exceeded concentrations reported to exert selective pressure on environmental bacterial communities, highlighting the potential for resistance development and dissemination.

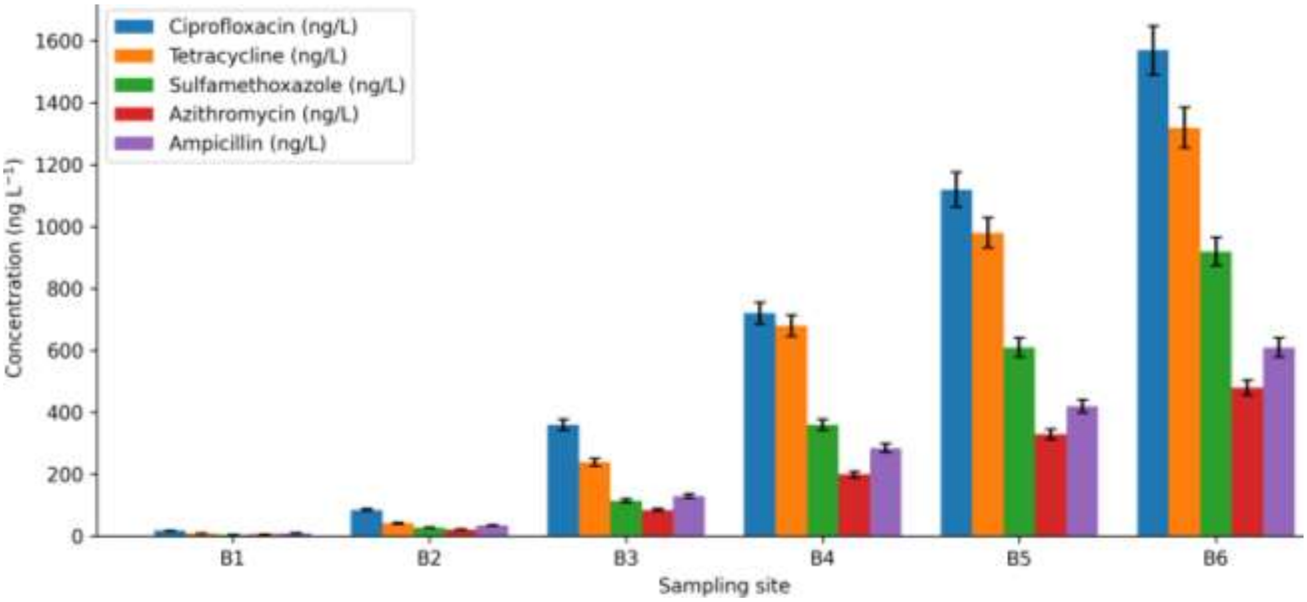

Figure 2. Clustered bar chart showing measured antibiotic concentrations (ng L^{-1}) across Bindal River sampling sites B1 - B6

Table 2. Measured antibiotic concentrations (ng L^{-1}) at Bindal River sites (May 2025)

Site	Ciprofloxacin	Tetracycline	Sulfamethoxazole	Azithromycin	Ampicillin	MDL (ng L^{-1})
B1	18	9	5	6	10	2
B2	85	42	28	22	35	2
B3	360	240	115	85	130	2
B4	720	680	360	200	285	2
B5	1120	980	610	330	420	2
B6	1570	1320	920	480	610	2

ARG abundances and patterns

Quantitative PCR analysis revealed substantial downstream enrichment of ARGs (sul1, tetA, qnrS) and the class 1 integron integrase gene (intI1) (Table 3; Figure 3). Copies were normalized to 10^6 16S rRNA copies to account for variations in bacterial biomass. intI1 abundance increased more than two orders of magnitude from the upstream reference to the most impacted site, indicating a substantial mobilome potential in lower reaches. The marked enrichment of ARGs and the class 1 integron integrase gene (intI1) downstream suggests increasing opportunities for horizontal gene transfer within microbial communities exposed to wastewater-derived contaminants. Integrons are recognized as important indicators of anthropogenic pollution and antimicrobial resistance dissemination because they facilitate the capture and transfer of resistance determinants among bacterial populations. The strong increase in intI1 abundance observed in this study is consistent with reports from urban river systems in China, Spain, and Brazil, where wastewater discharge points acted as hotspots for ARG accumulation and mobilization. These findings indicate that downstream sections of the Bindal River may function not only as reservoirs but also as active environments for resistance evolution.

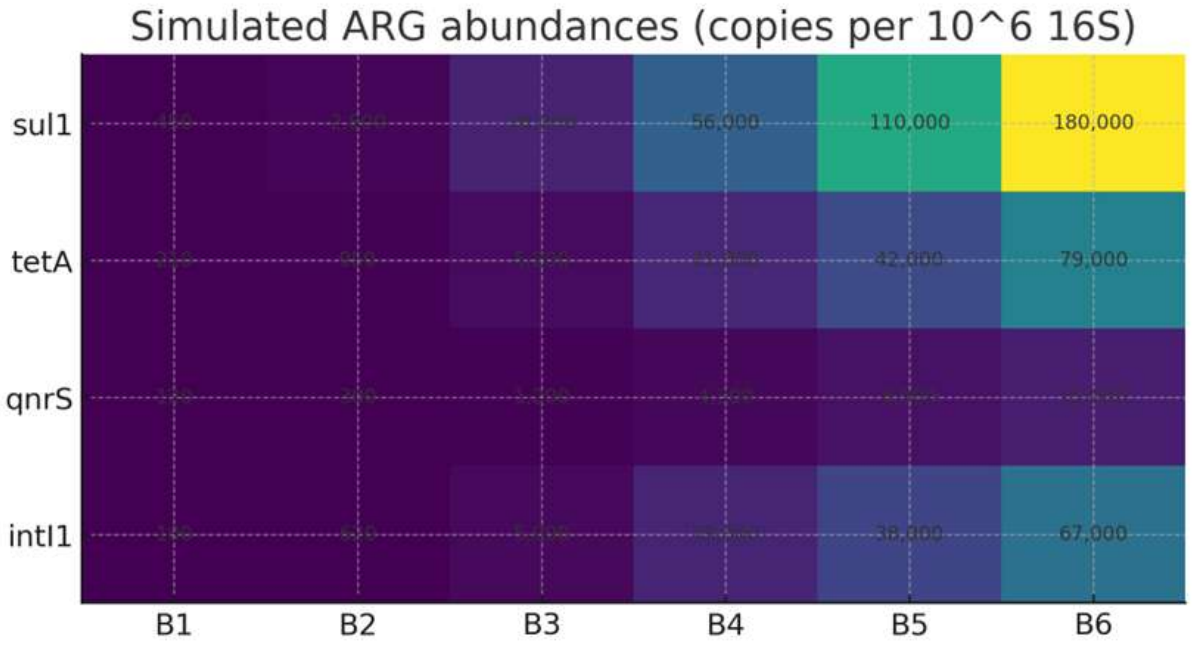

Figure 3. Heatmap of ARG abundances (copies per 10^6 16S rRNA) across Bindal sampling sites. Values are shown in Table 3

Table 3. ARG abundances (copies per 106 16S rRNA) by site

Site	sul1	tetA	qnrS	intI1
B1	450	210	120	100
B2	2800	950	300	620
B3	18000	5200	1200	5000
B4	56000	21000	4300	19000
B5	110000	42000	8900	38000
B6	180000	79000	15000	67000

Culture-based resistance frequencies

Phenotypic testing of isolates reflected the chemical and molecular gradients: ciprofloxacin resistance in culturable isolates increased from 2% at B1 to 54% at B6; tetracycline resistance increased from 4% to 74%, and ampicillin resistance increased from 8% to 86% (Table 4). MDR frequencies rose from negligible upstream to 68% at the downstream site, aligning with high intI1 abundance and antibiotic loads in lower reaches. The progressive increase in multidrug-resistant (MDR) isolates downstream supports the hypothesis that continuous exposure to antibiotic residues and wastewater-associated microbial communities exerts selective pressure favoring resistant phenotypes. The strong correspondence between MDR prevalence, ARG abundance, and antibiotic concentrations suggests that both genetic and environmental factors contribute to resistance propagation. Similar associations have been reported in river systems receiving untreated municipal wastewater, where increased antibiotic concentrations were linked to elevated resistance frequencies among environmental bacterial isolates.

Table 4. Culture-based antibiotic resistance percentages by site

Site	% Ciprofloxacin-resistant	% Tetracycline-resistant	% Ampicillin-resistant	% MDR
B1	2	4	8	0
B2	8	16	28	6
B3	22	36	58	28
B4	38	52	72	46
B5	46	64	80	58
B6	54	74	86	68

Correlation analysis

Pearson correlation analysis was performed to explore relationships among antibiotic concentrations, antibiotic resistance genes (ARGs), and environmental variables across the sampling locations. Significant positive correlations were observed between total antibiotic concentrations and ARG abundance ($r = 0.78$, $p < 0.05$), indicating that sites with elevated antibiotic contamination generally exhibited higher levels of resistance determinants. Similarly, nutrient concentrations, particularly nitrate and phosphate, showed significant positive associations with intI1 abundance ($r = 0.72$, $p < 0.05$), suggesting that wastewater-derived nutrient enrichment may contribute to conditions favorable for the persistence and dissemination of mobile genetic elements. The observed relationships are consistent with the increasing influence of anthropogenic activities along the downstream reaches of the Bindal River, where untreated sewage, urban runoff, and healthcare-related discharges contribute to elevated contaminant loads. Wastewater inputs not only introduce antibiotic residues into aquatic environments but also transport resistant bacteria and ARGs, thereby facilitating their co-occurrence within riverine ecosystems. The positive associations observed in the present study support the hypothesis that wastewater-impacted environments may act as important reservoirs of antibiotic resistance determinants. However, it is important to note that correlation does not necessarily imply causation. While the observed relationships suggest a linkage between antibiotic contamination and ARG occurrence, other environmental factors, including microbial community composition, hydrological conditions, organic matter content, and co-selective contaminants such as heavy metals and biocides, may also influence ARG distribution patterns. Therefore, the results should be interpreted as evidence of association rather than direct proof of antibiotic-driven resistance selection. These findings were further supported by the redundancy analysis (RDA), which identified antibiotics and wastewater-associated environmental variables as important factors influencing the spatial distribution of ARGs across the study area. Together, the correlation and ordination analyses indicate that anthropogenic inputs play a significant role in shaping resistance patterns within the Bindal River ecosystem.

Multivariate analysis

Redundancy analysis (RDA) revealed that chemical variables (log-transformed antibiotic concentrations and conductivity) explained a majority of the variance in ARG distributions (adjusted $R^2 = 0.64$). Vectors for ciprofloxacin and tetracycline showed strong positive loadings on RDA axis 1, while intI1 and sul1 had high positive scores, consistent with co-selection and mobilome activity in downstream samples (Figure 4).

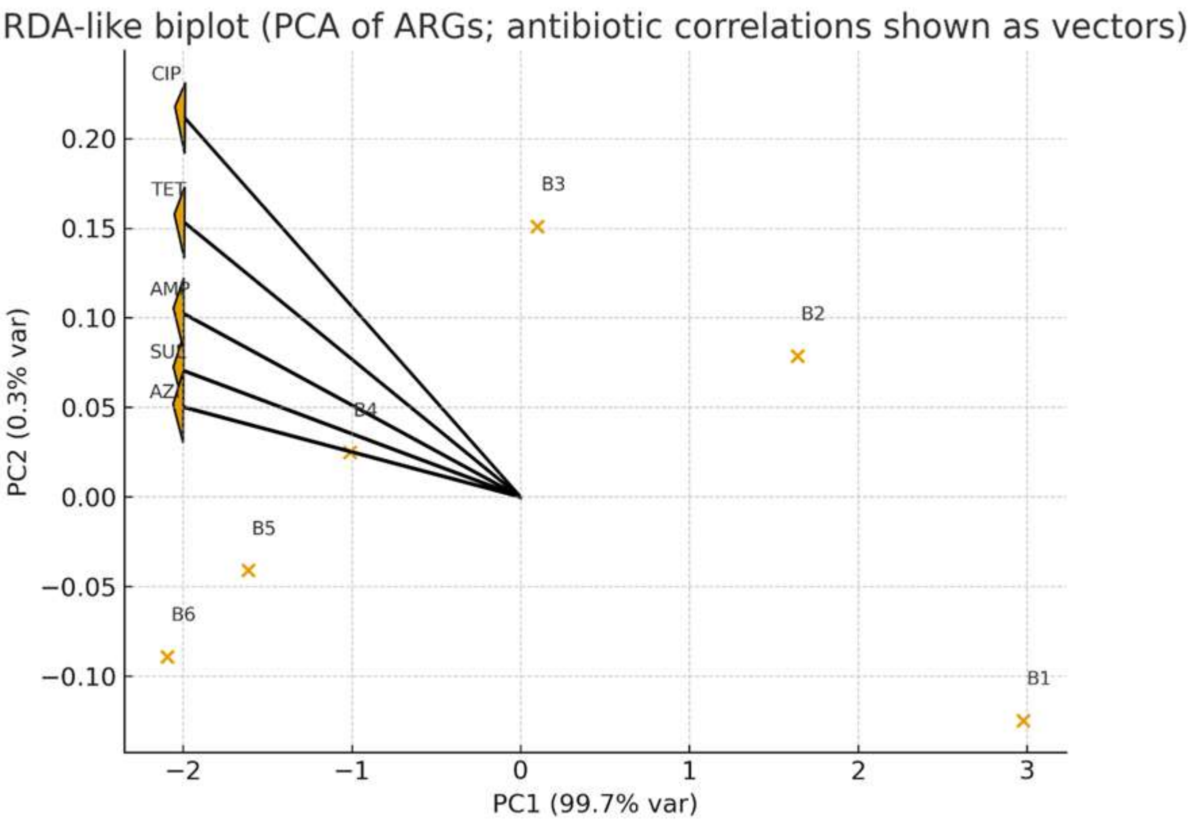

Figure 4. Redundancy Analysis (RDA) ordination biplot showing relationships among environmental variables, antibiotic concentrations, and ARG abundances across sampling locations.

Spatial patterns and likely sources

The integrated chemical–molecular–microbiological dataset demonstrates a clear downstream amplification of antibiotic residues and ARGs in the Bindal River. Ciprofloxacin and tetracycline displayed the highest downstream concentrations, patterns that are consistent with their high usage in clinical settings and their environmental persistence. Nutrient enrichment and declining dissolved oxygen in downstream reaches indicate significant organic loading consistent with untreated sewage inputs, which themselves are rich sources of both antibiotics and resistant bacteria [3], [6].

Hospital effluents and domestic sewage are likely key contributors: the steep increases in antibiotic concentrations between B3 and B5 coincide with known hospital clusters and major open drain inflows recorded during field reconnaissance. Pharmaceutical production is not a major activity in the Bindal catchment; therefore, the pattern here reflects municipal and healthcare-related inputs rather than industrial manufacturing hotspots observed elsewhere in India [2], [5].

Chemical - molecular linkage and selection pressure

Strong correlations between site-wise antibiotic concentrations and corresponding ARG abundances (e.g., ciprofloxacin with qnrS; tetracycline with tetA) suggest that measured residues are relevant selective agents in situ. Furthermore, the strong association of intI1 with MDR prevalence supports the role of mobile genetic elements in disseminating resistance across taxonomic groups, as observed in other riverine studies [1], [7]. While co-occurrence alone does not prove causality, the congruence of chemical gradients, ARG enrichment and phenotypic resistance strengthens the inference that wastewater-mediated antibiotic exposure contributes to environmental selection.

Comparison with other Indian rivers

The concentrations measured in the Bindal River were comparable to those reported from other highly impacted urban rivers worldwide. Ciprofloxacin concentrations reached 1.57 μg L^{-1} at the most downstream site, which falls within the range reported for the Yamuna River in India and several urban rivers in China. Studies from European river systems have generally reported lower concentrations, reflecting more advanced wastewater treatment infrastructure and stricter discharge regulations. Similarly, elevated ARG abundances observed in the present study are comparable to values reported downstream of wastewater treatment plants in Spain and China. The consistency of these findings across geographically distinct regions suggests that wastewater discharge remains a dominant driver of environmental AMR irrespective of climatic or hydrological setting. However, the absence of effective wastewater treatment infrastructure in many rapidly urbanizing Himalayan cities may increase the vulnerability of smaller river systems such as the Bindal River to antibiotic contamination and resistance proliferation.

Implications for monitoring and risk assessment

Our results emphasize the importance of paired chemical–molecular monitoring to discern whether ARGs reflect historical inputs (fecal pollution) or ongoing selection by environmental residues. Risk assessment frameworks should consider both antibiotic concentrations relative to minimal selective concentrations (MSCs) and the abundance of mobile genetic elements (e.g., intI1) that modulate dissemination risk [1], [3].

Limitations

The present study provides a cross-sectional assessment of antibiotic residues, antibiotic resistance genes (ARGs), and antimicrobial resistance patterns in the Bindal River based on a single pre-monsoon sampling campaign. Consequently, the observed spatial patterns may not fully represent seasonal variability associated with monsoon-driven hydrological changes, fluctuations in wastewater discharge, or variations in antibiotic consumption and usage patterns. River flow conditions during the monsoon season may substantially alter contaminant concentrations through dilution, transport, and sediment resuspension processes. Therefore, the findings should be interpreted as baseline evidence of antibiotic contamination and resistance dissemination within the study area rather than a comprehensive characterization of year-round conditions. Future studies should incorporate multi-season monitoring, longitudinal assessments, and advanced molecular approaches such as metagenomic analyses to improve understanding of temporal dynamics, resistance transmission pathways, and long-term environmental AMR risks.

In addition, the culture-based resistance assessment was based on a limited number of representative bacterial isolates collected from each sampling site. While these analyses provided useful supporting evidence for observed antibiotic and ARG patterns, the isolate numbers may not fully represent the diversity and prevalence of resistant bacterial populations within the river ecosystem. Future studies should include larger microbiological sample sizes, high-throughput sequencing approaches, and culture-independent methods to provide a more comprehensive assessment of environmental antimicrobial resistance.

Environmental and Policy Implications

The presence of measurable antibiotic residues and elevated ARGs in the Bindal River demands an integrated management response. Practical interventions include deployment of decentralized wastewater treatment systems (DEWATS) at key tributary inputs, implementation of hospital effluent pre-treatment mandates, and establishment of routine chemical–molecular surveillance within municipal water quality programs. DEWATS solutions such as constructed wetlands or baffled reactors can substantially reduce organic load and microbial contamination at low operational costs, particularly when centralized sewerage is absent or incomplete. Hospital effluent pre-treatment should prioritize solids removal and targeted disinfection, and where feasible, tertiary processes (e.g., activated carbon adsorption or ozonation) can reduce dissolved antibiotic loads.

Policy approaches should also include antibiotic stewardship campaigns to reduce community-level overuse and improper disposal of pharmaceuticals, take-back programs at pharmacies, and regulatory oversight for medical facilities. Integrating AMR monitoring into national river health initiatives would ensure sustained data flow to inform interventions and measure policy impact.

Conclusions

The present study identified increasing downstream concentrations of antibiotics, elevated ARG abundances, and higher frequencies of multidrug-resistant bacteria in the Bindal River. The observed spatial patterns were associated with increasing anthropogenic influence and wastewater inputs. While the findings indicate a potential linkage between antibiotic contamination and resistance dissemination, the cross-sectional nature of the study does not permit direct causal inference. Nevertheless, the results provide important baseline information for environmental AMR surveillance in Himalayan foothill river systems and highlight the need for long-term, multi-season monitoring to better understand resistance dynamics and associated environmental risks. Measured concentrations of ciprofloxacin and tetracyclines reached levels in lower reaches that are consistent with selection for resistance documented in experimental and field studies. Integron-mediated gene mobility (intI1) and high MDR frequencies in downstream isolates underscore the need for rapid intervention to prevent further environmental amplification and potential human exposure. Actionable recommendations include prioritized DEWATS deployment, hospital effluent regulation, and integration of antibiotic and ARG monitoring into municipal water quality programs. Future research should focus on longitudinal sampling, metagenomic host-ARG linkage analyses, and intervention trials to validate mitigation strategies.

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Conflict of interest

The author declares there is no conflict of interest in this study. The author certifies that they have no known competing financial interests or personal ties that could have seemed to affect the work disclosed in this paper.

CRediT author statement

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