Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 18 May 2026
Revised 12 June 2026
Accepted 01 July 2026

Natural Resources for Human Health 2026, Vol. 6 (4s): 190–212

KEYWORDS:
Ganga Water, Water Biochemical Test, Metal Sensitivity Test, Antibiotics Sensitivity Test, Bacteriophages

From Source to Estuary: Spatiotemporal Dynamics of Water Quality, Microbial Communities, Antimicrobial Resistance, and Bacteriophage Diversity in the Ganga River

Prashant Singh[1], Ranjan Singh[2]*, Rohit Kumar[2], Vinay Singh Baghel[3]

[1]Department of Microbiology, Dr Rammanohar Lohia Avadh University, Ayodhya, India prashantofficialmicro@gmail.com , ORCID: https://orcid.org/0009-0006-4257-4688

[2]* Department of Microbiology, Dr Rammanohar Lohia Avadh University, Ayodhya,India, ranjan.singh13@gmail.com ORCID: https://orcid.org/0000-0001-8830-9210

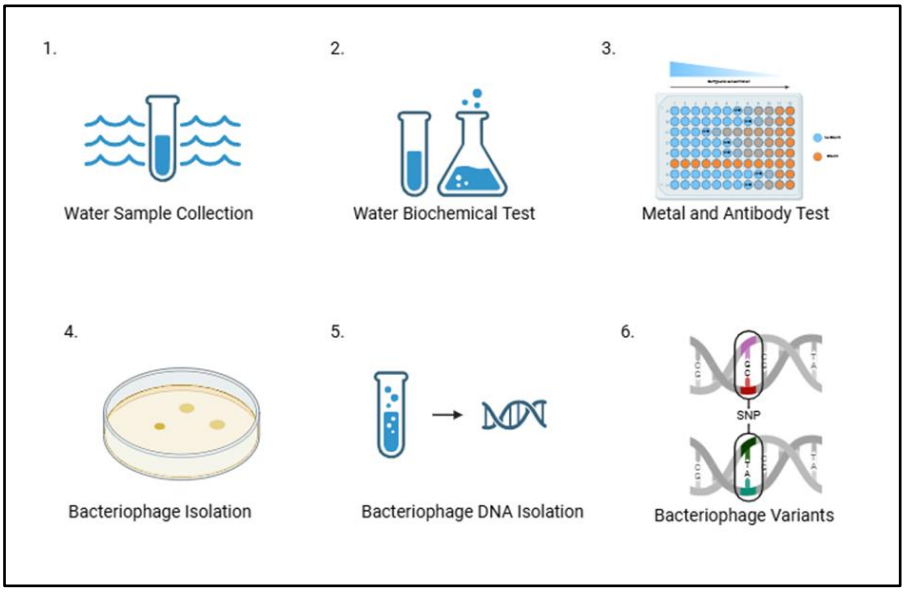
[3]Department of Biotechnology, SRM University, Delhi-NCR, Sonipat, Haryana, India, kumarrohitt121998@gmail.com

[4]Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow, India , baghelbbau@gmail.com , ORCID: https://orcid.org/0009-0008-4075-2545

ABSTRACT: The Ganga River is one of the world's most important freshwater ecosystems, sustaining millions of people while increasingly experiencing anthropogenic stress from urbanization, industrial discharge, agricultural runoff, and untreated sewage. This study investigated spatial and seasonal variations in water quality, microbial diversity, antimicrobial resistance, metal tolerance, and bacteriophage distribution along the river. Water samples were collected from 17 sites spanning the upper, middle, and lower Ganga during summer, monsoon, and winter, yielding 51 sampling events. Physicochemical parameters, coliform abundance, culture-based bacterial identification, antibiotic and metal tolerance assays, bacteriophage isolation, and phage-marker PCR screening were performed. Upstream sites exhibited comparatively low turbidity, TSS, BOD, COD, and microbial loads, whereas middle and downstream stretches showed significantly elevated values, particularly during the monsoon season. Coliform counts frequently exceeded 1000 MPN/100 mL, indicating substantial faecal contamination. The predominant culturable bacteria included Escherichia coli and Pseudomonas aeruginosa, together with other environmental and enteric taxa. These isolates displayed variable tolerance to tetracycline, levofloxacin, azithromycin, and selected metals, reflecting potential adaptation to persistent environmental stressors. Diverse bacteriophage populations and phage-associated genetic markers were detected across river stretches, indicating active phage–host interactions. Overall, the findings demonstrate that anthropogenic inputs and seasonal hydrological changes strongly influence microbial ecology and resistance patterns in the Ganga River, highlighting the need for continuous monitoring and improved wastewater management to safeguard ecosystem and public health.

Natural Resources for Human Health
eISSN: 2583-1194

Grapical Abstract



The graphical abstract depicts that the water samples were collected under sterile conditions to ensure the integrity of downstream microbiological and biochemical analyses. The collected samples were subsequently subjected to a series of biochemical assays to evaluate key physicochemical parameters and microbial activity. In parallel, the detection and quantification of metals and specific antibiotics were carried out to assess environmental contamination and associated immunological indicators. Bacteriophages were isolated from the water samples using appropriate bacterial host strains, primarily through standard plaque assay techniques. Following isolation, bacteriophage genomic DNA was extracted and purified for further molecular characterisation. Additionally, genetic variations, including the variants, within bacteriophage genomes were identified and analysed to investigate phage diversity, evolutionary dynamics, and their potential role in microbial adaptation.

INTRODUCTION

The Ganga River is a freshwater ecosystem of global ecological and socioeconomic importance, sustaining millions of people across India through agriculture, fisheries, and domestic water use (Clayton et al., 2025). Rivers continue to be the primary source of surface water, nonetheless. About 252.8 million hectares make up the whole catchment area of India's many big, medium and minor rivers (Singh & Singh, 2026b). Ganga River originated in the state of Uttarakhand and occupies more than one-fourth of the nation's land area (Singh & Singh, 2026a). The Ganga basin is home to around half of the country's class 1 and class 2 towns, most of which dispose of their waste in the Gangetic River system (Singh & Singh, 2026b). The increasing pollution of river systems poses a significant challenge to water quality management and public health (Singh & Singh, 2026). Key physicochemical indicators such as pH, turbidity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solids (TSS) serve as primary markers of freshwater ecosystem health, reflecting organic and inorganic load that affects dissolved oxygen balance and microbial dynamics (Das, 2025). Anthropogenic pressures have also resulted in metal contamination in river waters, particularly copper, cadmium, and chromium, which pose chronic toxicity risks to aquatic life and human health. Freshwater environments increasingly act as reservoirs and conduits for antimicrobial resistance (AMR), facilitating horizontal gene transfer among pathogenic and non-pathogenic bacteria (Hama Aziz et al., 2023). These genetic elements can be disseminated through human activities and wastewater treatment inefficiency, contributing to public health risks. Concurrently, bacteriophages, estimated to be the most abundant biological entities in aquatic ecosystems, govern microbial population dynamics, mediate horizontal gene transfer, and hold potential as ecological indicators or therapeutic agents against AMR pathogens (Kusi et al., 2022). Despite individual studies on water chemistry, antimicrobial prevalence, and microbial diversity, few investigations have integrated these dimensions with bacteriophage ecology within a longitudinal freshwater framework.

This research bridges that gap by combining physicochemical profiling, metal quantification, microbiological analysis,

antibiotic susceptibility testing, bacteriophage isolation, and PCR-based molecular mapping of resistance variants across multiple stretches of the Ganga River. Our study offers a targeted molecular perspective on pollution, microbial evolution, and AMR dissemination in a major freshwater ecosystem.

METHODOLOGY

Sample Collection

Water samples were collected from 17 geographically distributed sites along the Ganga River, covering the upper (UG), middle (MG), and lower (LG) stretches, during two hydrologically distinct seasons: summer (April–June 2025) and monsoon (August–October 2025), and winter (November-December 2025), resulting in a total of 51 site-season sampling events. The sampling locations were selected to represent varying degrees of anthropogenic influence, including urban settlements, industrial zones, agricultural regions, pilgrimage centres, and major tributary confluences. This stratified longitudinal sampling design enabled the assessment of spatial and seasonal variations in physicochemical characteristics and microbial communities across the river continuum.

At each sampling site, triplicate water samples were collected from the surface layer (approximately 15–30 cm below the water surface) using sterile 1 L polypropylene bottles. Samples were transported to the laboratory in insulated containers maintained at 4 °C and processed within 24 h for physicochemical and microbiological analyses. Aliquots intended for molecular investigations and bacteriophage studies were subsequently preserved at −20 °C and −80 °C, respectively, until further analysis.

Geographical coordinates (latitude and longitude; WGS84), sampling dates, collection times, ambient temperature, and river-stretch classifications were recorded for each site. A detailed description of all sampling locations is provided in Table 1. This sampling framework was designed to capture the influence of seasonal hydrological fluctuations and anthropogenic activities on water quality, microbial contamination, antimicrobial tolerance, and bacteriophage distribution throughout the river system.

Table 1. Description of sampling sites and temporal details of water sample collection. The table includes sample identification code, sampling dates during summer and monsoon seasons, geographic location, specific ghat name, geographic coordinates (latitude and longitude), and river stretch classification.

Sample Code	Date (Summer Season)	Date (Monsoon Season)	Date (Winter Season)	Location	Ghat	Latitude	Longitude	Stretch
UG-2	11/04/2025	12/08/2025	10/11/2025	Gangotri	Gangotri Mandir	30.993889°	78.941393°	Upper Stretch
UG-3	13/04/2025	15/08/25	18/11/25	Rishikesh	Triveni Ghat	30.102922°	78.300565°	
UG-4	13/04/2025	16/08/25	20/11/25	Haridwar	Har Ki Pauri	29.956725°	29.956725°	
UG-5	14/04/2025	16/08/25	20/11/25	Bijnor	Madhya Ganga Barrage	29.372842°	78.041388°	
MG-6	20/04/2025	08/08/25	24/11/25	Farrukhabad	Panchal Ghat	27.398728°	79.626912°	Middle Stretch
MG-7	27/04/2025	02/08/25	26/11/25	Kanpur	Brahmawat Ghat	26.613852°	80.274798°	
MG-8	23/04/2025	05/08/25	28/11/25	Dalmau (Raebareli)	Sankatmochan Ghat	26.062242°	81.028226°	
MG-9(A)	01/05/2025	04/09/25	30/11/25	Prayagraj	Before Confluence to Yamuna	25.435945°	81.880877°	
MG-					After	25.396543°	81.914816°	

9(B)					Confluence to Yamuna			
MG-10	13/05/2025	12/09/25	05/12/25	Varanasi	Tulsi Ghat	25.290474°	83.006431°	
MG-11	14/05/2025	12/09/25	07/12/25	Ballia	Mahavir Ghat	25.732978°	84.166192°	
LG-12	20/05/2025	06/09/25	10/12/25	Patna	Kangan Ghat	25.600565°	85.232026°	Lower Stretch
LG-13	15/06/2025	09/09/25	12/12/25	Bhagalpur	Vikramshila Setu	25.269224°	87.027322°	
LG-14	14/06/2025	22/09/25	14/12/25	Sahibganj	Munteshwar Ghat	25.248458°	87.640693°	
LG-15	14/06/2025	22/10/25	18/12/25	Farakka	Farakka Barrage Township	24.809212°	87.918348°	
LG-16	13/06/2025	21/10/25	20/12/25	Howrah	Howrah Bridge	22.584469°	88.344523°	
LG-17	13/06/2025	21/10/25	23/12/25	South 24 Parganas	Kakdwip	21.882205°	88.164143°	

Water sample testing

Physicochemical analyses were performed on all water samples within 24 h of collection following the procedures outlined in the APHA Standard Methods for the Examination of Water and Wastewater (24th Edition, 2023). All measurements were conducted in triplicate (n = 3), and the results are reported as mean ± standard deviation (SD). The pH of each sample was measured using a calibrated digital pH meter (BR Biochem (0 to 14.00 pH) Measurement Range pH Meter, PHS-3C) according to APHA Method 4500-H^+ B. Calibration was performed prior to analysis using standard buffer solutions of pH 4.0, 7.0, and 10.0. Turbidity was determined using a nephelometric turbidity meter (HTLP Digital Turbidity Meter HTLP-086) and expressed as nephelometric turbidity units (NTU) following APHA Method 2130 B. Total Suspended Solids (TSS) were quantified gravimetrically according to APHA Method 2540 D. Briefly, a known volume of water was filtered through pre-weighed glass-fiber filters, which were subsequently dried at 103–105 °C to constant weight. TSS concentrations were expressed as mg L^{-1}. Biochemical Oxygen Demand (BOD_5) was measured using the five-day incubation method described in APHA Method 5210 B. Samples were incubated at 20 ± 1 °C in the dark for five days, and dissolved oxygen concentrations were determined before and after incubation. BOD values were expressed as mg L^{-1} O_2. Chemical Oxygen Demand (COD) was determined using the closed reflux colorimetric method (APHA Method 5220 D). Samples were digested with potassium dichromate under acidic conditions and quantified spectrophotometrically. COD concentrations were expressed as mg L^{-1} O_2. Quality-control procedures included analysis of reagent blanks, calibration standards, and duplicate samples to ensure analytical accuracy and reproducibility. The measured physicochemical parameters were subsequently compared with the permissible limits recommended by the World Health Organization (WHO), Bureau of Indian Standards (BIS 10500:2012), and the Central Pollution Control Board (CPCB).

Most Probable Number Test

Microbiological quality of water samples was assessed using the Most Probable Number (MPN) method in accordance with APHA Standard Methods for the Examination of Water and Wastewater (24th Edition, 2023; APHA Method 9221 B, E, and F). The MPN assay was performed to estimate the abundance of viable coliform bacteria and to evaluate the extent of microbial contamination, faecal pollution, and associated public health risks across the sampling sites. For the presumptive coliform test, serial volumes of water samples (10 mL, 1 mL, and 0.1 mL) were inoculated into tubes containing Lauryl Tryptose Broth (LTB) supplemented with inverted Durham tubes and incubated at 35 ± 0.5 °C for 24–48 h. Tubes exhibiting gas production and turbidity were recorded as presumptive positive for coliform bacteria. Presumptive positive cultures were subsequently subjected to confirmatory testing using Brilliant Green Lactose Bile (BGLB) broth for the detection of total

coliforms. For faecal coliform enumeration, positive cultures were transferred to EC broth and incubated at 44.5 ± 0.2 °C for 24 h. Gas production in Durham tubes was considered indicative of faecal coliform presence. For the confirmation of *Escherichia coli*, cultures from positive EC broth tubes were streaked onto Eosin Methylene Blue (EMB) agar and incubated at 37 °C for 24 h. Colonies displaying a characteristic metallic green sheen were considered presumptive *Escherichia coli* and were further verified by Gram staining and standard biochemical tests. The MPN values were calculated using standard MPN probability tables and expressed as MPN/100 mL of water. All analyses were performed in triplicate (n = 3), and results were reported as mean ± standard deviation. The obtained values were compared with WHO (World Health Organization), BIS (Bureau of Indian Standards) 10500:2012, and CPCB (Central Pollution Control Board) guidelines to assess microbial water quality and the degree of faecal contamination across different stretches of the Ganga River

Bacterial Identification and Characterisation

Bacterial isolates recovered from water samples were subjected to morphological, microscopic, and biochemical characterisation to obtain preliminary taxonomic information. Pure bacterial colonies were isolated on appropriate culture media and examined for colony morphology, including size, shape, pigmentation, elevation, margin characteristics, and surface texture. Microscopic characterisation was performed using the Gram Staining Kit (HiMedia, K001-1KT, Mumbai, India) following the manufacturer's instructions. Briefly, bacterial smears were prepared on clean glass slides, heat-fixed, and sequentially stained with crystal violet, Gram's iodine, decolorizer, and safranin. Stained preparations were examined under an oil immersion objective (100×) using a light microscope. Bacterial isolates were categorised as Gram-positive or Gram-negative and further classified based on cellular morphology (cocci, bacilli, or coccobacilli) and cellular arrangement. Preliminary biochemical characterisation of Gram-negative enteric isolates was performed using the HiIMViC™ Biochemical Test Kit (HiMedia Laboratories Pvt. Ltd., Mumbai, India) according to the manufacturer's protocol. The test comprises Indole, Methyl Red, Voges–Proskauer, and Citrate (IMViC) assays, which were used to differentiate members of the family *Enterobacteriaceae* based on their metabolic characteristics. Positive and negative reactions were recorded following the recommended incubation period and interpreted using the manufacturer's identification chart.

Antibiotic and Metal Tolerance Test

The antibiotic and metal tolerance of the predominant culturable bacterial isolates, *Escherichia coli* and *Pseudomonas aeruginosa*, recovered from Ganga River water samples was evaluated using an agar dilution-based minimum inhibitory concentration (MIC) assay following the Clinical and Laboratory Standards Institute (CLSI) M07 (2024) guidelines. Representative isolates from each sampling site were purified and cultured overnight in nutrient broth at 37 °C under aerobic conditions. The bacterial suspension was standardised to a 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU mL^{-1}).

The susceptibility of the isolates to three commonly detected environmental antibiotics tetracycline, levofloxacin, and azithromycin and three metal compounds copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), cadmium chloride ($CdCl_2$), and potassium dichromate ($K_2Cr_2O_7$) was assessed. Sterile stock solutions were prepared in distilled water and incorporated into molten nutrient agar to generate two-fold serial dilutions. Antibiotic concentrations ranged from 0.0098 to 5 µg mL^{-1}, while metal concentrations ranged from 0.5 to 256 mg L^{-1}.

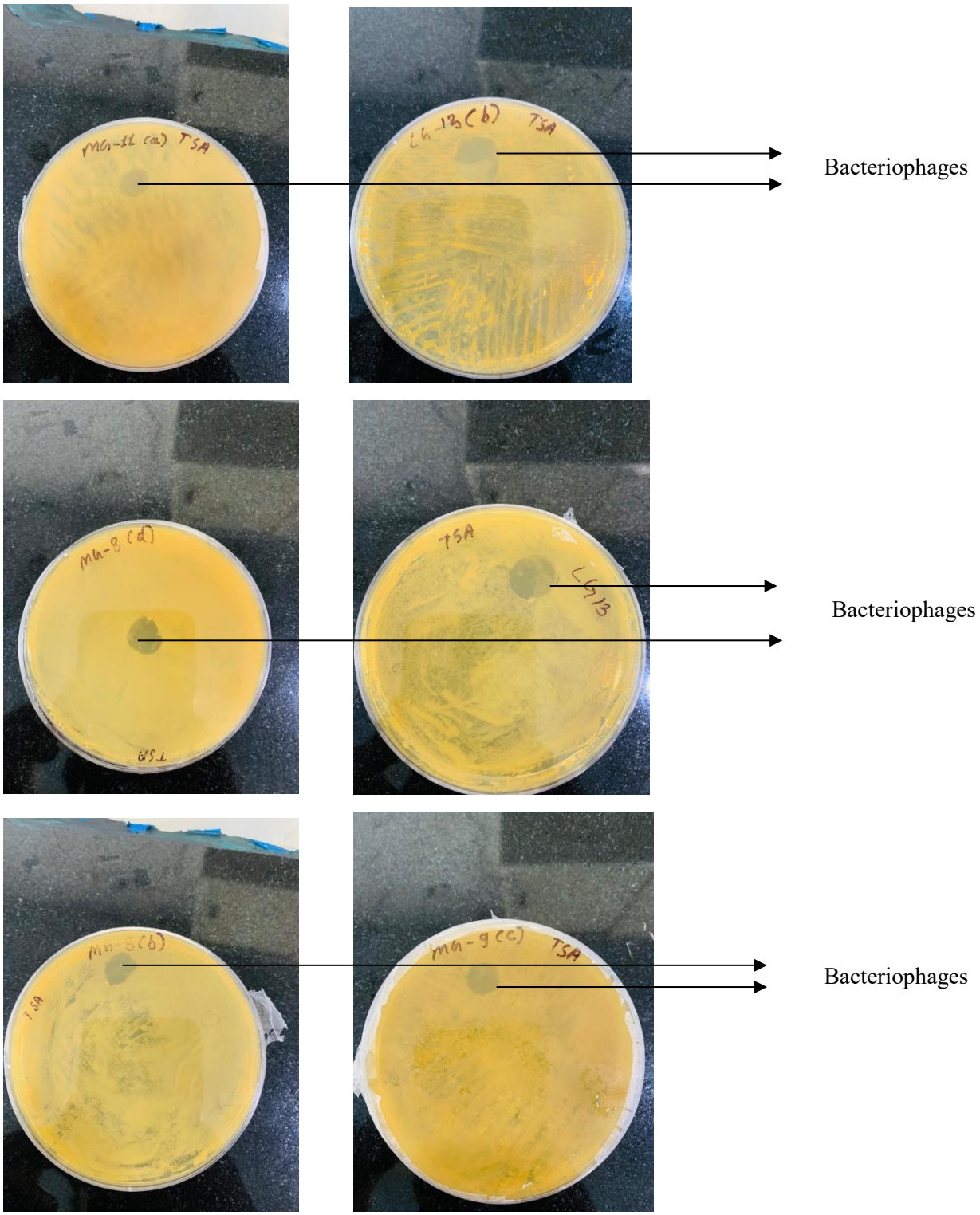
Standardized bacterial inoculate (5 µL per spot) were applied to the agar surface and incubated at 37 °C for 18–24 h. The MIC was recorded as the lowest concentration showing complete inhibition of visible growth. All assays were performed in triplicate, and the modal MIC value was used for subsequent analysis. The resulting MIC profiles were employed to compare the spatial distribution of antibiotic and metal tolerance among representative bacterial populations along different stretches of the Ganga River and to generate the corresponding heatmap visualizations.

Bacteriophage Isolation

Each bacterial host strain was cultivated in Luria Bertani (LB) broth (HiMedia) to mid-log phase (OD600=0.132) by incubation at 37 °C, 120 rpm, and overnight. 40 ml of water sample was centrifuged at 7000 rpm for 15 min and then filtered through a 0.22 µm filter. 10 ml of the filtered sample was added with 300 µl of each bacterial culture, were added into 30 mL of LB broth and incubated for 24 hrs at 30℃ at 85-100 rpm. The samples were also supplemented with 100 µL of 10 mM CaCl2 and 100 µl of 0.5 mM MgSO4 to enhance bacteriophage growth. After 24 hrs of incubation, the samples were again incubated at 4℃ for 48 hrs. The samples were then centrifuged at 10,000 rpm for 15 min. The supernatant was filtered through a 0.22 µm pore-size disposable syringe filter (HiMedia) to remove residual bacterial cells. The filtrate was centrifuged

again at 10,000 rpm for 10 min and tested for the presence of bacteriophages using the agar overlay assay. An agar overlay assay was done by pouring top agar Tryptic Soy Broth (TSB) (HiMedia) consist of 0.6% agar to the bottom agar TSB consist of 2% agar, where 150 µl of bacteriophage filtrate, 150 µl of mid-log phase bacterial host culture, 50 µL of 10 mM CaCl2, and 50 µL of 0.5 mM MgSO4 were mixed in 4 mL of molten LB soft agar and poured onto an LB agar plate, then followed by incubation at 37 °C overnight. Clear plaque formation was observed, as shown in Figure 1.

Figure 1.



Bacteriophages isolated on Tryptic Soy Broth (TSB) with 06% agar plates at a dilution of 10^{-4}, showing distinct and well-defined plaques indicative of phage activity against the host bacterial lawn.

PCR-based detection of the known variants

The PCR-based detection of the known variants is done by QIAquant 96 5plex. Determination of bacteriophage was done through the plaque technique and a TSB agar plate (HiMedia) spot. Further, their DNA was isolated through the salting-out method and visualised on 1.5% gel. The variant genes were designed through PerlPrimer v1.1.21 and were further BLAST through NCBI primer BLAST (Supplementary). 1 µl of 10 ng/µl DNA was used for the PCR amplification with the addition of 1 µl of 10 µm of each forward and reverse primer. 5 µl of 2x EmeraldAmp® GT PCR Master Mix, and 2 µl nucleus-free water. Followed by PCR cycles with 95 ℃ for 3 min (denaturation), 95 ℃ for 30 sec, 65 ℃ for 30 sec, 72 ℃ for 1 min, 72 ℃ for 5 min (annealing and extension), 35 cycles and 4 ℃ for hold.

Statistical Analysis

All the statistical analysis was performed using the R programming language (version 4.5.2).

RESULTS

Water sample testing

The collected water sample was aliquoted into separate autoclaved reagent bottles of 500ml under the Biosafety Level-3 cabinet (BSL-3) hood. The remaining water sample was again kept at -80℃ for other experimental work. The aliquoted water sample was then used for water testing analysis.

a. pH measurement

The water sample was thawed and brought to room temperature of 25℃, and measured with a thermometer (Easy-Read® thermometer). 5ml of water sample was used in triplicate to measure the pH through a pH meter (Avi Scientific), and then the average was taken as shown in Table 2.

b. Turbidity

Water samples were gently mixed to ensure uniform distribution of suspended particles while avoiding air bubble formation. An aliquot of 10ml water sample was transferred into a clean, dry glass cuvette, ensuring that no bubbles adhered to the inner surface. The exterior of the cuvette was wiped with a lint-free tissue to remove fingerprints or moisture. The cuvette was then placed in the turbidity meter (Microprocessor Nephol/Turbidity meter) and was measured at a 90° light-scattering angle. Readings were recorded in nephelometric turbidity units (NTU), and measurements were performed in triplicate to ensure accuracy and reproducibility. The average is shown in Table 2.

c. TSS

TSS in water samples were determined using the gravimetric method. A known volume of well-mixed water sample was filtered through a pre-weighed glass fiber filter with a nominal pore size of approximately 1.2 µm. Before filtration, the filter was washed with distilled water, dried at 103–105 °C for 1 hour, cooled in a desiccator, and weighed to obtain the initial weight. After filtration, the filter containing suspended solids was dried again at 103–105 °C to constant weight, cooled in a desiccator to prevent moisture absorption, and reweighed. The increase in filter weight represented the mass of suspended solids. TSS was calculated by dividing the mass difference by the volume of the sample filtered and expressed as milligrams per litre (mg/L). The experiment was performed in triplicate, and the average is shown in Table 2.

d. BOD

BOD was determined using the 5-day incubation method. Appropriate dilutions of the water sample were prepared using BOD dilution water saturated with dissolved oxygen. The initial dissolved oxygen (DO) concentration was measured immediately using a calibrated DO meter (Orion Pro Star DO213 Dissolved Oxygen Bench Meter). The bottles were then incubated at 20°C in the dark for 5 days to prevent photosynthetic oxygen production. After incubation, the final DO was measured, and BOD was calculated as the difference between the initial and final DO. Results were expressed as milligrams of oxygen consumed per litre (mg/L). The experiment was performed in triplicate, and the average is shown in Table 2.

e. COD

COD was determined using the closed reflux titrimetric method. A measured volume of water sample was digested with a known excess of potassium dichromate ($K_2Cr_2O_7$) solution in the presence of concentrated sulfuric acid containing silver sulfate as a catalyst. Mercuric sulfate was added where necessary to eliminate chloride interference. The mixture was refluxed at 150 °C for 2 hours in a COD digestion apparatus. After cooling, the remaining unreduced dichromate was titrated with standardized ferrous ammonium sulfate (FAS) using ferroin indicator. COD was calculated from the amount of dichromate consumed during oxidation and expressed as milligrams of oxygen equivalent per litre (mg/L). The experiment was performed in triplicate, and the average is shown in Table 2.

Table 2.

Sample Code	Sampling Site	pH (S)	pH (M)	pH (W)	Turbidity (NTU) S	M	W	TSS (mg/L) S	M	W	BOD (mg/L) S	M	W	COD (mg/L) S	M	W
UG-2	Gangotri	6.5	6.9	6.7	7	8	7.5	0.12	0.13	0.12	3	4	3.5	10	12	11
UG-3	Rishikesh	7.6	8	7.8	46	98	72	126.8	156.8	142	11.7	15.7	13.7	18.5	23.5	21
UG-4	Haridwar	7.2	7.5	7.4	110	150	130	368	468	418	12.8	16.8	14.8	15.2	25.2	20.2
UG-5	Bijnor	7	7.3	7.1	121	171	146	213.6	273.6	243.6	21.4	27.4	24.4	21	41	31
MG-6	Farrukhabad	8.2	8.5	8.3	228	268	248	318.8	428.8	373.8	32.9	42.9	37.9	54.3	64.3	59.3
MG-7	Kanpur	8.4	8.6	8.5	250	303	276	324.8	484.8	404.8	38.5	48.5	43.5	52.7	72.7	62.7
MG-8	Dalmau	7.8	8	7.9	150	202	176	203.2	323.2	263.2	22.3	32.3	27.3	28.5	48.5	38.5
MG-9A	Prayagraj (Before Yamuna Confluence)	6.8	7	6.9	15	23	19	16.8	36.8	26.8	1.7	3.7	2.7	3.5	5.5	4.5
MG-9B	Prayagraj (After Yamuna Confluence)	7.1	7.3	7.2	206	276	241	341.6	441.6	391.6	24.2	44.2	34.2	46.2	66.2	56.2
MG-10	Varanasi	8	8.2	8.1	14	24	19	18.4	38.4	28.4	1.8	3.8	2.8	2.8	5.8	4.3
MG-11	Ballia	7.6	8	7.8	146	196	171	213.6	313.6	263.6	11.4	31.4	21.4	27	47	37
LG-12	Patna	7.2	7.5	7.3	13	23	18	16.8	36.8	26.8	1.7	3.7	2.7	2.5	5.5	4
LG-13	Bhagalpur	7	7.2	7.1	49	79	64	56.4	126.4	91.4	9.6	12.6	11.1	11.9	18.9	15.4
LG-14	Sahibganj	7.1	7.3	7.2	15	25	20	17	40	28.5	2	4	3	4	6	5
LG-15	Farakka	7.3	7.5	7.4	14	24	19	18.4	38.4	28.4	2.8	3.8	3.3	2.8	5.8	4.3
LG-16	Howrah	7.4	7.6	7.5	29	59	44	44.4	94.4	69.4	4.4	9.4	6.9	10.2	14.2	12.2
LG-17	Kakdwip	7.6	7.8	7.7	24	54	39	36.4	86.4	61.4	3.6	8.6	6.1	10.9	13.9	12.4

Abbreviations: S = Summer, M = Monsoon, W = Winter; TSS = Total Suspended Solids; BOD = Biochemical Oxygen Demand; COD = Chemical Oxygen Demand. Values are presented as mean ± SD (n = 3). Physicochemical parameters were analysed according to the standard methods described by the American Public Health Association. pH was measured using a calibrated digital pH meter (APHA 4500-H^+ B), turbidity by nephelometric method (APHA 2130 B), TSS by gravimetric analysis (APHA 2540 D), BOD by the 5-day incubation method (APHA 5210 B), and COD by the closed reflux dichromate method (APHA 5220 D). Statistical significance among seasons and river stretches was evaluated using two-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

Most Probable Test

The Most Probable Number (MPN) test of coliform bacteria was determined using the multiple-tube fermentation technique. Serial dilutions of the water samples were prepared and inoculated into lactose broth tubes containing inverted Durham tubes for gas detection. A set of three or five tubes per dilution (10 mL, 1 mL, and 0.1 mL) was incubated at 37 °C for 24-48 hours for total coliform analysis. Tubes showing acid production and gas formation were recorded as positive in the presumptive test. Positive tubes were further confirmed using Brilliant Green Lactose Bile (HiMedia BGLB) broth for total coliforms and EC broth (HiMedia) incubated at 44.5 °C for faecal coliforms. The number of positive tubes at each dilution was compared with standard MPN probability tables to estimate bacterial density. Results were expressed as MPN per 100 mL of water sample. All analyses were performed in triplicate and shown as MPN index in Table 3.

Table 3. Most Probable Number (MPN) Analysis of Ganga River Water Samples

Sample Code	Sampling Site	Presumptive MPN (MPN/100 mL)	Total Coliform MPN (MPN/100 mL)	95% CI (Presumptive)	95% CI (Coliform)	Water Quality Category
UG-2	Gangotri	<1	1	0–0.9	0.1–0.9	Excellent
UG-3	Rishikesh	28	35	16.8–50.4	21–63	Suspicious
UG-4	Haridwar	7451	7451	5961–8941	5961–8941	Highly Contaminated
UG-5	Bijnor	7451	7451	5961–8941	5961–8941	Highly Contaminated
MG-6	Farrukhabad	7451	7451	5961–8941	5961–8941	Highly Contaminated
MG-7	Kanpur	7451	7451	5961–8941	5961–8941	Highly Contaminated
MG-8	Dalmau	7451	7451	5961–8941	5961–8941	Highly Contaminated
MG-9A	Prayagraj (Before Yamuna)	120	185	96–144	148–222	Unsatisfactory
MG-9B	Prayagraj (After Yamuna)	7451	7451	5961–8941	5961–8941	Highly Contaminated
MG-10	Varanasi	7451	35	5961–8941	21–63	Mixed Contamination
MG-11	Ballia	14	20	8.4–25.2	12–36	Suspicious

LG-12	Patna	7451	7451	5961–8941	5961–8941	Highly Contaminated
LG-13	Bhagalpur	7451	7451	5961–8941	5961–8941	Highly Contaminated
LG-14	Sahibganj	35	35	21–63	21–63	Suspicious
LG-15	Farakka	7451	7451	5961–8941	5961–8941	Highly Contaminated
LG-16	Howrah	15	7451	9–27	5961–8941	Highly Contaminated
LG-17	Kakdwip	7451	7451	5961–8941	5961–8941	Highly Contaminated

Water quality categories were assigned according to APHA (2023) and WHO guidelines: Excellent (<1 MPN/100 mL), Satisfactory (1–10 MPN/100 mL), Suspicious (11–100 MPN/100 mL), Unsatisfactory (101–1000 MPN/100 mL), and Highly Contaminated (>1000 MPN/100 mL). Values exceeding the upper detection limit of the MPN assay are reported as >7451 MPN/100 mL. Confidence intervals (CI) represent 95% limits derived from standard MPN statistical tables. All analyses were performed in triplicate and expressed as mean values.

Biochemical Tests

a. Gram-staining

Gram staining was performed using a commercially available Gram Staining Kit (HiMedia Laboratories, India; SKU: K001) according to the manufacturer's instructions to classify bacterial isolates based on cell wall structure and cellular morphology. Briefly, pure bacterial colonies obtained from water samples were used to prepare thin smears on clean glass slides, which were air-dried and heat-fixed. The smears were stained with crystal violet for 1 min, followed by Gram's iodine for 1 min to facilitate formation of the crystal violet–iodine complex. Slides were then decolorized with 95% ethanol for 10–20 s and immediately rinsed with distilled water. Counterstaining was performed using safranin for 30–60 s, followed by washing and air drying. Stained preparations were examined under a light microscope using an oil immersion objective (100×). Based on staining characteristics and cell morphology, isolates were categorised as Gram-positive cocci, Gram-positive bacilli, Gram-negative cocci, or Gram-negative bacilli. Gram-positive organisms retained the crystal violet stain and appeared purple, whereas Gram-negative organisms appeared pink to red following safranin counterstaining. The results were used for preliminary phenotypic characterisation and assessment of bacterial diversity across sampling sites (Table 4). As Gram staining alone does not permit definitive taxonomic identification, the observed morphological groups were reported as presumptive bacterial populations and interpreted in conjunction with biochemical characterisation results.

Table 4. Morphological and Gram-Staining Characterisation of Bacterial Isolates from the Ganga River

Sample Code	Sample Site	No. Of Isolates	Gram Positive Cocci	Gram Positive Bacilli	Gram Negative Cocci	Gram Negative Bacilli	Presumptive Dominant Bacterial Groups
UG-2	Gangotri	3	0	3	0	0	Environmental spore-forming bacilli
UG-3	Rishikesh	3	2	1	0	0	Cocci and environmental bacilli
UG-4	Haridwar	4	1	2	0	1	Mixed Gram-positive and

							enteric bacteria
UG-5	Bijnor	2	2	0	0	0	Gram-positive cocci
MG-6	Farrukhabad	6	1	1	2	2	Diverse mixed bacterial community
MG-7	Kanpur	2	1	0	1	0	Cocci-dominated community
MG-8	Dalmau	4	4	0	0	0	Gram-positive cocci
MG-9A	Prayagraj (Before Yamuna)	4	3	0	0	1	Cocci with enteric bacteria
MG-9B	Prayagraj (After Yamuna)	4	2	2	0	0	Mixed Gram-positive community
MG-10	Varanasi	5	2	2	1	0	Mixed cocci and bacilli
MG-11	Ballia	2	0	2	0	0	Environmental bacilli
LG-12	Patna	2	0	2	0	0	Environmental bacilli
LG-13	Bhagalpur	8	3	4	0	1	Mixed bacterial assemblage
LG-14	Sahibganj	4	1	3	0	0	Bacilli-dominated community
LG-15	Farakka	8	7	1	0	0	Gram-positive cocci dominant
LG-16	Howrah	8	4	3	1	0	Diverse mixed bacterial population
LG-17	Kakdwip (South 24 Parganas)	4	1	1	2	0	Mixed Gram-positive and Gram-negative cocci

Bacterial groups were assigned based on Gram-staining morphology and preliminary biochemical characterisation (IMViC test). These results represent presumptive bacterial groups only and should not be considered definitive genus- or species-level identification. Molecular confirmation by 16S rRNA gene sequencing was not performed in the present study.

b. Metal Sensitivity Test

1. Potassium

The heatmap illustrates the inhibitory response of the predominant culturable bacterial isolates, primarily *Escherichia coli* and *Pseudomonas aeruginosa*, recovered from different Ganga River sampling sites following exposure to increasing potassium concentrations (0.5–256 mg/mL). A modest concentration-dependent increase in growth inhibition was observed, although the response varied considerably among locations. Most sites displayed low to moderate inhibition (0–50%), indicating a relatively high tolerance of the resident bacterial populations to potassium. Comparatively higher inhibition was recorded for isolates from Haridwar (UG4), Farrukhabad (MG6), and Ballia (MG11) at intermediate concentrations (8–32 mg/mL), whereas isolates from Varanasi (MG10), Dalmau (Raebareli) (MG8), and Bijnor (UG5) exhibited limited inhibition throughout the tested range. These findings suggest spatial variation in potassium tolerance among environmental bacterial communities (Figure 2A).

2. Copper Sulphate

Copper sulphate exhibited the strongest antimicrobial activity among the tested metals. The heatmap indicates that the dominant bacterial isolates, particularly *E. coli* and *P. aeruginosa*, showed substantial growth inhibition at low to intermediate concentrations (0.5–32 mg/mL), with inhibition values exceeding 50% in several sampling sites. Isolates from Haridwar (UG4), Farrukhabad (MG6), and Prayagraj demonstrated consistently high sensitivity to copper sulphate, while those from Howrah (LG16) and Kanpur (MG7) retained comparatively greater tolerance at higher concentrations. The overall pattern indicates that copper sulphate exerts a marked bacteriostatic effect, although the degree of susceptibility differs according to site-specific microbial composition and local environmental conditions (Figure 2B).

3. Cadmium

The inhibitory response to cadmium was comparatively weak across the studied locations. The heatmap reveals that the majority of *E. coli* and *P. aeruginosa* isolates maintained low to moderate inhibition levels across the concentration gradient, suggesting the prevalence of cadmium-tolerant bacterial populations. Moderate inhibition was observed only sporadically, particularly in isolates obtained from Sahibganj and Gangotri, whereas those from Varanasi, Dalmau (Raebareli), and Howrah exhibited minimal changes in growth. Unlike copper sulphate, cadmium did not display a clear concentration-dependent inhibitory trend, indicating a widespread distribution of cadmium-resistant environmental bacteria within the Ganga River system (Figure 2C).

c. Antibiotic Sensitivity Test

1. Tetracycline

The heatmap depicts the growth response of the dominant bacterial isolates (*Escherichia coli and Pseudomonas aeruginosa*) to increasing tetracycline concentrations. At lower concentrations (≤0.15625 mg/mL), isolates from most sampling sites, including Sahibganj (LG14), Prayagraj Before Confluence (MG9B), Haridwar (UG4), and Gangotri (UG2), maintained active growth, indicating limited antibiotic stress. Partial inhibition was observed at intermediate concentrations (0.3125–1.25 mg/mL), reflecting variability in tetracycline susceptibility among sites. At higher concentrations (≥2.5–5 mg/mL), growth inhibition became pronounced, particularly for isolates from Gangotri (UG2), South 24 Parganas (Kakdwip) (LG17), Howrah (LG16), and Bijnor (UG5). Nevertheless, a subset of isolates, especially from Kanpur and Prayagraj, retained measurable growth at intermediate concentrations, suggesting the presence of tetracycline-tolerant bacterial populations. Overall, the results indicate site-specific differences in tetracycline resistance, potentially reflecting differential exposure to anthropogenic antibiotic inputs (Figure 3A).

2. Levofloxacin

Levofloxacin displayed a progressive concentration-dependent inhibitory effect on the predominant *E. coli* and *P. aeruginosa* isolates. Most bacterial populations showed little or no inhibition at concentrations ≤0.15625 mg/mL, while intermediate concentrations (0.3125–1.25 mg/mL) resulted in partial growth suppression, particularly in isolates from Prayagraj After Confluence (MG9A), Howrah (LG16), and Farrukhabad (MG6). At concentrations of 2.5–5 mg/mL, marked inhibition was observed across most sites, with isolates from Bijnor (UG5), Howrah (LG16), and South 24 Parganas (Kakdwip) (LG17) exhibiting the highest sensitivity. In contrast, isolates from Kanpur (MG7) and Haridwar (UG4) demonstrated relatively greater tolerance, maintaining limited growth under moderate antibiotic pressure. The overall response pattern suggests that levofloxacin effectively suppresses environmental bacterial populations at higher concentrations, although resistance profiles differ among sampling sites (Figure 3B).

3. Azithromycin

The heatmap shows the response of the dominant bacterial isolates to increasing azithromycin concentrations. At lower concentrations (≤0.078125 mg/mL), most isolates from Varanasi (MG10), Prayagraj Before Confluence (MG9B), Howrah (LG16), and Farrukhabad (MG6) displayed active growth, indicating limited susceptibility. A gradual reduction in growth was observed at intermediate concentrations (0.15625–0.625 mg/mL), with isolates from Gangotri (UG2) exhibiting comparatively earlier inhibition. At higher concentrations (1.25–5 mg/mL), substantial growth suppression was evident across the majority of sites, especially in isolates from Bijnor (UG5), Patna (LG12), Haridwar (UG4), and South 24 Parganas (Kakdwip) (LG17). Although some isolates maintained partial growth at intermediate doses, the overall trend demonstrates a clear dose-dependent inhibitory effect of azithromycin, with site-specific differences reflecting the heterogeneous distribution of antibiotic-resistant bacterial populations within the Ganga River ecosystem (Figure 3C).

Bacteriophage DNA Isolation

Bacteriophage DNA was isolated by the following standard purification procedures with minor modifications. Water samples were incubated at 37 ℃ overnight in LB (HiMedia). The next day, the bacteriophage isolation was done through the plaque assay protocol. After isolation, 5 ml of the bacteriophage lysates was treated with 2 µl each DNase I and RNase A (Servicebio G3057-100T, G3405-1ML), respectively, to eliminate contaminating host nucleic acids, followed by incubation at 37°C for 30 min. 500 µl of 1M NaCl (HiMedia) and 10 µl of 10% Polyethylene Glycol (PEG) were added to the samples, which were then followed by incubation at 4℃ overnight. The next day, the samples were then processed by centrifugation for 20 min at 10000 rpm. The pellets were then resuspended in 500 µl SM buffer. Followed by the addition of 50 µl of 10% SDS, 5µl of proteinase K and 1 hr of incubation at 56 ℃. An equal amount of phenol: chloroform was added, mixed gently, followed by centrifugation for 15 min at 10,000 rpm. DNA was then precipitated using cold isopropanol of 500 µl and again washed with 70% ethanol of 500 µl, air-dried, and resuspended in TE buffer. The quality and quantity of the isolated bacteriophage DNA were assessed using 1.5% agarose gel electrophoresis and further visualised on GeL Doc (Bio-Rad) (Figure 4).

Variants Identification

PCR-based screening of bacteriophage diversity across Ganga River sampling sites (UG2–UG17) was performed using a broad panel of phage-specific primers targeting conserved structural and functional genes. We observed the genes ΦEF24C, Felix O1, PBS1, Φ29, ICP3 (Vibrio phages), gp028 (T2-like phages), Sf6, g23 (T4-like capsid protein), and, P68 with clear visible bands after successful PCR run (Figure 5). These variants were strategically selected to represent a wide taxonomic and ecological spectrum of bacteriophages infecting diverse bacterial hosts (including *Escherichia*, *Salmonella*, *Vibrio*, *Pseudomonas*, and *Staphylococcus*), thereby enabling comprehensive profiling of phage diversity in a complex aquatic ecosystem like the Ganga River.

DISCUSSION

The findings of this study clearly establish that the Ganga River functions as a dynamic and highly impacted aquatic ecosystem, where physicochemical conditions, microbial load, antimicrobial resistance, and bacteriophage diversity are tightly interconnected and primarily driven by anthropogenic pressures and seasonal variability (Rout et al., 2023).

The near-pristine conditions observed at the upstream site (UG-2, Gangotri), characterised by low turbidity, negligible TSS, low BOD and COD, and almost absent coliform load (<1 MPN/100 ml), can be attributed to minimal human interference, low population density, and limited exposure to external contaminants. The microbial population at this site is dominated by environmental Gram-positive bacilli, which are naturally occurring and adapted to oligotrophic (low nutrient) conditions (Naylor et al., 2022).

As the river progresses downstream, a sharp deterioration in water quality is observed, particularly at urban and industrial hotspots such as Haridwar (UG-4), Bijnor (UG-5), Farrukhabad (MG-6), and Kanpur (MG-7). The extremely high MPN values (>1000 MPN/100 ml), along with elevated BOD, COD, and TSS, are a direct consequence of untreated sewage discharge, industrial effluents, and agricultural runoff. These inputs introduce large quantities of organic matter and nutrients into the river, which stimulate rapid microbial growth. The increased BOD values reflect enhanced microbial respiration and oxygen consumption, while high COD indicates the presence of chemically oxidizable pollutants (Qi et al., 2024). The elevated turbidity and TSS further facilitate microbial survival by providing particulate surfaces for attachment and protection from environmental stress.

The drastic increase in contamination observed at Prayagraj after the Yamuna confluence (MG-9B) compared to before (MG-9A) provides strong evidence for tributary-driven pollution loading. The Yamuna River, already heavily polluted, contributes significantly to the microbial and chemical burden of the Ganga, leading to a cumulative deterioration in water quality. Downstream fluctuations in contamination levels (e.g., partial reduction at Varanasi and Patna followed by increases at Ballia and lower Ganga sites) can be explained by dilution effects, sedimentation, and reintroduction of pollutants from local sources (Jaiswal et al., 2022).

The Gram staining results provide critical insight into microbial ecology and pollution sources. The dominance of Gram-negative bacilli such as *Escherichia*, *Klebsiella*, *Salmonella*, and *Pseudomonas* in highly contaminated sites is a strong indicator of faecal pollution and sewage contamination, as these organisms are commonly associated with the gastrointestinal tract (Cruz et al., 2021). Their presence confirms that the high MPN values are primarily due to faecal inputs rather than natural environmental bacteria. On the other hand, the widespread occurrence of Gram-positive cocci (*Staphylococcus*, *Streptococcus*, *Enterococcus*) and spore-forming bacilli (*Bacillus*, *Clostridium*) reflects both environmental persistence and contamination (Ginn et al., 2021). Gram-positive bacteria are more resistant to environmental stress due to their thick peptidoglycan cell wall, and in the case of spore-formers, the ability to survive extreme conditions through endospore formation. This explains their presence even in sites with fluctuating or adverse conditions, such as low pH (LG-13 to LG-17) and high pollutant loads (Rohde, 2019).

The observed antimicrobial resistance patterns are a direct outcome of continuous environmental exposure to sub-lethal concentrations of antibiotics and metals. Wastewater from hospitals, pharmaceutical industries, and agricultural fields introduces antibiotics such as tetracycline, levofloxacin, and azithromycin into the river. At sub-inhibitory concentrations, these compounds do not kill bacteria but instead exert selective pressure, allowing resistant strains to survive and proliferate (Mutuku et al., 2022). Similarly, metals such as cadmium and copper act as co-selective agents by promoting resistance mechanisms like efflux pumps and metal-binding proteins, which can also confer cross-resistance to antibiotics. This explains the site-specific variability in resistance patterns, with more polluted sites showing higher tolerance (Kumar et al., 2025).

The high diversity and abundance of bacteriophages observed across all sampling sites are closely linked to the availability of bacterial hosts. In heavily contaminated regions with high bacterial density, page proliferation occurs due to increased opportunities for infection. The detection of multiple phage variants targeting diverse bacterial hosts indicates a complex and active phage host interaction network (Sutton & Hill, 2019). Importantly, bacteriophages contribute not only to bacterial population control through lytic cycles but also to horizontal gene transfer via transduction. In environments with high microbial density and stress, such as polluted river systems, this process may facilitate the spread of antimicrobial resistance genes, further amplifying the resistance problem (Ranveer et al., 2024).

Seasonal variation, particularly during the monsoon, plays a crucial role in shaping these outcomes. Increased rainfall leads to runoff from agricultural fields, urban areas, and open defecation sites, introducing additional nutrients, pollutants, and microbes into the river. This results in elevated turbidity, TSS, BOD, and COD, along with increased microbial load. At the same time, higher water flow may cause partial dilution in some regions, leading to localised variability in results.

LIMITATION

A limitation of this study is the absence of direct source-tracking, metagenomic validation, and high-resolution temporal monitoring, which may further refine the understanding of microbial dynamics and AMR dissemination in the Ganga River.

CONCLUSION

This study demonstrates that the Ganga River is a highly dynamic ecosystem shaped by spatial, seasonal, and anthropogenic influences, leading to substantial variations in water quality and microbial communities. Upstream sites generally exhibited better water quality, whereas midstream and downstream regions showed elevated physicochemical parameters and high coliform loads (>1000 MPN/100 mL), indicative of significant faecal contamination. The predominance of environmental and enteric bacterial groups, particularly *Escherichia coli* and *Pseudomonas aeruginosa*, reflects both anthropogenic pollution and microbial adaptation to stressed aquatic environments.

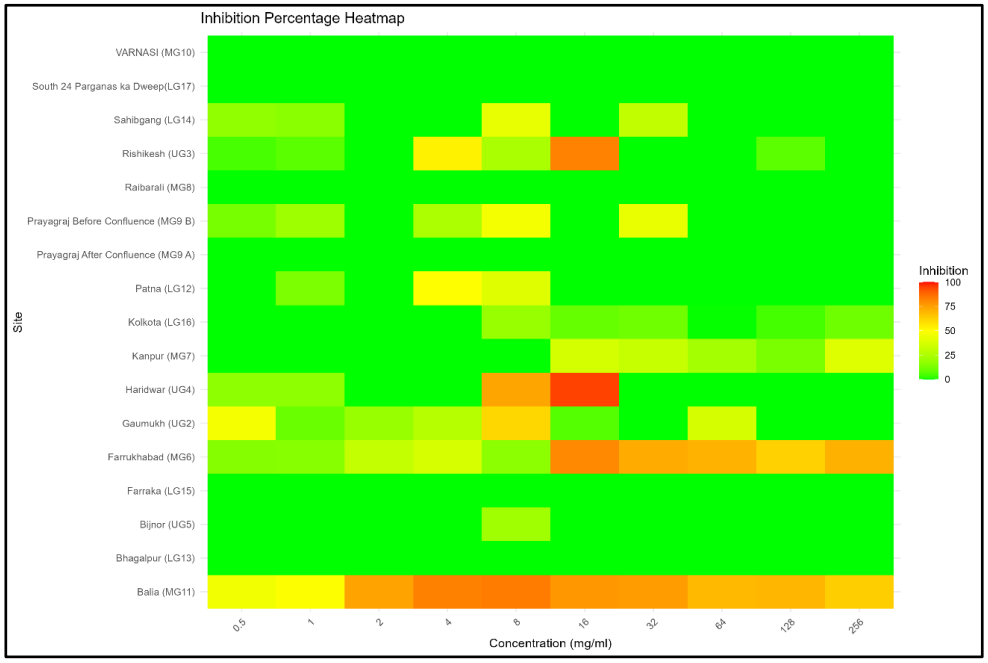
The observed patterns of antimicrobial and metal tolerance among these bacterial populations suggest the widespread occurrence of resistant environmental microbiota, likely driven by long-term exposure to untreated sewage, industrial discharges, agricultural runoff, and associated contaminants. In addition, the detection of diverse bacteriophage populations

highlights their potential ecological role in shaping bacterial community structure and influencing the dissemination of antimicrobial resistance determinants.

Overall, the findings underscore the need for strengthened wastewater treatment infrastructure, effective pollution control strategies, and long-term integrated monitoring of microbial and environmental indicators. Future studies incorporating source-tracking approaches, metagenomic analyses, and high-resolution temporal sampling will further improve our understanding of microbial ecology and antimicrobial resistance dynamics in the Ganga River basin.

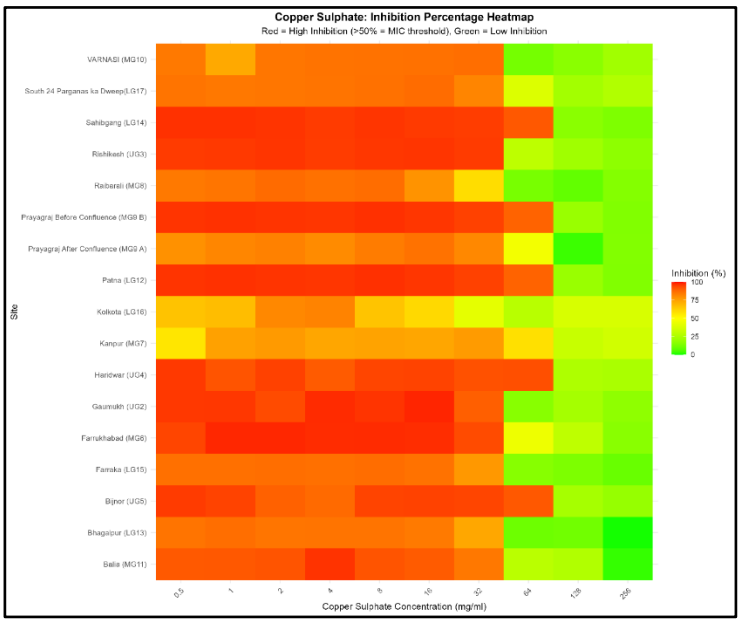
Figure 2.

A.



Inhibition percentage of microbial growth (or activity) across different sampling sites of the Ganga River at varying concentrations (mg/ml). The colour gradient ranges from green (low/no inhibition) to red (high inhibition, ~100%)

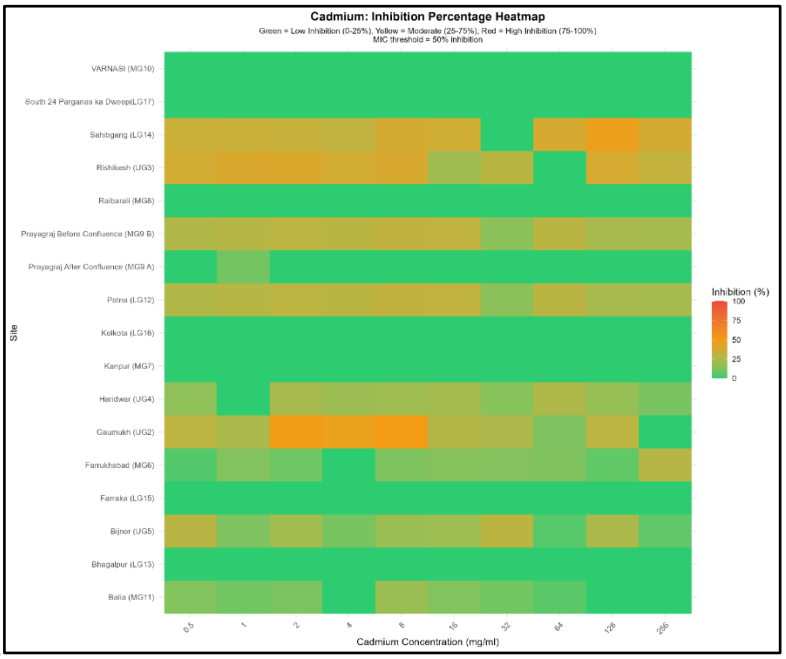
B.



Natural Resources for Human Health
eISSN: 2583-1194

The percentage inhibition of microbial isolates across different sampling sites along the Ganga River in response to increasing concentrations of copper sulphate (0.5–256 mg/ml). Colour gradient ranges from green (low inhibition) to red (high inhibition), with values above 50% indicating the minimum inhibitory concentration (MIC) threshold. Rows represent sampling locations, and columns represent copper sulphate concentrations.

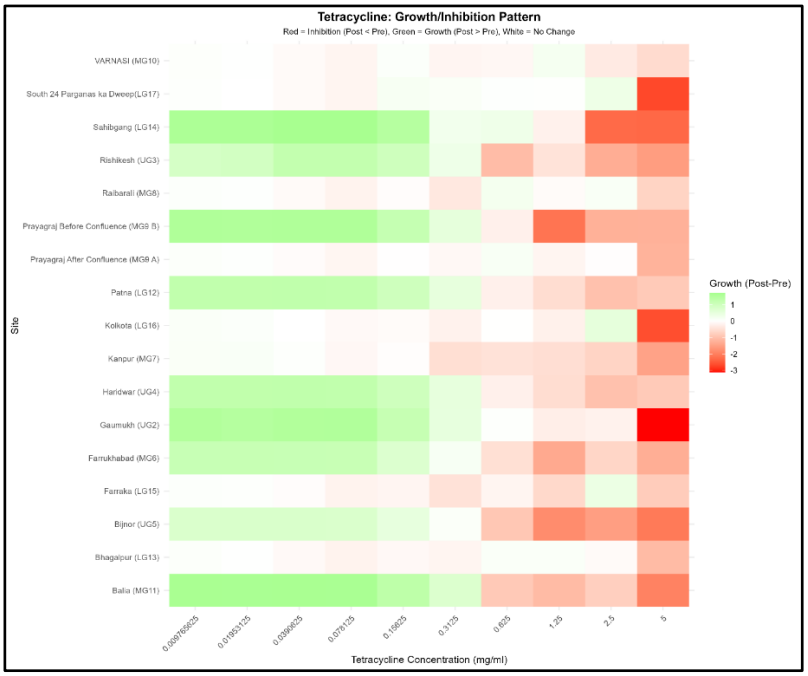
C.



Percentage inhibition of microbial isolates from various Ganga River sampling sites exposed to increasing concentrations of cadmium (0.5–256 mg/ml). The colour gradient indicates inhibition levels: green (0–25%, low), yellow (25–75%, moderate), and red (75–100%, high). A threshold of 50% inhibition is considered the minimum inhibitory concentration (MIC). Rows represent sampling sites, and columns correspond to cadmium concentrations.

Figure 3.

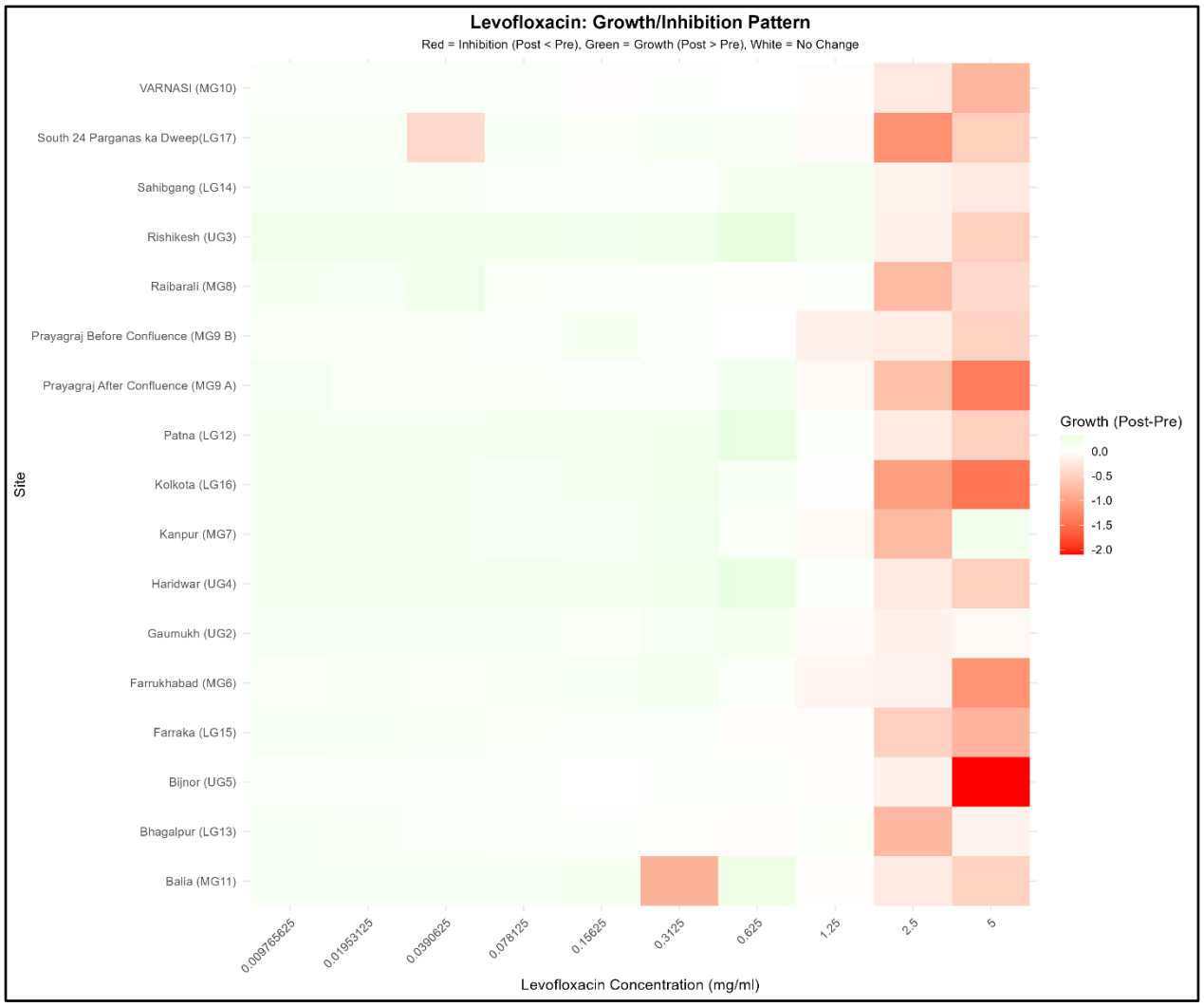
A.



Natural Resources for Human Health
eISSN: 2583-1194

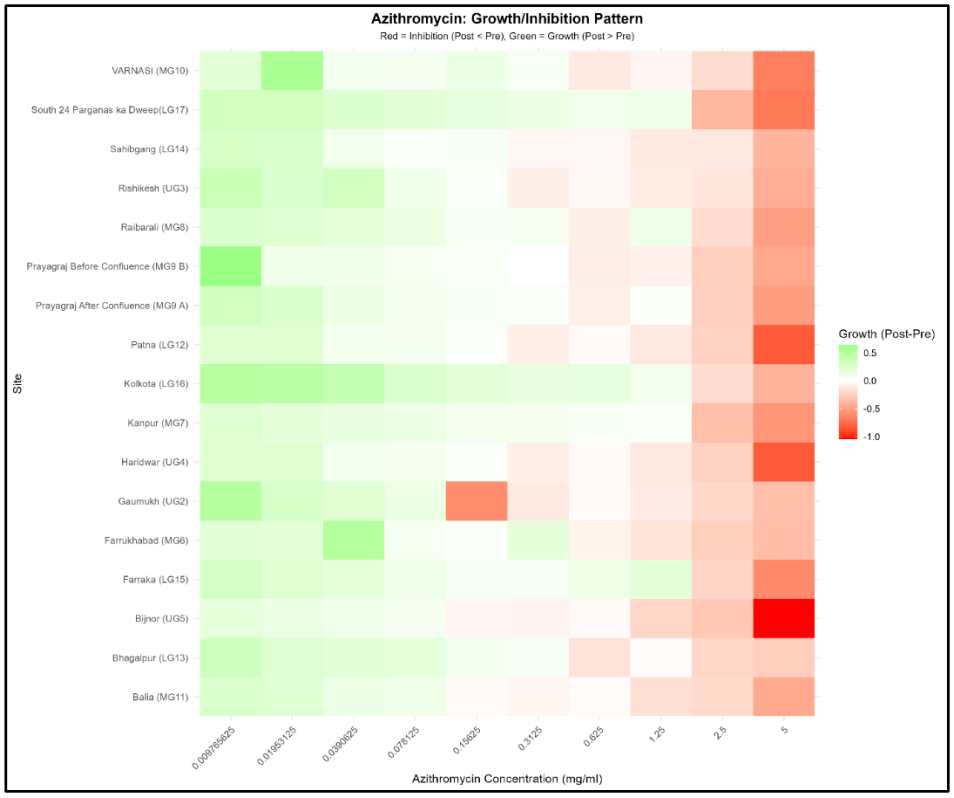
Microbial growth response to varying concentrations of tetracycline across different sampling sites of the Ganga River. The x-axis denotes tetracycline concentrations (mg/ml), while the y-axis lists sampling locations. Colour intensity represents the change in growth (post-treatment vs pre-treatment): green indicates growth (positive response), red indicates inhibition (negative response), and white indicates no significant change. The gradient scale ranges from +1 (maximum growth) to −3 (maximum inhibition), reflecting the degree of microbial sensitivity or resistance to tetracycline.

B.



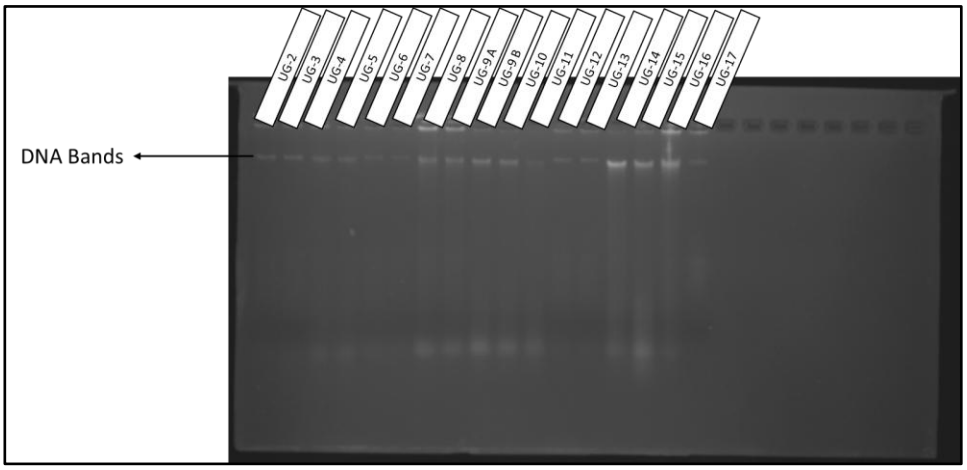
Microbial growth response to varying concentrations of levofloxacin across multiple Ganga River sampling sites. The x-axis represents levofloxacin concentrations (mg/ml), and the y-axis lists the sampling locations. Colour coding indicates growth changes (post-treatment vs pre-treatment), where green denotes growth, red denotes inhibition, and white indicates no significant change. The colour scale ranges from 0 (no change or minimal growth) to −2 (maximum inhibition), highlighting the degree of microbial sensitivity or resistance to levofloxacin across different environmental sites.

C.



Microbial growth response to increasing concentrations of azithromycin across different Ganga River sampling sites. The x-axis indicates azithromycin concentrations (mg/ml), and the y-axis lists the sampling locations. Colour coding reflects growth changes (post-treatment vs pre-treatment), where green denotes growth, red denotes inhibition, and white indicates no significant change. The colour scale ranges from +0.5 (maximum growth) to −1.0 (maximum inhibition), illustrating the extent of microbial sensitivity or resistance to azithromycin across diverse environmental samples.

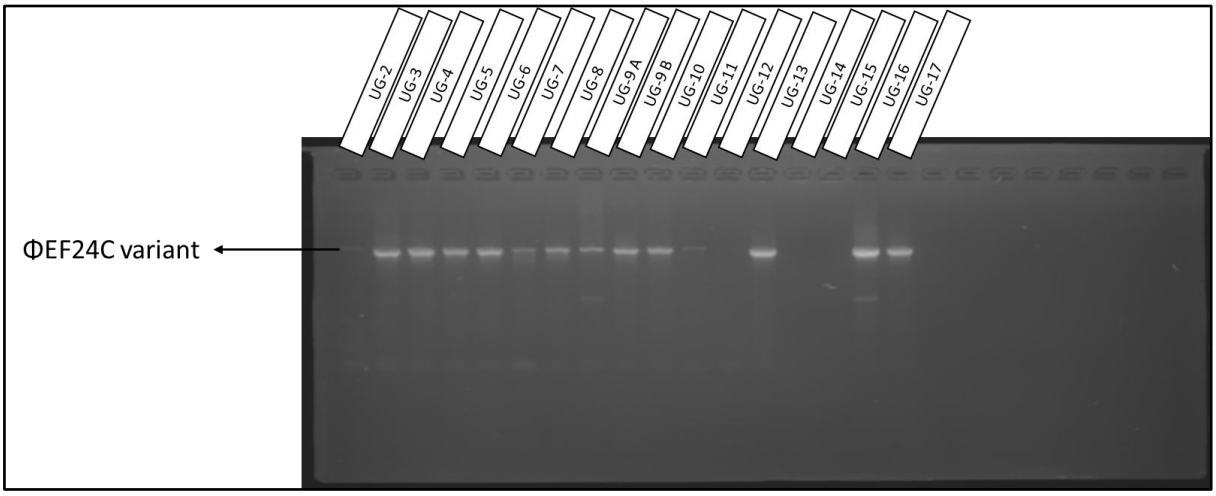
Figure 4.

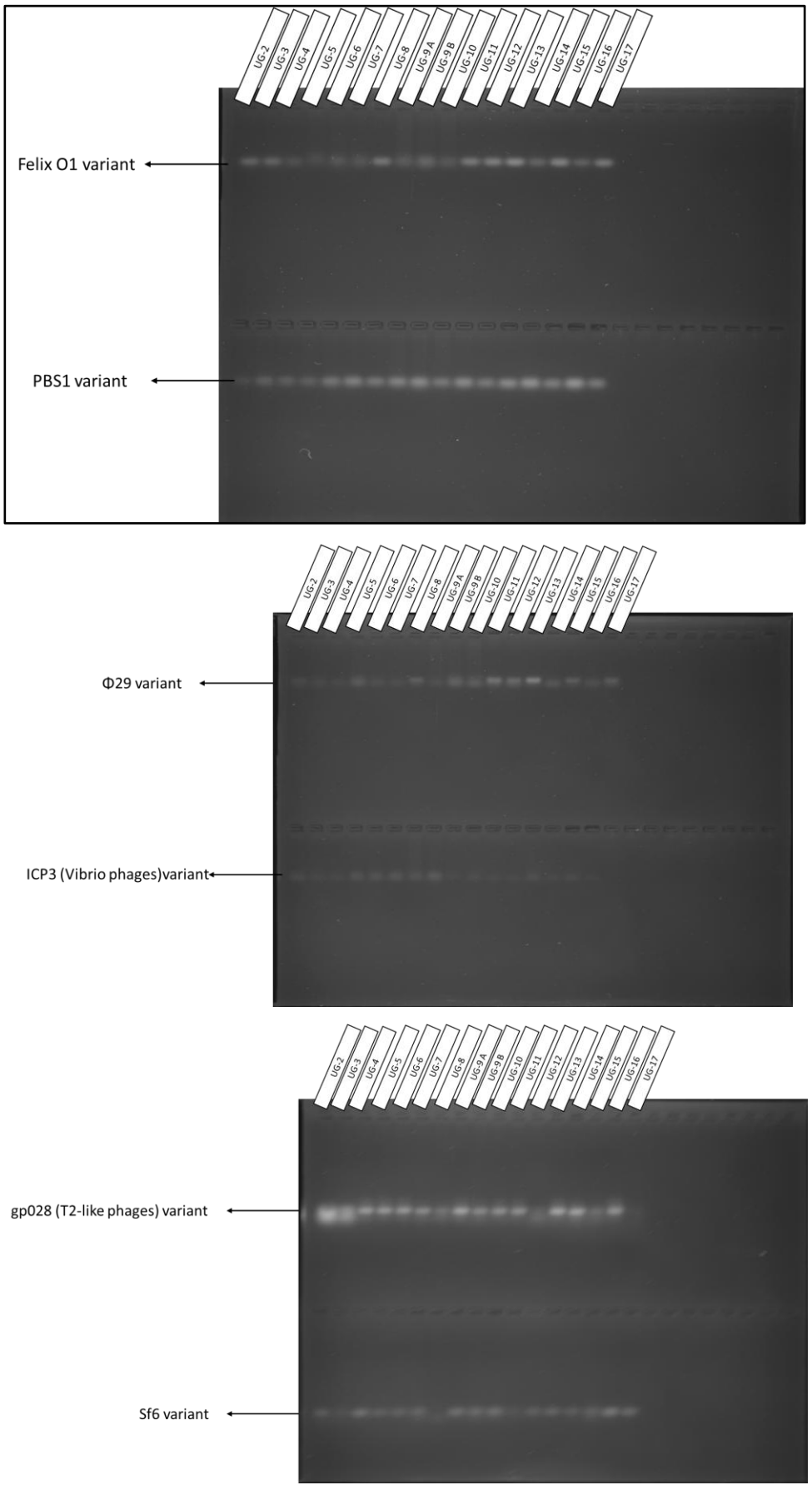


Agarose gel electrophoresis of isolated bacteriophage DNA from different sites of Ganga River. Each lane represents a distinct phage isolate (UG-2 to UG-17) as labelled above the gel. The presence of visible DNA bands near the wells indicates successful extraction of high-molecular-weight bacteriophage genomic DNA. Variations in band intensity reflect differences in DNA yield among samples. Faint smearing observed in some lanes may indicate partial degradation or residual impurities. The gel confirms the successful isolation and integrity of bacteriophage DNA suitable for downstream molecular analyses.

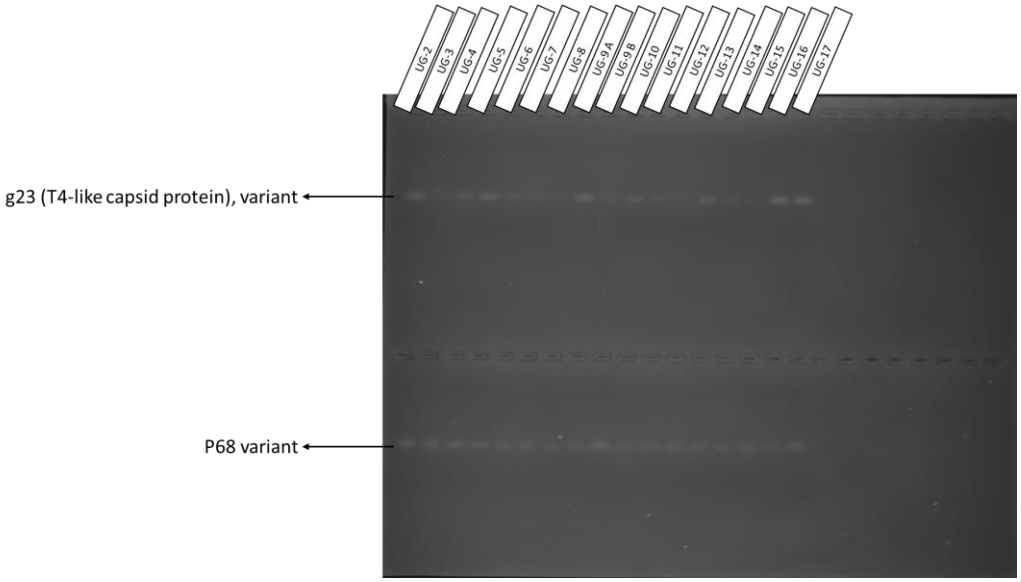
Sample Code	Location
UG-2	Gangotri
UG-3	Rishikesh
UG-4	Haridwar
UG-5	Bijnor
MG-6	Farrukhabad
MG-7	Kanpur
MG-8	Raebareli
MG-9(A)	Prayagraj
MG-9(B)	
MG-10	Varanasi
MG-11	Ballia
LG-12	Patna
LG-13	Bhagalpur
LG-14	Sahibganj
LG-15	Farakka
LG-16	Howrah
LG-17	South 24 Parganas

Figure 5.




UG-2
UG-3
UG-4
UG-5
UG-6
UG-7
UG-8
UG-9A
UG-9B
UG-10
UG-11
UG-12
UG-13
UG-14
UG-15
UG-16
UG-17
Felix O1 variant
PBS1 variant
Φ29 variant
ICP3 (Vibrio phages)variant
gp028 (T2-like phages) variant
Sf6 variant

Natural Resources for Human Health
eISSN: 2583-1194



PCR products were resolved on an agarose gel and visualised under UV illumination. Each lane corresponds to individual samples labelled UG-2 through UG-17. The expected amplicon size is indicated by the horizontal reference mark on the left side of each gel. Multiple panels represent independent PCR assays or different target genes analysed under similar conditions. Bands appearing at the expected size indicate successful amplification of the target sequence, whereas the absence of bands indicates no amplification.

Supplementary

Sl No	Variants Name	Primer Sequence
1	g23 T4 primers MZIA1bis (F)	5'-GAT ATT TGI GGI GTT CAG CCI ATG A-3'
	g23 T4 primers MZIA1bis (R)	5'-CGC GGT TGA TTT CCA GCA TGA TTT C-3'
2	gp028 T2 (F)	5'-ctcgcggaacgttaaaataccca-3'
	gp028 T2 (R)	5'-ccggctagtcgagcatttgg-3'
3	ICP3(F)	5'-TCAGGTAAAGGTCAATCAGGT-3'
	ICP3(R)	5'-AAGACTACCGCTGAGTTGACC-3'
4	Felix O1(F)	5'-CCTTCATGTCACGTCTTGCC-3'
	Felix O1(R)	5'-GGAGTAGTTGGCACTTGGGC-3'
5	Sf6(F)	5'-CCA TGG CGA CTG AAC CAA AAG-3'
	Sf6(R)	5'-TTA CCA ACC GGA GGA TGA GGG-3'
6	P68(F)	5'-GGAATTCATATGAAACCTG AAGGTAACAGTCAACGC-3'
	P68(R)	5'-CCGCTCGAGTTACCCGCG GCTGCTGGCAC-3'
7	ΦEF24C(F)	5'-AGAGTTTGATCCTGGCTCAG-3'
	ΦEF24C(R)	5'-GACGGGCGGTGTGTACAAG-3'
8	Φ29(F)	5'-AATTGAATGGCATGAAGGCGCATCATCAATGCCGCGTAAAATGTATAGCT

		GT-3'
	Φ29(R)	5'- TTCTGCGCTTCAAAAATATCGTTCAGGCCATGGCTGCCGCGCGGCAC -3'
9	PBS1(F)	5'-GATACGACAGCTAGCTGGAAGGGCTAATTCACTCCC-3'
	PBS1(R)	5'-AACGTCTGGCTCGAGTTCAGGTCCCTGTTCGGGCGCCACTGCTAGAGATTTTCC-3'

ACKNOWLEDGEMENT

All authors contributed significantly to the study. RS conceptualised the work and designed the study. PS conducted sample collection, prepared the original draft of the manuscript and experiments. RK performed the experiments, while RK also carried out data analysis. VSB critically reviewed and proofread the manuscript. All authors read and approved the final version for publication.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this study.

FUNDING

The authors declare that no financial support or funding was received for the conduct of this study.

REFERENCES

[1] Clayton, G. E., Richards, L. A., Fox, B. G., Thorn, R. M. S., Bowes, M. J., Read, D. S., Tipper, H. J., Khamis, K., Dutta, T. K., Kumar, A., Hazra, M., Howard, B., Schneidewind, U., Armstrong, L. K., Nicholls, D. J. E., Davies, H., Hannah, D., Nel, H. A., Ghosh, A., … Reynolds, D. M. (2025). Associations of anthropogenic activity and tributaries with the physicochemical, nutrient and microbial composition of the Ganga (Ganges) River, India. Water Research, 278. https://doi.org/10.1016/j.watres.2025.123374

[2] Cruz, D., Cisneros, R., Benítez, Á., Zúñiga-Sarango, W., Peña, J., Fernández, H., & Jaramillo, A. (2021). Gram-negative bacteria from organic and conventional agriculture in the hydrographic basin of loja: Quality or pathogen reservoir? Agronomy, 11(11). https://doi.org/10.3390/agronomy11112362

[3] Das, D. A. (2025). Harnessing hydro chemical characterization of surface water using water quality indices and machine learning – Driven water quality modelling with special emphasis on side – Stream pollution. Desalination and Water Treatment, 324. https://doi.org/10.1016/j.dwt.2025.101592

[4] Ginn, O., Rocha-Melogno, L., Bivins, A., Lowry, S., Cardelino, M., Nichols, D., Tripathi, S. N., Soria, F., Andrade, M., Bergin, M., Deshusses, M. A., & Brown, J. (2021). Detection and Quantification of Enteric Pathogens in Aerosols near Open Wastewater Canals in Cities with Poor Sanitation. Environmental Science and Technology, 55(21), 14758–14771. https://doi.org/10.1021/acs.est.1c05060

[5] Hama Aziz, K. H., Mustafa, F. S., Omer, K. M., Hama, S., Hamarawf, R. F., & Rahman, K. O. (2023). Heavy metal pollution in the aquatic environment: efficient and low-cost removal approaches to eliminate their toxicity: a review. In RSC Advances (Vol. 13, Number 26, pp. 17595–17610). Royal Society of Chemistry. https://doi.org/10.1039/d3ra00723e

[6] Jaiswal, M., Gupta, S. K., Chabukdhara, M., Nasr, M., Nema, A. K., Hussain, J., & Malik, T. (2022). Heavy metal contamination in the complete stretch of Yamuna river: A fuzzy logic approach for comprehensive health risk assessment. PLoS ONE, 17(8 August). https://doi.org/10.1371/journal.pone.0272562

[7] Kumar, R., Vasić, T. P., Živković, S. P., Panneerselvam, P., Santoyo, G., de los Santos Villalobos, S., Olatunbosun, A. N., Pandit, A., Koolman, L., Mitra, D., & Gautam, P. (2025). Mechanistic Role of Heavy Metals in Driving Antimicrobial Resistance: From Rhizosphere to Phyllosphere. In Applied Microbiology (Switzerland) (Vol. 5, Number

3). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/applmicrobiol5030079

[8] Kusi, J., Ojewole, C. O., Ojewole, A. E., & Nwi-Mozu, I. (2022). Antimicrobial Resistance Development Pathways in Surface Waters and Public Health Implications. In Antibiotics (Vol. 11, Number 6). MDPI. https://doi.org/10.3390/antibiotics11060821

[9] Mutuku, C., Gazdag, Z., & Melegh, S. (2022). Occurrence of antibiotics and bacterial resistance genes in wastewater: resistance mechanisms and antimicrobial resistance control approaches. In World Journal of Microbiology and Biotechnology (Vol. 38, Number 9). Springer Science and Business Media B.V. https://doi.org/10.1007/s11274-022-03334-0

[10] Naylor, D., McClure, R., & Jansson, J. (2022). Trends in Microbial Community Composition and Function by Soil Depth. In Microorganisms (Vol. 10, Number 3). MDPI. https://doi.org/10.3390/microorganisms10030540

[11] Qi, Y., Cao, X., Cao, R., Cao, M., Yan, A., Li, E., & Xu, D. (2024). Research on the Analysis of and Countermeasures for the Eutrophication of Water Bodies: Waihu Reservoir as a Case Study. Processes, 12(4). https://doi.org/10.3390/pr12040796

[12] Ranveer, S. A., Dasriya, V., Ahmad, M. F., Dhillon, H. S., Samtiya, M., Shama, E., Anand, T., Dhewa, T., Chaudhary, V., Chaudhary, P., Behare, P., Ram, C., Puniya, D. V., Khedkar, G. D., Raposo, A., Han, H., & Puniya, A. K. (2024). Positive and negative aspects of bacteriophages and their immense role in the food chain. In npj Science of Food (Vol. 8, Number 1). Nature Research. https://doi.org/10.1038/s41538-023-00245-8

[13] Rohde, M. (2019). The Gram-Positive Bacterial Cell Wall. Microbiology Spectrum, 7(3). https://doi.org/10.1128/microbiolspec.gpp3-0044-2018

[14] Rout, A. K., Tripathy, P. S., Dixit, S., Behera, D. U., Behera, B., Das, B. K., & Behera, B. K. (2023). Unveiling the Microbiome Landscape: A Metagenomic Study of Bacterial Diversity, Antibiotic Resistance, and Virulence Factors in the Sediments of the River Ganga, India. Antibiotics, 12(12). https://doi.org/10.3390/antibiotics12121735

[15] Singh, P., & Singh, R. (2026a). Antimicrobial Resistance in the Ganga River Ecosystem: Linking Heavy Metal Pollution,Microbial Communities,and Environmental Resistance. Life Science Review, 10(1), 139–173. https://doi.org/10.51470/LSR.2026.10.01.139

[16] Singh, P., & Singh, R. (2026b). Unveiling the Bacteriophage Reservoir of the Ganga River: Ecological Roles, Environmental Drivers, and Biotechnological Potential. Journal of Diversity Studies, 5(1), 272–288. https://doi.org/10.51470/JOD.2026.5.1.272

[17] Sutton, T. D. S., & Hill, C. (2019). Gut Bacteriophage: Current Understanding and Challenges. In Frontiers in Endocrinology (Vol. 10). Frontiers Media S.A. https://doi.org/10.3389/fendo.2019.00784