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Antimicrobial Resistance in the Ganga River Ecosystem: Linking Heavy Metal Pollution, Microbial Communities, and Environmental Resistance

Prashant Singh, and Ranjan Singh*

Department of Microbiology, Dr Rammanohar Lohia Avadh University, Ayodhya (224001), India

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*Corresponding Author: **Ranjan Singh** | Email Address: **Ranjan.singh13@gmail.com**

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Abstract

Antimicrobial resistance (AMR) has emerged as a major global public health and environmental challenge, with freshwater ecosystems increasingly recognized as important reservoirs and dissemination pathways for antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), and metal resistance genes (MRGs). Among these ecosystems, the Ganga River represents a unique and globally significant model for investigating the interactions between microbial ecology, environmental pollution, heavy metal contamination, and resistance evolution. The river receives substantial inputs of municipal sewage, industrial effluents, hospital wastewater, agricultural runoff, pharmaceutical residues, and emerging contaminants, creating strong selective pressures that influence microbial community structure and function.This review synthesizes current knowledge regarding riverine microbial communities, the occurrence and dissemination of ARB and ARGs, heavy metal pollution, microbial metal tolerance mechanisms, and the role of co-selection in maintaining environmental resistomes, with particular emphasis on the Ganga River. Recent metagenomic and resistome-based investigations have revealed widespread distributions of ARGs, MRGs, virulence factors, and mobile genetic elements (MGEs) throughout both water and sediment environments. Sediments frequently harbor higher microbial diversity and greater abundances of resistance determinants than overlying waters, highlighting their importance as long-term environmental reservoirs. Particular attention is given to the mechanisms linking heavy metal contamination and antimicrobial resistance, including cross-resistance, co-resistance, and co-regulation pathways that facilitate the persistence and dissemination of resistance determinants.The review further discusses environmental and public health implications associated with resistance dissemination, including human exposure pathways, emergence of multidrug-resistant pathogens, and One Health concerns. Modern approaches for resistance monitoring, including metagenomics, shotgun sequencing, resistome analysis, bioinformatics, and GIS-based surveillance, are evaluated. Finally, key mitigation strategies, including wastewater treatment improvements, industrial discharge management, environmental surveillance, and policy interventionsapproaches, are highlighted. Further, the evidence demonstrates that the Ganga River functions as a significant environmental reservoir of antimicrobial resistance and underscores the urgent need for integrated One Health strategies to mitigate resistance dissemination and protect ecosystem and public health.

Keywords: *Ganga River, Antibiotic-resistant bacteria, Antibiotic resistance genes, Heavy metals, Co-selection, Riverine microbiome, Freshwater ecosystems, Bacteriophage.*

1. Introduction

Antimicrobial resistance (AMR) has emerged as one of the most significant environmental and public health challenges worldwide. Although the development of resistance has traditionally been associated with clinical settings, increasing evidence suggests that natural ecosystems serve as important reservoirs and transmission pathways for antibiotic resistance genes (ARGs). Among environmental compartments, freshwater ecosystems are particularly vulnerable because they receive continuous inputs of municipal sewage, industrial effluents, agricultural runoff, pharmaceutical residues, and other anthropogenic contaminants that can exert selective pressure on microbial communities [1], [2]. Consequently, rivers have become critical interfaces where environmental pollution, microbial ecology, and antimicrobial resistance intersect.The emergence and dissemination of antibiotic-resistant bacteria (ARB) and ARGs have become a growing concern due to their potential impacts on ecosystem functioning and public health. Environmental dissemination of resistance determinants is facilitated through wastewater discharge, urban runoff, hospital effluents, livestock waste, and industrial activities. Riverine ecosystems act as conduits that transport resistant microorganisms across large geographical regions while simultaneously providing conditions favorable for horizontal gene transfer and resistance evolution [3], [4]. Metagenomic investigations conducted along different stretches of the Ganga River have revealed the widespread occurrence of multidrug resistance genes, β-lactam resistance genes, tetracycline resistance determinants, aminoglycoside resistance genes, and vancomycin resistance pathways, indicating that river ecosystems are increasingly important reservoirs of environmental AMR [5], [6]. Freshwater ecosystems are increasingly recognized as hotspots for the emergence and maintenance of antimicrobial resistance. Rivers integrate pollutants originating from multiple sources and therefore represent ideal environments for the accumulation of resistant microorganisms and resistance genes. The Ganga River is among the most densely populated river basins in the world and receives substantial inputs of municipal sewage, industrial effluents, agricultural runoff, and emerging contaminants. These anthropogenic pressures have significantly altered the physicochemical characteristics and microbial ecology of the river [2], [7].Recent metagenomic investigations have revealed the widespread occurrence of antibiotic resistance genes (ARGs), metal resistance genes (MRGs), and mobile genetic elements (MGEs) throughout different stretches of the river, indicating that the Ganga acts as an important environmental reservoir of antimicrobial resistance [3], [4], [5]. Comparative analyses of water and sediment microbiomes further demonstrated that sediments harbor greater microbial richness and a higher abundance of ARGs than overlying waters, emphasizing their role as long-term repositories of resistance determinants [5], [8]. The persistence of ARGs within these environments increases the likelihood of their dissemination through microbial interactions and horizontal gene transfer. Microorganisms constitute the foundation of aquatic ecosystem functioning and play indispensable roles in nutrient cycling, organic matter decomposition, pollutant degradation, and biogeochemical transformations. Riverine microbial communities contribute significantly to carbon, nitrogen, sulfur, and phosphorus cycling and thereby regulate ecosystem productivity and stability [8], [9], [10]. Studies on the Ganga River have revealed diverse microbial communities dominated by Proteobacteria, Firmicutes, Acidobacteria, Chloroflexi, and Bacteroidota, many of which are associated with biodegradation and ecological resilience under polluted conditions [8], [9], [10].Several bacterial genera, including *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Hydrogenophaga*, *Nitrospira*, and *Thauera*, have been identified as important members of the Ganga microbiome and are associated with thedegradation of organic pollutants and xenobiotic compounds [9], [11]. However, increasing anthropogenic pollution can alter microbial community composition, promoting the proliferation of opportunistic pathogens and resistant bacterial populations. The occurrence of biofilm-forming bacteria and multidrug-resistant microorganisms in both water and sediments further highlights the environmental and public health significance of riverine microbial communities [12].Heavy metal pollution has also emerged as a major environmental stressor influencing microbial community structure and function in freshwater ecosystems. Industrial discharges, tannery effluents, mining activities, municipal wastewater, and agricultural runoff contribute significant quantities of arsenic, cadmium, chromium, copper, lead, nickel, mercury, and zinc to riverine environments [4], [7]. Unlike many organic contaminants, heavy metals are persistent and non-biodegradable, allowing them to accumulate in sediments where they remain biologically available for extended periods. Several studies conducted in the Ganga River have reported elevated concentrations of arsenic, cadmium, copper, and other metals in water and sediment environments [4], [13]. Investigations have also identified diverse populations of metal-tolerant and metal-transforming bacteria capable of surviving under extreme contaminant stress [13], [14]. The widespread occurrence of metal resistance genes within Ganga microbial communities suggests that heavy metal contamination plays an important role in shaping environmental resistomes and microbial adaptation processes [3], [4].The relationship between heavy metal contamination and antimicrobial resistance has attracted considerable attention due to the phenomenon of co-selection. Co-selection refers to the maintenance and dissemination of ARGs under selective pressure imposed by heavy metals when resistance determinants for both stressors are genetically linked or physiologically associated. Because heavy metals persist in the environment for considerably longer periods than antibiotics, they may continuously select for microbial populations carrying both metal resistance genes and ARGs [3], [4]. Metagenomic studies of the lower Ganga River have revealed strong associations among ARGs, MRGs, and MGEs, indicating potential interactions between metal pollution and antimicrobial resistance dissemination [4]. High abundances of arsenic- and copper-resistance genes have been observed alongside multidrug resistance determinants, suggesting that co-selection mechanisms may contribute to the persistence of environmental resistomes. However, recent investigations have also indicated that untreated wastewater discharge and fecal contamination remain major contributors to ARG dissemination in river systems, highlighting the complexity of environmental resistance dynamics [15]. Considering the increasing evidence linking heavy metal pollution with antimicrobial resistance, a comprehensive understanding of their interactions in freshwater ecosystems is urgently needed.

The Ganga River provides an ideal model system because of its ecological importance, extensive anthropogenic pressures, diverse microbial communities, and documented occurrence of ARGs, MRGs, and MGEs. Therefore, this review aims to synthesize current knowledge regarding riverine microorganisms, heavy metal contamination, antimicrobial resistance, and co-selection mechanisms in freshwater ecosystems with particular emphasis on the Ganga River. The review further evaluates ecological and public health implications, highlights existing knowledge gaps, and identifies future research priorities for monitoring and mitigating environmental AMR.

2. Riverine Microbial Communities and Their Ecological Functions

Riverine microbial communities represent one of the most dynamic and ecologically significant biological components of freshwater ecosystems. These microorganisms regulate nutrient cycling, organic matter decomposition, pollutant degradation, primary productivity, and comprehensive ecosystem stability. Rivers continuously receive microorganisms from terrestrial, atmospheric, and aquatic sources, resulting in highly diverse microbial assemblages that respond rapidly to environmental disturbances and anthropogenic pressures. Recent advances in metagenomics and high-throughput sequencing have substantially improved the understanding of riverine microbial ecology, revealing the remarkable diversity and functional capabilities of microbial communities inhabiting the Ganga River and other freshwater ecosystems [4], [8], [16]. As anthropogenic pollution intensifies across river basins, understanding the structure and ecological functions of riverine microorganisms has become increasingly important for evaluating ecosystem health and environmental sustainability.

2.1. Diversity of Riverine Microorganisms

River ecosystems harbor diverse assemblages of bacteria, archaea, fungi, protists, algae, and viruses that collectively contribute to ecosystem functioning. Among these groups, bacteria constitute the dominant microbial distinct and play critical roles in biogeochemical transformations. Chettri and Joshi (2021) [17], has identified 245 bacterial isolates representing 69 genera distributed among five major bacterial phyla,like the Proteobacteria, Actinobacteria and Firmicutes, followed by Bacteroidetes andDeinococcus-Thermus. These phyla were represented by various classes belonging to Alpha-peoteobacteria, Betapeoteobacteria, Gamma-proteobacteria, Actinobacteria, Bacilli, Flavobacteriia, Deinococci, Sphingobacteriia andCytophagia, demonstrating significant spatial variability along the river continuum. Metagenomic investigations conducted along various stretches of the Ganga River have revealed a rich microbial diversity dominated by Proteobacteria, Firmicutes, Acidobacteria, Chloroflexi, Bacteroidota, Actinobacteria, Verrucomicrobiota, and Gemmatimonadota[5], [10], [16]. Sediment habitats generally exhibit greater microbial richness than overlying waters due to their higher organic matter content and stable environmental conditions [5].Several genera including *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Hydrogenophaga*, *Nitrospira*, *Thauera*, *Gemmatimonas*, and *Arenimonas*, have been reported from different regions of the Ganga River [6], [16]. Proteobacteria frequently dominate both water and sediment environments, reflecting their remarkable metabolic versatility and adaptability to fluctuating environmental conditions [10]. Studies from the lower stretch of the Ganga further demonstrated the ubiquity of Gammaproteobacteria and the presence of rare microbial taxa exhibiting site-specific distributions influenced by local environmental conditions [4]. Seasonal investigations have also highlighted temporal variations in microbial community composition, indicating that riverine microbiomes remain highly responsive to hydrological and physicochemical changes [3].Similarly, metagenomic investigations of the Brahmaputra River revealed more than 3,600 microbial species belonging to 31 phyla and over 1,000 genera, highlighting the extraordinary richness of river sediment [18].Microbial diversity differs substantially between water and sediment compartments. River sediments generally support more diverse and metabolically active microbial assemblages because they provide stable habitats enriched with organic matter and nutrients. These sediment-associated communities act as reservoirs of genetic diversity and contribute significantly to ecosystem functioning.

2.2. Microbial Roles in Nutrient Cycling

Microorganisms are fundamental drivers of nutrient cycling within river ecosystems. Through diverse metabolic activities, they mediate the transformation and recycling of carbon, nitrogen, phosphorus, sulfur, and other essential elements. These processes sustain ecosystem productivity and maintain biogeochemical equilibrium[8]. In the Ganga River, microbial communities have been shown to participate actively in carbon mineralization and degradation of complex organic compounds. Sediment-associated bacterial populations possess substantial genetic potential for degradation of xenobiotic compounds, aromatic hydrocarbons, benzoates, aminobenzoates, and nitrotoluene derivatives [16]. Such functional capabilities are particularly important in polluted environments where microorganisms contribute to natural attenuation and remediation of contaminants. Nitrogen cycling processes, including nitrification, denitrification, and ammonification are also strongly influenced by riverine microbial communities. The occurrence of nitrifying bacteria such as *Nitrospira* suggests active nitrogen transformation pathways in the Ganga ecosystem [16]. Similarly, microbial taxa involved in phosphorus mobilization and nutrient regeneration contribute to maintaining nutrient availability for primary producers.

Functional metagenomic analyses have revealed numerous genes associated with metabolic pathways linked to nutrient turnover, environmental adaptation, and ecosystem resilience [8].Metagenomic studies have revealed extensive genetic potential for amino acid metabolism, energy production, carbohydrate degradation, and nutrient transformation pathways in river sediments[8], [18]. Microorganisms also participate in metal and metalloid cycling. Arsenic-transforming bacteria isolated from river environments can oxidize toxic arsenite [As(III)] into the less toxic arsenate [As(V)], thereby influencing contaminant mobility and reducing ecological risk [13], [19].

2.3. Biofilm Formation in Aquatic Systems

Biofilms represent structured microbial communities embedded within extracellular polymeric substances and attached to biotic or abiotic surfaces. In river ecosystems, biofilms develop on sediments, rocks, aquatic vegetation, and anthropogenic surfaces. Recent investigations on culturable bacteria from the Ganga River demonstrated that a substantial proportion of isolates possess moderate to strong biofilm-forming capabilities, indicating their potential role in persistence and dissemination of resistance determinants. In the Ganga River, approximately 43% of isolated bacterial strains exhibited moderate biofilm-forming capabilities, indicating the ecological importance of biofilm-mediated adaptation and persistence[12]. Biofilms also promote horizontal gene transfer, facilitating the dissemination of antibiotic resistance genes (ARGs), metal resistance genes (MRGs), and virulence factors. Verma et al (2024)[13], reported that arsenic-resistant bacteria isolated from the Ganga River exhibited significant biofilm formation under arsenic stress, demonstrating the role of biofilms in enhancing microbial survival under adverse environmental conditions.

2.4. Environmental Factors Shaping Microbial Communities

Riverine microbial communities are strongly influenced by environmental variables that regulate microbial growth, survival, and community structure. Physicochemical parameters including temperature, pH, dissolved oxygen, nutrient concentrations, conductivity, salinity, turbidity, and hydrological conditions significantly affect microbial diversity and distribution patterns [1], [4].Spatial variations in water quality across the Ganga River have been associated with distinct microbial assemblages and functional profiles. Elevated concentrations of nitrate and phosphate in downstream urban stretches have been linked with increased abundance of heterotrophic and pollution-associated bacterial taxa [6]. Similarly, dissolved oxygen, nutrient availability, and organic matter content influence microbial metabolic pathways and ecological functions[2], [4].Heavy metals and emerging organic contaminants represent additional environmental factors capable of shaping microbial communities. Elevated concentrations of arsenic, cadmium, copper, chromium, and other contaminants can selectively favor resistant microbial populations while suppressing sensitive taxa [2], [20]. Metagenomic analyses have demonstrated strong associations between environmental variables and the distribution of ARGs, metal resistance genes (MRGs), and mobile genetic elements (MGEs), indicating that environmental conditions play a crucial role in determining microbial community composition and resistance profiles [4].

2.5. Impact of Anthropogenic Activities on Microbial Ecology

Anthropogenic activities are among the most significant drivers of microbial ecological change in river systems. Rapid urbanization, industrialization, agricultural intensification, population growth, and inadequate wastewater management have substantially altered microbial habitats throughout the Ganga basin [1], [2].Comparative analyses between upstream and downstream sections of the Ganga River revealed significantly greater abundance of Gram-negative bacteria, chemo-organo-heterotrophs, and ARGs in urbanized downstream regions, demonstrating the profound influence of human activities on microbial ecology [6].Numerous studies have demonstrated that anthropogenic pollution modifies microbial diversity and promotes the proliferation of antibiotic-resistant bacteria and resistance genes. Metagenomic investigations of the lower Ganga River identified widespread distributions of ARGs, MRGs, and mobile genetic elements, particularly in regions affected by dense human populations and sewage discharge [3], [4]. Multidrug resistance genes, copper and arsenic-resistance determinants, and virulence-associated genes have been reported in several riverine microbial communities, indicating substantial environmental selection pressures [21].Studies have consistently reported increased abundance of ARGs, MRGs, and MGEs in heavily impacted stretches of the Ganga River, indicating that anthropogenic pollution is a major driver of environmental resistome expansion [3], [4], [8].Untreated wastewater has been identified as a primary source of antimicrobial resistance dissemination in urban rivers. Bagra et al (2024)[15], has demonstrated that continuous wastewater discharge contributed more significantly to river resistomes than heavy-metal-mediated co-selection. Similarly, studies on the Ganga and Yamuna rivers reported elevated abundances of antibiotic-resistant *Escherichia coli* and multiple antibiotic resistance phenotypes associated with deteriorating water quality [20].

3. Antibiotic-Resistant Bacteria (ARB) In River Ecosystems

The increasing prevalence of ARB in river ecosystems poses significant ecological and public health concerns because rivers serve as important sources of drinking water, irrigation, fisheries, recreation, and cultural activities.

Recent metagenomic investigations of the Ganga River have demonstrated widespread occurrence of ARGs, multidrug-resistant bacteria, and mobile genetic elements (MGEs), highlighting the importance of river ecosystems as environmental reservoirs of antimicrobial resistance [3], [4], [16], [3], [4], [22]. Similar studies conducted in the Brahmaputra, Saryu, Yamuna, and other river systems have revealed widespread occurrence of multidrug-resistant bacteria and resistance determinants associated with human activities. These findings highlight the ecological and public health significance of river ecosystems in the global AMR crisis [8], [18].

3.1. Major ARB Reported in Freshwater Environments

Several clinically important bacterial species have been repeatedly detected in the rive Ganga and freshwater environments worldwide. These organisms are of particular concern because they possess significant pathogenic potential and frequently harbor multiple resistance determinants,Table1.

3.1.1. Escherichia coli

Escherichia coli(*E. coli*) is widely used as an indicator of fecal contamination and environmental antimicrobial resistance. Although many strains are harmless commensals, pathogenic variants can cause gastrointestinal infections, urinary tract infections, septicemia, and meningitis. Environmental studies of the Ganga and Yamuna rivers have reported elevated abundances of antibiotic-resistant *E. coli* exhibiting resistance to penicillin, vancomycin, ampicillin, and multiple other antibiotics [23]. Studies also investigating urban river systems in India identified fecal contamination as a major driver of environmental resistomes, emphasizing the importance of *E. coli* as an indicator organism for monitoring environmental AMR [15].High Most Probable Number (MPN) values and multiple antibiotic resistance indices indicate extensive fecal contamination and anthropogenic pressure in these river systems. Resistant *E. coli* populations are particularly important because they serve as reservoirs of transferable ARGs capable of spreading resistance throughout aquatic microbial communities[24].

3.1.2. Enterococcus spp.

*Enterococcus spp.*often carries resistance determinants against tetracyclines, aminoglycosides, macrolides, and glycopeptides. Their ability to survive in sediments and biofilms enhances long-term persistence and facilitates the dissemination of ARGs through conjugative plasmids and transposons. Consequently, *Enterococcus spp.*plays a crucial role in maintaining environmental resistomes and transmitting resistance among bacterial populations[25].

3.1.3. Pseudomonas aeruginosa

Pseudomonas aeruginosa possesses intrinsic resistance mechanisms including efflux pumps, low membrane permeability, antibiotic-modifying enzymes, and biofilm-forming capabilities. Metagenomic investigations of the Ganga River consistently reported the dominance of *Pseudomonas* species in both water and sediment samples [5], [26]. Correlation analyses revealed significant associations between *Pseudomonas* populations and multiple antibiotic resistance pathways, including β-lactam resistance, multidrug resistance, tetracycline resistance, aminoglycoside resistance, and vancomycin resistance [5].

3.1.4. Acinetobacter baumannii

Acinetobacter baumannii exhibits remarkable adaptability to adverse environmental conditions and possesses a strong capacity to acquire resistance determinants through horizontal gene transfer. Environmental metagenomic studies have detected Acinetobacter-associated resistance genes in polluted river systems where anthropogenic pressures are high. Resistance to carbapenems, aminoglycosides, fluoroquinolones, and tetracyclines is frequently reported, making *A. baumannii* a significant contributor to environmental antimicrobial resistance reservoirs[27], [28], [29].

3.1.5. Klebsiella pneumoniae

Klebsiella pneumoniae is another major opportunistic pathogen commonly associated with wastewater contamination. The bacterium possesses diverse resistance mechanisms, including extended-spectrum β-lactamase (ESBL) production and carbapenem resistance. Although metagenomic studies of the Ganga River primarily emphasize community-level resistomes, the occurrence of Enterobacteriaceae members suggests the environmental presence of clinically relevant pathogens such as *K. pneumoniae*[12].

3.1.6. Other ESKAPE Pathogens

The ESKAPE pathogens—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.—represent some of the most problematic antibiotic-resistant bacteria worldwide. Investigations of the Ganga River identified bacterial communities containing genera associated with pathogenicity, biofilm formation, virulence factors, and antimicrobial resistance, indicating the potential environmental persistence of ESKAPE-related organisms [8], [12].

Table 1: Major antibiotic-resistant bacteria (ARB) reported in freshwater ecosystems, with evidence from the Ganga River

Bacterial Taxon	Ecological Role in River Ecosystems	Resistance Characteristics	Environmental Significance	Evidence from the Ganga River	Ref.
Pseudomonas spp.	Organic matter degradation, xenobiotic transformation, nutrient cycling	Multidrug resistance (MDR), β-lactam, tetracycline, aminoglycoside and efflux-mediated resistance	Important environmental reservoir of ARGs; capable of biofilm formation and horizontal gene transfer	Frequently detected in water and sediment microbiomes; strongly associated with ARG abundance	[5], [8]
Acinetobacter spp.	Adaptation to polluted aquatic environments	MDR phenotype, carbapenem-associated resistance determinants, ARG carrier	Opportunistic pathogen with high environmental persistence	Associated with antibiotic resistance pathways in river microbiomes	[3], [5]
Flavobacterium spp.	Organic matter decomposition and carbon cycling	Reservoir of environmental ARGs and stress-response genes	Contributes to dissemination of resistance determinants in aquatic habitats	Detected in both water and sediment microbial communities	[5]
Hydrogenophaga spp.	Nitrogen transformation and nutrient cycling	Potential carrier of ARGs and metal resistance genes	Frequently associated with polluted freshwater environments	Commonly reported in Ganga microbiome datasets	[8], [16]
Nitrospira spp.	Nitrification and nitrogen cycling	Environmental resistance reservoir; adaptation to contaminant stress	Important contributor to ecosystem functioning under pollution pressure	Abundant in Ganga sediment microbiomes	[16]
Kurthiagibsonii	Metal transformation and detoxification	Arsenic resistance, metal tolerance mechanisms	Indicative of microbial adaptation to heavy metal contamination	Isolated and characterized from Ganga River samples	[20]
Gram-negative chemo-organo-heterotrophs	Degradation of organic pollutants and nutrient turnover	Elevated ARG abundance and multidrug resistance pathways	Indicators of anthropogenic contamination and wastewater influence	Significantly enriched in downstream urban stretches of the Ganga River	[6]
Biofilm-forming bacterial communities	Surface colonization, nutrient retention and microbial interactions	Enhanced antibiotic tolerance, increased horizontal gene transfer and persistence of ARGs	Major reservoirs of environmental resistance determinants	Multiple resistant biofilm-forming isolates recovered from the Ganga River	[12]
Pseudomonas–Acinetobacter complex	Dominant taxa in contaminated freshwater ecosystems	Multidrug resistance and metal tolerance traits	Frequently associated with ARG–MRG co-occurrence and co-selection processes	Commonly reported in metagenomic studies of the Ganga River	[3], [4]
Potential ESKAPE-related environmental bacteria	Opportunistic colonizers of polluted aquatic environments	Multidrug resistance, virulence-associated traits and biofilm formation	Public health concern due to potential transfer of resistance determinants	Resistance-associated taxa detected in Ganga water and sediments	[8], [12]

Abbreviations: ARB, antibiotic-resistant bacteria; ARGs, antibiotic resistance genes; MDR, multidrug resistance; ESKAPE, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp.

3.2. Sources of ARB Contamination

3.2.1. Municipal Wastewater

Municipal wastewater is considered one of the primary sources of ARB and ARGs in river ecosystems. Untreated and partially treated sewage contains resistant bacteria originating from human microbiota, household waste, and community antibiotic consumption.Bagra et al (2024)[15], demonstrated that continuous wastewater discharge was the primary driver of river resistomes in urban Indian rivers, exceeding the influence of heavy metal contamination alone. The Ganga River receives substantial quantities of municipal wastewater throughout its course, contributing significantly to environmental resistome expansion [1], [6].

3.2.2. Hospital Effluents

Hospitals represent concentrated sources of antibiotics, disinfectants, pathogens, and resistant microorganisms. Hospital wastewater frequently contains high densities of multidrug-resistant bacteria and ARGs. The discharge of inadequately treated hospital effluents into freshwater systems facilitates dissemination of clinically relevant resistance determinants and pathogenic bacteria into natural environments [3], [4].

3.2.3. Agricultural Runoff

Agricultural activities contribute antibiotics, animal wastes, fertilizers, and resistant microorganisms to river systems. Runoff from livestock production areas introduces resistant enteric bacteria and ARGs into aquatic environments. The extensive agricultural land use throughout the Ganga basin has been identified as an important contributor to river pollution and microbial community alteration [1], [6].

3.2.4. Religious and Mass Gathering Activities

Religious activities and mass gatherings along riverbanks contribute significantly to microbial contamination. During events such as the Kumbh Mela, increased human activity introduces fecal microorganisms, pathogens, and resistant bacteria into river water[30]. Comparative metagenomic analyses demonstrated higher bacterial diversity, greater ARG abundance, and increased multidrug resistance during mass gathering periods than under normal conditions Maurya[31]. Such events may therefore facilitate the environmental dissemination of antimicrobial resistance.The Ganga River holds immense cultural and religious significance and hosts large-scale religious gatherings involving millions of participants. Ritual bathing, disposal of offerings, mass congregations, and temporary sanitation infrastructure can substantially increase microbial loading and fecal contamination in river waters[32].

Such activities contribute to fluctuations in microbial community composition and may facilitate thedissemination of ARB and ARGs, particularly when combined with inadequate wastewater management and high population density [1], [6], Figure 1.

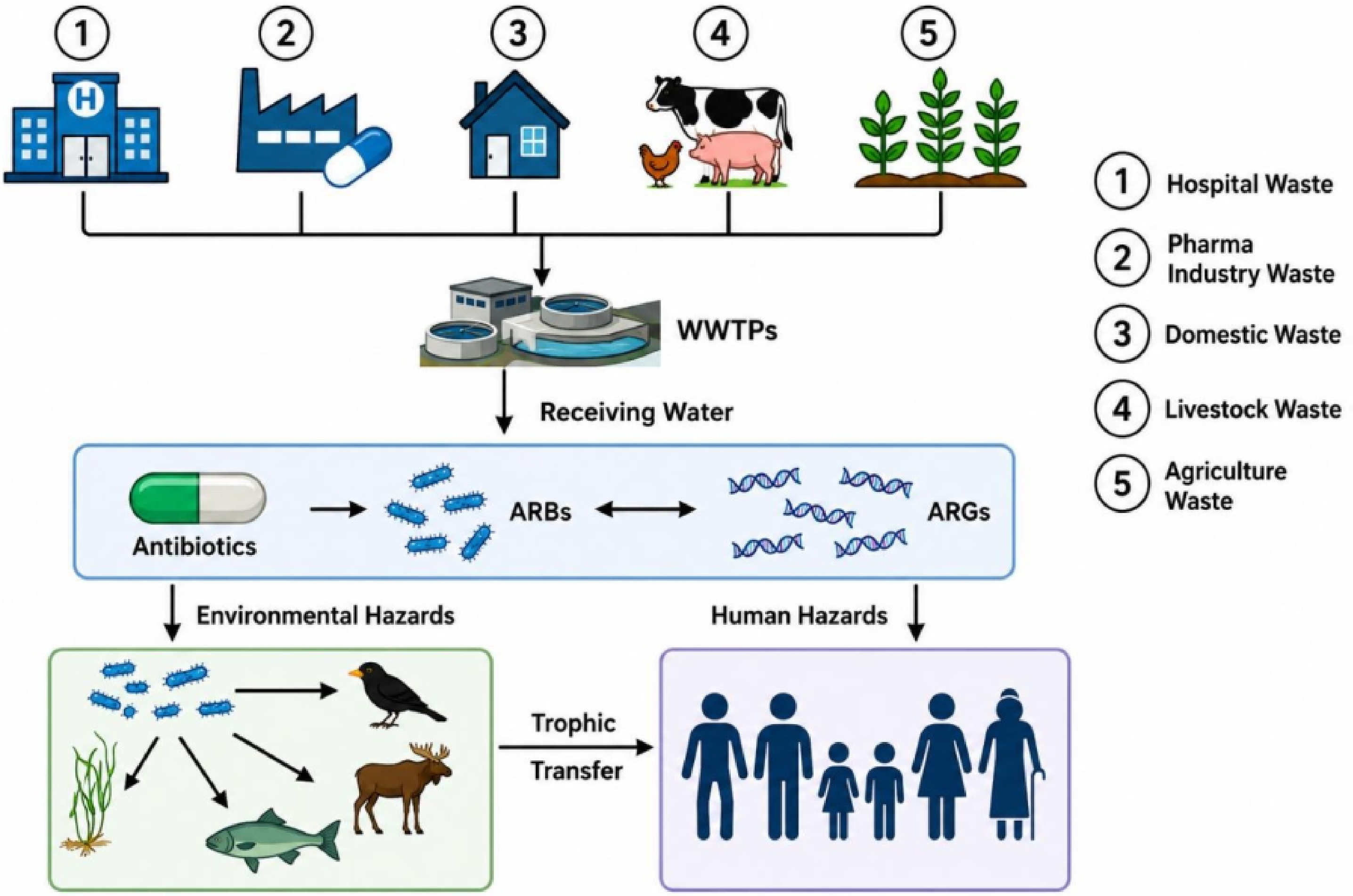

Figure 1: Environmental dissemination pathways of antibiotics, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs) from wastewater sources to ecological and human health systems.

Figure Description: Pathways of antimicrobial resistance dissemination from wastewater sources to aquatic ecosystems and human populations. Wastewater originating from pharmaceutical, domestic, hospital, livestock, and agricultural sources introduce antibiotics, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs) into wastewater treatment plants (WWTPs). Incomplete removal during treatment facilitates their release into receiving waters, where they persist, spread through aquatic food webs, and ultimately pose environmental and human health risks through trophic transfer and multiple exposure pathways.

3.3. Distribution of ARB in River Systems Worldwide

Antibiotic-resistant bacteria have been documented in river systems across Asia, Europe, Africa, and the Americas. Rivers receiving untreated wastewater generally exhibit higher abundances of ARB and ARGs than relatively pristine systems. In South Asia, extensive studies of the Yamuna, Brahmaputra, Teesta, and the river of Bangladesh networks have revealed widespread multidrug resistance, ARG hotspots, and strong associations between anthropogenic contamination and resistance dissemination [4], [33].

Studies conducted along the Ganga River have demonstrated widespread distribution of multidrug resistance genes, β-lactam resistance determinants, vancomycin resistance pathways, tetracycline resistance genes, and metal resistance genes across both water and sediment environments [3], [4], [5]. Spatial investigations revealed that downstream urbanized sections generally harbor higher ARG abundance than upstream regions due to increased anthropogenic influence [6]. Similarly, sediment environments frequently contain greater microbial diversity and higher resistance gene abundance than water columns, emphasizing their importance as environmental reservoirs of ARB [5], [8]. Collectively, these findings demonstrate that river ecosystems function as critical nodes in the environmental dissemination of antimicrobial resistance and highlight the need for comprehensive monitoring and mitigation strategies to reduce ARB transmission through freshwater environments.Metagenomic investigations have identified resistance determinants against β-lactams, tetracyclines, macrolides, aminoglycosides, sulfonamides, fluoroquinolones, rifamycins, and polymyxins in river water and sediments.

Furthermore, resistance genes frequently co-occur with metal resistance genes, suggesting that heavy metal pollution may contribute to ARG persistence through co-selection mechanisms [3], [4].

4. Antibiotic Resistance Genes (ARGs): Occurrence and Dissemination

Antibiotic resistance genes (ARGs) have emerged as critical environmental contaminants due to their ability to disseminate antimicrobial resistance among microbial populations. The widespread occurrence of ARGs in aquatic ecosystems has transformed rivers into important environmental reservoirs of antimicrobial resistance. River systems integrate contaminants from urban settlements, hospitals, industrial zones, agricultural lands, aquaculture facilities, and religious activities, creating favorable conditions for the selection and dissemination of ARGs. Advances in molecular biology, metagenomics, and next-generation sequencing have revealed extensive ARG diversity in riverine ecosystems worldwide, particularly in densely populated regions experiencing significant anthropogenic pressure[3], [4], [22].The Ganga River represents one of the most extensively studied freshwater ecosystems regarding environmental antimicrobial resistance. Metagenomic investigations conducted in different stretches of the river have consistently demonstrated the widespread occurrence of ARGs in both water and sediment environments. These studies have identified resistance determinants associated with β-lactams, tetracyclines, aminoglycosides, macrolides, sulfonamides, vancomycin, CAMP resistance pathways, and multidrug resistance mechanisms [3], [4], [5], [6]. The coexistence of ARGs with metal resistance genes (MRGs) and mobile genetic elements (MGEs) further emphasizes the complexity of resistance dissemination within river ecosystems.Recent studies conducted in the Brahmaputra, Teesta, Saryu, Yamuna, Padma, Jamuna, Bongshai, Kumar, and other South Asian river systems have documented extensive resistomes comprising clinically important resistance determinants associated with human pathogens and environmental microorganisms. The coexistence of ARGs, metal resistance genes (MRGs), virulence factors, and mobile genetic elements (MGEs) highlights the ecological complexity of riverine antimicrobial resistance and underscores the importance of rivers as hotspots for resistance evolution and dissemination [8], [10], [18], [33]. Comprehensive informationis presented in Table 2.

4.1. Overview of ARGs

ARGs are naturally occurring components of microbial genomes; however, anthropogenic activities have dramatically increased their abundance, diversity, and dissemination in environmental systems. Environmental ARGs can be present within chromosomes, plasmids, transposons, integrons, bacteriophages, and other mobile genetic elements that facilitate their transfer between microorganisms. The continuous introduction of antibiotics and resistant bacteria into aquatic ecosystems creates selective pressure that favors the proliferation of resistant populations and the persistence of ARGs[22], [29], [34], [35]. Studies conducted in the Ganga River have demonstrated that ARGs are widely distributed across both upstream and downstream regions, although their abundance generally increases in heavily urbanized stretches receiving untreated wastewater [6]. Comparative analyses of water and sediment microbiomes further revealed that sediment environments harbor significantly higher ARG richness and abundance than water columns, highlighting their role as long-term environmental reservoirs of resistance determinants [5], [8]. Metagenomic investigations also revealed strong associations between ARGs, microbial community composition, and environmental variables, suggesting that ecological processes play an important role in shaping environmental resistomes[4]. Metagenomic investigations across the Ganga River basin have revealed a broad spectrum of ARGs associated with multidrug resistance, β-lactams, tetracyclines, macrolides, sulfonamides, aminoglycosides, rifamycins, polymyxins, and fluoroquinolones [3], [4], [26]. Similar observations were reported from the Saryu River, where rifampin, tetracycline, macrolide, and multidrug resistance genes dominated the resistome profile
[21].

4.2. Major classes of ARGs

River ecosystems contain a wide diversity of ARGs reflecting the extensive use of antibiotics in human medicine, veterinary practices, agriculture, and aquaculture. Several ARG classes have been repeatedly reported in freshwater environments.

4.2.1. β-lactam resistance genes

β-lactam resistance genes encode mechanisms that neutralize β-lactam antibiotics through enzymatic degradation or modification of target proteins.Resistance to these antibiotics is primarily mediated through β-lactamase enzymes encoded by genes such as *bla*TEM, *bla*SHV, *bla*CTX-M, *bla*NDM, and *bla*OXA[5], [6], [36].Several river studies have documented widespread occurrence of β-lactam resistance genes[11], [37]. Ganga River identified β-lactam resistance pathways as the dominant ARG category in both water and sediment samples [5], [6]. Comparative investigations showed that β-lactam resistance genes were widely distributed throughout the river and frequently associated with bacterial genera such as *Pseudomonas*, *Acinetobacter*, and *Flavobacterium*[5].Swain et al(2025)[38], detected *bla*OXA-48, *bla*TEM, and *bla*SHV genes in carbapenem-resistant *Klebsiella pneumoniae* isolated from river systems receiving urban wastewater. Metagenomic survey conducted in the Brahmaputra River further revealed abundant β-lactam resistance determinants associated with *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp. [8], [18].

The occurrence of extended-spectrum β-lactamase (ESBL)-associated genes in river water highlights a growing environmental health concern.

4.2.2. Tetracycline Resistance Genes

Tetracycline resistance genes represent another important ARG group commonly detected in freshwater ecosystems. These genes confer resistance through efflux pumps, ribosomal protection proteins, or enzymatic inactivation mechanisms. Resistance is generally mediated through efflux pumps and ribosomal protection proteins encoded by *tetA*, *tetB*, *tetM*, *tetO*, *tetW*, and related genes [8], [39]. Studies conducted along the Ganga River identified tetracycline resistance genes among the most abundant ARGs in both sediment and water samples [3]. Metagenomic studies of the Ganga River identified tetracycline resistance pathways in both water and sediment samples, although their abundance was generally lower than β-lactam resistance genes [5]. Seasonal investigations further indicated significant variations in tetracycline resistance gene abundance, suggesting environmental influences on their persistence and dissemination [3].Bagra et al (2024)[15], detected *tetW* in urban rivers receiving untreated wastewater, while Maurya et al (2025)[21], reported*tetB(P)* among the dominant ARGs in the Saryu River. Similar findings from Bangladesh rivers demonstrated the widespread distribution of tetracycline resistance genes across freshwater environments [33].

4.2.3. Sulfonamide Resistance Genes

Sulfonamide resistance is commonly associated with *sul1*, *sul2*, and *sul3* genes, which encode modified dihydropteroate synthase enzymes with reduced antibiotic affinity. These genes frequently occur within class 1 integrons and other mobile genetic elements, facilitating horizontal transfer[40], [41], [42].Urban river studies consistently report sulfonamide resistance genes among the most abundant environmental ARGs. Bagra et al(2024)[15], detected *sul1* and *sul2* genes at all wastewaterimpacted river sites, confirming municipal sewage as a major source of sulfonamide resistance dissemination. Similar observations have been reported in the Ganga and Bangladeshi river systems
[33], [35].

4.2.4. Macrolide Resistance Genes

Macrolide resistance genes including *ermB*, *ermF*, *mefA*, and *mphA*, confer protection against antibiotics such as erythromycin, azithromycin, and clarithromycinthrough ribosomal methylation, efflux pumps, and antibiotic modification mechanisms. These genes are commonly detected in freshwater systems receiving municipal sewage and hospital effluents[8], [31]. Metagenomic analyses of the Ganga River identified macrolide resistance pathways among the dominant ARG categories associated with bacterial communities inhabiting both water and sediment environments [5]. Likewise, studies from the lower Ganga River reported substantial abundances of macrolide resistance genes associated with wastewater-derived bacterial populations [4].Another Metagenomic investigations of the Saryu River identified macrolide resistance as one of the dominant ARG categories [31].Network analyses further suggested strong associations between macrolide resistance pathways and dominant bacterial genera such as *Pseudomonas* and *Acinetobacter*.

4.2.5. Fluoroquinolone Resistance Genes

Fluoroquinolone resistance genes have become increasingly important due to the extensive use of fluoroquinolones in clinical and veterinary settings. These genes confer resistance through target modifications, efflux mechanisms, and protection proteins. Thsese genes occur through chromosomal mutations and plasmid-mediated mechanisms involving *qnrA*, *qnrB*, *qnrS*, *oqxAB*, and *aac(6′)-Ib-cr* genes [4], [8], [18], [19], [33], [43].Faruk et al (2025)[33], documented widespread fluoroquinolone resistance determinants in Bangladeshi river systems, while Sharma et al (2024)[18], reported multiple fluoroquinolone resistance-associated genes in Brahmaputra sediments. Their occurrence alongside multidrug resistance determinants increases the environmental risk posed by these resistomes. Althoughfluoroquinoloneresistance genes are generally reported at lower abundance than β-lactam resistance genes in river ecosystems, their environmental persistence remains a significant concern because of their association with multidrug-resistant bacterial populations and mobile genetic elements [3], [4].

4.3. Horizontal Gene Transfer Mechanisms

The dissemination of ARGs in environmental systems is largely facilitated through horizontal gene transfer (HGT), which enables genetic exchange among phylogenetically diverse microorganisms. HGT plays a crucial role in accelerating resistance evolution and environmental dissemination.

4.3.1. Transformation

Transformation involves the uptake of extracellular DNA released from lysed bacterial cells. River sediments and biofilms often contain abundant extracellular DNA, providing opportunities for naturally competent bacteria to acquire ARGs from their surroundings. The high microbial density and organic matter content of sediment environments create favorable conditions for transformation-mediated gene transfer.The high microbial density observed in Ganga and Brahmaputra sediments enhances opportunities for transformation-mediated gene exchange [8],[18].

4.3.2. Transduction

Transduction is mediated by bacteriophages that transfer genetic material between bacterial hosts. Recent metagenomic studies of the Ganga River revealed substantial phage diversity associated with both pathogenic and environmental bacterial populations [3]. Because bacteriophages can carry ARGs and transfer them across bacterial communities, transduction represents an important mechanism contributing to environmental resistome dissemination.

4.3.3. Conjugation

Conjugation is considered the most efficient mechanism for ARG dissemination in aquatic ecosystems. Conjugative plasmids, integrons, and transposons carrying ARGs can move directly between bacterial cells through cell-to-cell contact. Several studies identified abundant mobile genetic elements in the Ganga, Saryu, and Bangladeshi river systems, indicating active conjugative transfer processes [4], [31], [33].

4.4. Environmental Reservoirs of ARGs

Environmental reservoirs play a critical role in maintaining and spreading ARGs. River water, sediments, wastewater treatment plants, agricultural soils, aquaculture systems, animal manure, and groundwater have all been recognized as important ARG repositories. Municipal wastewater is considered the dominant source of ARG contamination in urban rivers[15]. Hospital discharges introduce clinically important resistance determinants, while agricultural runoff contributes veterinary-associated ARGs[37]. The Ganga River has been repeatedly identified as an important environmental reservoir of ARGs due to continuous inputs of municipal sewage, industrial discharges, agricultural runoff, and urban waste [1], [6]. Studies conducted along different stretches of the river demonstrated widespread occurrence of multidrug resistance genes and antibiotic resistance pathways, indicating extensive environmental dissemination of ARGs [4], [5]. The coexistence of ARGs with MRGs and MGEs further contributes to their persistence and mobility within environmental microbial communities.

4.5. River Sediment as an ARG Hotspot

River sediments are increasingly recognized as major hotspots for ARG accumulation and dissemination. Compared with water columns, sediments provide relatively stable environmental conditions, higher nutrient availability, greater microbial abundance, and elevated concentrations of pollutants that favor ARG persistence. Sediments also accumulate antibiotics, heavy metals, organic contaminants, and extracellular DNA, creating conditions conducive to resistance evolution and horizontal gene transfer. Metagenomic studies of Ganga sediments revealed hundreds of ARG subtypes representing resistance to at least 28 antibiotic classes [3]. Comparative metagenomic analyses of the Ganga River revealed significantly higher ARG abundance and microbial richness in sediment samples than in water samples [5]. Several antibiotic resistance pathways, including vancomycin resistance, multidrug resistance, tetracycline resistance, aminoglycoside resistance, and macrolide resistance, were found to be more abundant in sediments [5]. The lower Ganga River exhibited hotspots characterized by elevated abundances of multidrug resistance genes, arsenic resistance genes, copper resistance genes, and mobile genetic elements [4]. Studies on the Saryu River, Bangladeshi rivers, and Teesta River further support the concept that sediments act as longterm reservoirs of resistance determinants [17], [31], [33]. Similarly, Brahmaputra sediments contained more than 50 ARG categories and numerous plasmid-associated genes, demonstrating the importance of sediment ecosystems in ARG maintenance [18]. Additional investigations identified extensive distributions of ARGs, virulence factors, and resistant microbial taxa within river sediments, emphasizing their importance as long-term environmental reservoirs of antimicrobial resistance [8]. The accumulation of ARGs, MRGs, MGEs, and diverse microbial populations within sediments creates an ideal environment for resistance maintenance and dissemination. Consequently, sediment compartments should be considered critical targets for environmental monitoring programs and future mitigation strategies aimed at reducing the spread of antimicrobial resistance in freshwater ecosystems.

Table 2: Major antibiotic resistance genes (ARGs) reported in freshwater environments and the Ganga River

ARG Class	Representative Genes	Antibiotic Class Affected	Major Resistance Mechanism	Environmental Significance	Evidence from the Ganga River	Ref.
β-lactam resistance genes	blaTEM, blaSHV, blaCTX-M, ampC	Penicillins, cephalosporins, carbapenems	β-lactamase-mediated antibiotic degradation and target modification	Most prevalent ARG category in aquatic environments; associated with multidrug-resistant pathogens	Widely detected in water and sediment resistomes; dominant ARG category	[3], [4], [5]
Tetracycline resistance genes	tetA, tetB, tetM, tetW	Tetracyclines	Efflux pumps and ribosomal protection proteins	Common indicator of anthropogenic pollution and agricultural inputs	Frequently detected in river microbiomes and resistome analyses	[3], [5]
Sulfonamide resistance genes	sul1, sul2, sul3	Sulfonamides	Alteration of target enzymes involved in folate synthesis	Often associated with wastewater contamination and mobile genetic elements	Reported in freshwater resistomes and linked with anthropogenic activities	[3], [15]
Macrolide resistance genes	ermB, ermF, mefA	Macrolides	Ribosomal target modification and active efflux	Frequently enriched in sewage-impacted environments	Detected among dominant resistance pathways in the Ganga River	[4], [5]

Aminoglycoside resistance genes	aadA, aph, aac	Aminoglycosides	Enzymatic antibiotic modification	Commonly associated with clinical and environmental resistance dissemination	Identified in metagenomic analyses of Ganga sediments and water	[4], [8]
Fluoroquinolone resistance genes	qnrA, qnrB, qnrS	Fluoroquinolones	Target protection and reduced drug binding	Emerging concern because of widespread clinical use of fluoroquinolones	Associated with urban and wastewater-impacted stretches	[3], [15]
Vancomycin resistance genes	vanA, vanB	Glycopeptides	Modification of cell wall precursors	Clinically important resistance determinant linked to opportunistic pathogens	Identified within environmental resistomes of the Ganga River	[5]
Multidrug resistance genes	mexAB-oprM, acrAB-tolC, mdtK	Multiple antibiotic classes	Broad-spectrum efflux systems	Promote cross-resistance and environmental persistence of resistant bacteria	Highly abundant in anthropogenically impacted regions	[3], [4]
CAMP resistance genes	Multiple CAMP-associated resistance determinants	Cationic antimicrobial peptides	Membrane modification and stress adaptation	Enhance bacterial survival under environmental stress	Detected in metagenomic studies of the lower Ganga River	[4]
Mobile ARG-associated determinants	Integron-associated gene cassettes, plasmid-borne ARGs	Multiple antibiotic classes	Horizontal gene transfer via MGEs	Critical drivers of ARG dissemination and co-selection	Frequently co-occur with MRGs and MGEs in Ganga microbiomes	[3], [4], [8]

Abbreviations: ARGs, antibiotic resistance genes; MGEs, mobile genetic elements; CAMP, cationic antimicrobial peptide; MDR, multidrug resistance.

5. HEAVY METAL POLLUTION IN RIVER SYSTEMS

Heavy metal contamination has emerged as one of the most persistent environmental challenges affecting freshwater ecosystems worldwide. Unlike many organic pollutants, heavy metals are nonbiodegradable, can accumulate in sediments and biota, and remain biologically available for extended periods. River systems are particularly vulnerable because they receive contaminants from diverse anthropogenic and natural sources, including industrial effluents, mining activities, agricultural runoff, municipal wastewater, and urban stormwater discharges. The Ganga River, one of the largest and most densely populated river systems in the world, has experienced substantial deterioration in water and sediment quality due to increasing anthropogenic pressures associated with rapid urbanization, industrialization, and agricultural intensification [1], [2]. Several investigations conducted along different stretches of the Ganga River have reported elevated concentrations of heavy metals in both water and sediment environments. These contaminants not only pose direct toxicological risks to aquatic organisms but also influence microbial community composition, ecological functions, and the dissemination of antimicrobial resistance [3], [4]. Consequently, understanding the sources, environmental behavior, and ecological impacts of heavy metals is essential for effective river management and ecosystem restoration.Heavy metals reported from the Ganga River and their associated microbial resistance mechanisms are summarized in Table 3.

5.1. Sources of Heavy Metals

Heavy metals enter river ecosystems through multiple pathways, with anthropogenic activities representing the dominant sources in most contaminated river basins. The Ganga River receives heavy metal inputs from industrial zones, urban settlements, agricultural landscapes, and natural geological processes, resulting in complex contamination patterns along its course Figure 2.

5.1.1. Industrial Discharge

Industrial activities are among the most significant contributors of heavy metals to river ecosystems. Numerous industries located along the Ganga basin, including tanneries, textile factories, paper mills, electroplating units, chemical manufacturing facilities, and metal-processing industries, discharge effluents containing various heavy metals into the river system [1], [16]. Industrial wastewater frequently contains chromium, cadmium, lead, copper, nickel, zinc, and arsenic, many of which persist in aquatic environments and accumulate in sediments. The high density of grossly polluting industries in urban stretches of the Ganga has been identified as a major factor contributing to metal contamination and ecological degradation [6].The lower Ganga River and several urban river systems in India have been reported to receive untreated or partially treated industrial effluents containing elevated metal concentrations [4]. Similarly, Faruk et al (2025)[33], observed that rivers connected to industrialized and densely populated regions of Bangladesh exhibited significantly greater abundances of metal resistance genes and heavy metal-associated microbial communities. Continuous industrial discharge not only increases metal accumulation but also exerts strong selective pressure on aquatic microorganisms.

5.1.2. Mining Activities

Mining and mineral processing operations contribute substantial quantities of heavy metals to freshwater environments through acid mine drainage, tailings disposal, ore processing residues, and erosion of exposed mineral deposits. Although direct mining activities are less extensive within some stretches of the Ganga basin than industrial sources, geological weathering and mining-related discharges can release arsenic, lead, cadmium, nickel, and other metals into tributaries that ultimately drain into the river system [1]. These inputs contribute to long-term accumulation of metals within sediments and aquatic habitats.Several Himalayan and sub-Himalayan river systems experience metal enrichment associated with geological formations and mining activities. Weathering of mineral-rich rocks contributes natural metal inputs; however, mining significantly accelerates metal mobilization and environmental contamination [17]. Long-term exposure to mining-derived metals can alter microbial community composition and disrupt ecosystem functioning.

5.1.3. Agricultural Practices

Agricultural activities represent an important non-point source of heavy metal contamination in river systems. Fertilizers, pesticides, livestock wastes, irrigation return flows, and soil erosion contribute metals such as cadmium, copper, zinc, and arsenic to aquatic environments. Approximately two-thirds of the Ganga basin is utilized for agricultural production, making agricultural runoff a significant contributor to pollutant loading [1], [5].Agricultural runoff transports these contaminants into rivers, particularly during monsoon periods. Studies from the Ganga basin have indicated that agricultural landscapes contribute substantially to metal loading in freshwater ecosystems, thereby increasing selective pressure on environmental microbial communities [2]. Continuous agricultural inputs may facilitate the enrichment of metal-tolerant and antibioticresistant bacteria within river ecosystems.

5.1.4. Urban Runoff

Urban runoff represents another major pathway for heavy metal entry into rivers. Road dust, vehicle emissions, tire wear, construction materials, domestic waste, stormwater runoff, and municipal wastewater contribute significant quantities of metals to aquatic environments. Studies conducted in highly urbanized stretches of the Ganga River have demonstrated strong relationships between urban development, wastewater discharge, and contaminant accumulation, including heavy metals [1], [4]. Rapid population growth and inadequate wastewater infrastructure further exacerbate contamination levels.Urban rivers receiving untreated sewage and runoff typically exhibit elevated concentrations of lead, copper, zinc, chromium, and nickel. Swain et al (2025)[38], reported substantial heavy metal contamination in river stretches receiving wastewater from densely populated urban centers. The accumulation of metals in sediments and biofilms further enhances ecological risks and long-term environmental persistence.

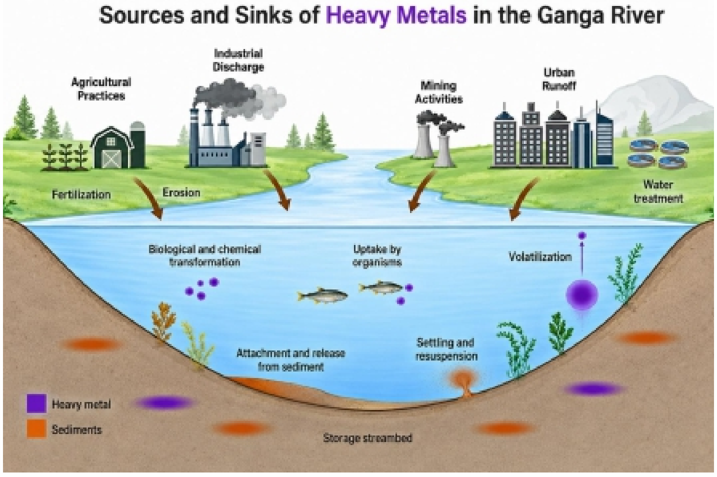

Figure 2: Sources, Transport Pathways, and Environmental Sinks of Heavy Metals in the Ganga River Ecosystem

Figure Description: Major sources, transport pathways, transformation processes, and environmental sinks of heavy metals in the Ganga River. Heavy metals originating from agricultural, industrial, mining, urban, and wastewater sources undergo biological uptake, sediment interaction, transformation, volatilization, and long-term storage within aquatic ecosystems.

5.2. Common Metals Detected in Rivers

Numerous heavy metals have been reported from river ecosystems worldwide, with several consistently detected in the Ganga River. These metals vary in toxicity, mobility, bioavailability, and ecological impact.

5.2.1. Mercury (Hg)

Mercury is among the most toxic environmental contaminants due to its ability to bioaccumulate and biomagnify through food webs. Industrial discharges, coal combustion, mining activities, and contaminated tributaries represent major sourcesof mercury contamination in the Ganga. In aquatic environments, mercury can be transformed into methylmercury by microorganisms, increasing its toxicity and ecological significance. Mercury contamination has been associated with alterations in microbial community structure and enrichment of mercury resistance genes[44], [45], [46].

5.2.2. Cadmium (Cd)

Cadmium is a highly toxic metal commonly introduced through industrial activities, phosphate fertilizers, and mining operations. Even at low concentrations, cadmium can adversely affect microbial metabolism, enzymatic activity, and cellular integrity. Environmental studies consistently identify cadmium as a major contaminant in polluted river systems and sediments [38].Investigations of the lower Ganga River reported elevated cadmium concentrations in surface waters, indicating significant anthropogenic influence [4].

5.2.3. Lead (Pb)

Lead contamination primarily originates from industrial emissions, urban runoff, batteries, paints, and vehicular sources. Lead accumulates in sediments and can persist for extended periods due to limited degradation[47], [48], [49].Elevated lead concentrations have been associated with reduced microbial diversity and shifts toward metal-resistant bacterial populations.

5.2.4. Chromium (Cr)

Chromium contamination is frequently linked to leather tanning, electroplating, textile industries, and metal finishing operations. Hexavalent chromium [Cr(VI)] is particularly toxic and poses significant ecological and human health risks. River systems receiving industrial wastewater often exhibit elevated chromium concentrations that influence microbial community composition and resistance development[48], [49], [50], [51].

5.2.5. Copper (Cu)

Copper is an essential micronutrient but becomes toxic at elevated concentrations. Major sources include industrial effluents, pesticides, urban runoff, and mining activities. Copper contamination exerts strong selective pressure on aquatic microorganisms and is frequently associated with the occurrence of copper resistance genes.The Ganga River basin contains numerous tannery industries that discharge chromium-containing wastes into aquatic environments [6]. Metagenomic analyses of the Ganga River have identified abundant copper resistance determinants co-occurring with ARGs [4].

5.2.6. Zinc (Zn)

Zinc enters river ecosystems through industrial discharges, galvanized materials, fertilizers, and municipal wastewater. Although essential for biological functions, excessive zinc concentrations can disrupt microbial metabolic processes and alter community composition. Zinc resistance genes are commonly detected in polluted aquatic environments[44], [47], [52].

5.2.7. Nickel (Ni)

Nickel contamination originates from metallurgical industries, mining activities, electroplating operations, and urban runoff. Elevated nickel concentrations may inhibit microbial growth and interfere with enzymatic functions. Several metal-tolerant bacterial isolates recovered from the Ganga River have demonstrated significant nickel resistance, reflecting adaptation to contaminated environments [14].

5.3. Environmental Persistence of Heavy Metals

One of the most significant characteristics of heavy metals is their environmental persistence. Unlike organic contaminants, heavy metals cannot be degraded into harmless compounds through biological or chemical processes. Instead, they remain within aquatic ecosystems and continuously cycle among water, sediments, suspended particles, and biological organisms. River sediments act as major sinks for heavy metals because particulate-bound contaminants settle and accumulate over time. Investigations of the Ganga River have repeatedly demonstrated the importance of sediment environments as repositories of contaminants, including heavy metals and associated resistance determinants [4], [16].Studies from the Ganga, Brahmaputra, and Bangladeshi river systems have demonstrated that sediments frequently contain higher concentrations of heavy metals than overlying water, making them critical repositories of contamination [4], [18], [33]. Persistent metal contamination maintains continuous selective pressure on microbial communities, promoting the evolution and maintenance of resistance traits.

5.4. Ecotoxicological Effects on Microbial Communities

Heavy metals exert profound effects on microbial communities by influencing cellular physiology, metabolic processes, community structure, and ecological interactions. Toxic metals can damage cell membranes, interfere with enzyme systems, induce oxidative stress, and disrupt nutrient acquisition pathways. Sensitive microorganisms may be eliminated from contaminated habitats, whereas resistant taxa become increasingly dominant. Recent metagenomic studies have demonstrated strong associations between heavy metal contamination and shifts in riverine microbial communities [18], [33]. Studies conducted along the Ganga River have demonstrated strong associations between heavy metal contamination and shifts in microbial community composition [3], [4].Metagenomic analyses revealed widespread occurrence of metal resistance genes (MRGs), particularly those associated with arsenic and copper resistance, indicating adaptive responses of microbial populations to chronic metal exposure [4]. Investigations involving arsenic-resistant bacteria further demonstrated the ability of indigenous microbial communities to transform, detoxify, and tolerate elevated metal concentrations [20]. Similar observations were reported from Bangladeshi rivers and Odisha river systems, where heavy metal contamination correlated positively with clinically relevant ARGs including blaOXA-48, blaTEM, and blaSHV[33], [38].Heavy metal contamination also has important implications for antimicrobial resistance. Co-occurrence of MRGs, antibiotic resistance genes (ARGs), and mobile genetic elements (MGEs) has been widely reported in the Ganga River, suggesting that metal pollution may contribute to the maintenance and dissemination of environmental resistomes through co-selection mechanisms [3], [4]. Heavy metal exposure may also stimulate biofilm formation, horizontal gene transfer, and the proliferation of multidrug-resistant bacteria. Studies investigating metal-tolerant bacteria from the Ganga River identified numerous isolates capable of tolerating arsenic, cadmium, chromium, copper, nickel, and iron concentrations exceeding 100 ppm, demonstrating substantial microbial adaptation to contaminated environments [14]. Such adaptations contribute to the development of complex environmental resistomes and may pose significant risks to ecosystem integrity and public health.

Table 3: Heavy metals reported from the Ganga River and associated microbial resistance mechanisms

Heavy Metal	Major Anthropogenic Sources in the Ganga Basin	Major Resistance Genes/Mechanisms	Microbial Adaptation Strategy	Potential Role in ARG Co-selection	Ref
Arsenic (As)	Industrial effluents, groundwater inputs, geogenic weathering, municipal wastewater	*arsR, arsB, arsC* operon; arsenite oxidase; arsenate reductase	Transformation of As(III) to less toxic As(V), active efflux and intracellular detoxification	Strong; arsenic resistance genes frequently co-occur with ARGs and MGEs	[3], [4], [20]
Copper (Cu)	Electroplating industries, metal-processing units, urban runoff, industrial wastewater	Copper efflux systems (*copA*, *cueO*), P-type ATPases, multicopper oxidases	Active metal extrusion and intracellular homeostasis	Strong; copper resistance determinants commonly linked with multidrug resistance genes	[3], [4]
Cadmium (Cd)	Industrial discharge, phosphate fertilizers, sewage sludge, battery waste	Cadmium efflux pumps (*cadA*), sequestration proteins, metal-binding peptides	Cellular detoxification and metal exclusion	Moderate to strong; chronic exposure promotes resistant microbial populations	[4]
Chromium (Cr)	Tanneries, leather-processing industries, electroplating units, industrial wastewater	Chromate transporters, chromate reductases (*chrA*), efflux systems	Reduction of toxic Cr(VI) to Cr(III) and active extrusion	Strong; chromium-contaminated sites frequently harbor ARG-rich microbiomes	[1], [4]
Zinc (Zn)	Urban runoff, fertilizers, industrial effluents, galvanized materials	Zinc transport proteins (*zntA*, *czcABC*), metal efflux systems	Regulation of intracellular zinc homeostasis	Moderate; often detected with copper and multidrug resistance pathways	[3], [4]
Nickel (Ni)	Metal-processing industries, combustion residues, industrial wastewater	Nickel transporters, intracellular sequestration proteins, efflux systems	Metal sequestration and detoxification	Moderate; contributes to environmental selection pressure	[4]
Lead (Pb)	Industrial discharge, municipal wastewater, urban runoff, battery waste	Biosorption, extracellular sequestration, efflux-mediated exclusion	Surface adsorption and reduced intracellular accumulation	Moderate; promotes persistence of resistant microbial communities	[1], [3]
Mercury (Hg)	Industrial waste, chemical manufacturing, municipal discharge	*mer* operon, mercuric reductase, volatilization pathways	Reduction of Hg^{2+} to elemental Hg^{0}	Very strong; mercury resistance genes are often linked with ARG-bearing plasmids	[3], [4]

6. HEAVY METAL TOLERANCE MECHANISMS IN MICROORGANISMS

Heavy metals are naturally occurring elements that become toxic when present at elevated concentrations in aquatic ecosystems. The continuous release of industrial effluents, municipal wastewater, agricultural runoff, mining residues, and urban discharges has significantly increased heavy metal concentrations in many river systems, including the Ganga River [1], [4]. Because heavy metals are non-biodegradable and persist in sediments for prolonged periods, riverine microorganisms are continuously exposed to metal stress. Such exposure exerts strong selective pressure on microbial communities, favoring the survival and proliferation of metal-tolerant populations. Consequently, microorganisms inhabiting contaminated aquatic environments have evolved diverse physiological, biochemical, and genetic mechanisms that enable them to tolerate, transform, detoxify, and remove toxic metals from their surroundings [3], [20].Metagenomic investigations conducted along different stretches of the Ganga River have revealed widespread occurrence of metal resistance genes (MRGs), particularly those associated with arsenic, copper, zinc, and other heavy metals [3], [4]. The identification of metal-resistant microbial taxa and functional resistance pathways highlights the adaptive capabilities of riverine microorganisms under chronic contaminant exposure. Understanding these tolerance mechanisms is essential for elucidating microbial adaptation processes and evaluating the ecological consequences of metal pollution in freshwater ecosystems.

6.1. Cellular Responses to Metal Stress

Exposure to heavy metals induces a variety of cellular responses aimed at minimizing toxicity and maintaining cellular homeostasis. Toxic metals interfere with essential biological processes by disrupting enzyme function, damaging membranes, altering protein structure, and generating reactive oxygen species (ROS). In response, microorganisms activate stress-response pathways that regulate gene expression, cellular metabolism, membrane transport systems, and protective biochemical mechanisms. Microbial communities inhabiting contaminated sections of the Ganga River exhibit remarkable adaptive capacities under metal stress conditions. Metagenomic analyses have revealed enrichment of functional genes involved in stress adaptation, metal transport, detoxification, and environmental resilience [4], [8]. These adaptive responses enable microorganisms to survive in habitats contaminated with arsenic, copper, cadmium, chromium, zinc, nickel, and other toxic metals. Long-term exposure to metal contamination often results in the selection of specialized microbial populations possessing enhanced tolerance mechanisms and resistance determinants [3].Studies involving arsenic-resistant and heavy metal-tolerant bacteria isolated from the Ganga River demonstrated that microbial populations exposed to contaminated environments possess enhanced physiological adaptations that enable survival under elevated concentrations of arsenic, cadmium, chromium, copper, nickel, and iron [13], [14].

Metagenomic analysis of river sediments further indicate that microbial communities inhabiting metalcontaminated environments possess enriched functional pathways associated with stress tolerance, membrane transport, and detoxification processes [10], [18].

6.2. Efflux Pumps

Efflux pumps constitute one of the most important microbial defense mechanisms against heavy metal toxicity. These membrane-associated transport proteins actively remove toxic metal ions from the cytoplasm and export them into the extracellular environment, thereby reducing intracellular metal concentrations to non-toxic levels.Many bacterial species inhabiting contaminated aquatic environments possess energy-dependent transport systems capable of exporting copper, zinc, cadmium, arsenic, nickel, and other toxic metals.Metagenomic investigations of the Ganga River identified numerous metal resistance genes associated with transport and efflux functions, indicating that active metal extrusion plays a major role in microbial survival under metal stress [3], [4].Other metagenomic analysis of the Saryu River revealed abundant metal resistance genes associated with membrane transport systems, including arsB, ruvB, and other transporter-related genes involved in metal detoxification [4], [31]. The prevalence of copper and arsenic resistance determinants throughout the river ecosystem suggests that efflux-mediated resistance mechanisms are widespread among indigenous microbial populations. These transport systems not only contribute to metal tolerance but also influence microbial community structure by providing competitive advantages under contaminated conditions.

6.3. Metal Sequestration Proteins

Another important mechanism of metal tolerance involves intracellular sequestration of toxic metal ions by specialized proteins. Metal sequestration proteins bind free metal ions and prevent their interaction with sensitive cellular components such as enzymes, nucleic acids, and membranes. By reducing the concentration of bioavailable metal ions within the cytoplasm, these proteins minimize toxic effects and maintain cellular function. Studies investigating metal-tolerant bacterial isolates from the Ganga River demonstrated that strains exhibiting high resistance to cadmium, chromium, copper, nickel, and arsenic possess enhanced metal-binding capacities, suggesting an important role for sequestration mechanisms in environmental adaptation [14]. Microorganisms isolated from contaminated stretches of the Ganga River have demonstrated remarkable capacities to tolerate elevated concentrations of arsenic and other metals, suggesting the presence of efficient intracellular sequestration systems [14], [20]. Similar observations have been reported from Teesta River isolates and metal-resistant microbial communities identified through metagenomic analyses [17].

6.4. Metallothioneins and Biosorption

Metallothioneins are low-molecular-weight, cysteine-rich proteins that bind heavy metals through thiol groups and play critical roles in metal homeostasis and detoxification. These proteins exhibit high affinity for metals such as cadmium, copper, zinc, mercury, and arsenic, enabling microorganisms to sequester toxic ions and reduce their biological availability.In addition to metallothioneins, microbial cells employ biosorption mechanisms involving cell wall components such as peptidoglycan, lipopolysaccharides, teichoic acids, extracellular polysaccharides, and biofilm matrices. These structures provide numerous binding sites for metal ions and reduce metal entry into the cytoplasm.In addition to intracellular binding mechanisms, microorganisms can remove metals through biosorption processes occurring at the cell surface. Biosorption involves passive binding of metal ions to functional groups present on microbial cell walls, extracellular polymeric substances (EPS), and biofilm matrices. Biofilms are particularly effective in accumulating and immobilizing heavy metals because of their large surface area and abundance of negatively charged binding sites [12]. Biofilm-mediated biosorption is particularly important in aquatic ecosystems because biofilms accumulate substantial quantities of heavy metals from surrounding environments. Environmental isolates from the Ganga River have demonstrated strong biofilm-forming capabilities under metal stress conditions, suggesting that biosorption contributes significantly to microbial survival in contaminated habitats [12], [13].The occurrence of biofilm-forming bacterial populations in the Ganga River suggests that biosorption may contribute significantly to microbial adaptation under metal-contaminated conditions. Microbial biofilms not only provide protection against metal toxicity but also act as sinks for heavy metal accumulation within aquatic ecosystems. Consequently, metallothionein-mediated sequestration and biosorption processes play crucial roles in determining metal mobility and bioavailability in riverine environments.

6.5. Enzymatic Detoxification Pathways

Many microorganisms possess enzymatic systems capable of transforming toxic metal species into less toxic or less mobile forms. These biochemical transformations represent important mechanisms of microbial adaptation and contribute significantly to natural attenuation processes in contaminated environments. Arsenic transformation provides a well-documented example of microbial enzymatic detoxification. Investigations conducted in the Ganga River identified arsenicresistant bacterial strains capable of oxidizing arsenite [As(III)] to arsenate [As(V)] through the activity of arsenite oxidase enzymes [20]. This transformation reduces toxicity because arsenate is generally less mobile and less toxic than arsenite. Similarly, microbial arsenate reductases participate in arsenic cycling and detoxification processes within aquatic environments.

Similarly, certain microorganisms reduce toxic hexavalent chromium [Cr(VI)] to the less toxic trivalent chromium [Cr(III)], while mercury-resistant bacteria possess mercuric reductase enzymes that convert ionic mercury into volatile elemental mercury. Metagenomic investigations of river ecosystems have identified numerous genes associated with metal transformation and detoxification pathways, highlighting the importance of enzymatic mechanisms in microbial adaptation to contaminated environments [4], [33].

6.6. Oxidative Stress Response Systems

Heavy metal exposure frequently leads to excessive production of reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals. These reactive molecules can damage proteins, lipids, nucleic acids, and cellular membranes, resulting in oxidative stress and cellular dysfunction.To mitigate oxidative stress, microorganisms possess sophisticated antioxidant defense systems. Key enzymatic antioxidants include superoxide dismutase (SOD), catalase, glutathione reductase, peroxidases, and thioredoxin-dependent enzymes. These systems neutralize reactive oxygen species and maintain intracellular redox balance. To counteract oxidative damage, microorganisms activate antioxidant defense systems composed of enzymes such as superoxide dismutase, catalase, peroxidases, and various redox-regulating proteins. These systems neutralize ROS and maintain cellular redox balance under stressful conditions. The persistence of microbial communities in heavily contaminated sections of the Ganga River indicates the effectiveness of oxidative stress response mechanisms in promoting survival under chronic metal exposure [3], [4].Metagenomic analyses of microbial communities inhabiting contaminated river sediments have identified numerous oxidative stress response genes associated with antioxidant defense pathways [10], [18]. The enrichment of these genes in metal-polluted environments suggests that oxidative stress management is a critical component of microbial metal tolerance.

6.7. Genetic Determinants of Metal Resistance

The ability of microorganisms to tolerate heavy metals is ultimately governed by specific genetic determinants collectively referred to as metal resistance genes (MRGs). These genes encode proteins involved in metal transport, sequestration, enzymatic transformation, stress response, and regulatory functions. MRGs may be located on chromosomes, plasmids, transposons, integrons, and other mobile genetic elements, facilitating their dissemination among microbial populations. Metagenomic studies conducted along the Ganga River consistently identified abundant MRGs associated with arsenic, copper, zinc, nickel, cadmium, and other metals [3], [4]. Among these, arsenic and copper resistance genes were particularly dominant, reflecting the selective pressures imposed by environmental contamination. Importantly, many MRGs coexist with antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs), creating opportunities for co-selection and horizontal gene transfer [4].In the Saryu River, arsB and ruvB were among the dominant metal resistance genes detected through shotgun metagenomic sequencing [31]. The ars operon, which includes arsR, arsB, arsC, and related genes, is particularly important for arsenic detoxification and has been widely detected in contaminated aquatic environments. Other commonly reported MRGs include copA (copper resistance), czcA (cadmium-zinc-cobalt resistance), merA (mercury resistance), and chrA (chromium resistance).A notable feature of many MRGs is their localization on plasmids, transposons, integrons, and other mobile genetic elements. This facilitates horizontal gene transfer between environmental and pathogenic microorganisms, contributing to the rapid dissemination of resistance traits. Several studies have demonstrated strong correlations between ARGs and MRGs in river ecosystems, suggesting that heavy metal contamination promotes co-selection of antimicrobial resistance even in the absence of antibiotic exposure [4], [33], [38]. The widespread occurrence of MRGs within riverine microbial communities demonstrates the long-term adaptive responses of microorganisms to metal pollution. Furthermore, the coexistence of MRGs and ARGs suggests that heavy metal contamination may indirectly contribute to the maintenance and dissemination of antimicrobial resistance in freshwater ecosystems. Therefore, understanding the genetic basis of metal tolerance is essential for assessing environmental risks and developing effective strategies for pollution management and ecosystem restoration.

7. CO-SELECTION OF ANTIBIOTIC RESISTANCE BY HEAVY METALS

The increasing prevalence of antimicrobial resistance (AMR) in aquatic environments is no longer considered solely a consequence of antibiotic contamination. Growing evidence suggests that heavy metals play a crucial role in the maintenance, enrichment, and dissemination of antibiotic resistance genes (ARGs) in environmental microbial communities. Unlike antibiotics, which may degrade relatively rapidly under environmental conditions, heavy metals are persistent contaminants that accumulate in water, sediments, and biota. Consequently, heavy metals can exert long-term selective pressure on microorganisms, favoring populations carrying both metal resistance genes (MRGs) and ARGs. This phenomenon, known as co-selection, has emerged as a major mechanism linking environmental pollution with the evolution and persistence of antimicrobial resistance [3], [4]. The Ganga River provides a particularly relevant model for studying co-selection because it receives substantial inputs of municipal sewage, industrial effluents, agricultural runoff, and heavy metal contaminants while simultaneously harboring diverse microbial communities enriched with ARGs, MRGs, and mobile genetic elements

(MGEs) [1], [5]. Metagenomic investigations conducted throughout the river have consistently revealed strong associations between antibiotic resistance determinants and metal resistance pathways, indicating that heavy metal contamination may contribute significantly to environmental resistome expansion [3], [4]. Table 4, representsthe evidence demonstrating co-selection of antibiotic resistance genes (ARGs) and metal resistance genes (MRGs) in the Ganga River.

7.1. Concept of Co-selection

Co-selection refers to the process by which one selective agent indirectly promotes the persistence and dissemination of resistance traits against another selective agent. In the context of environmental AMR, heavy metals can select for microbial populations carrying ARGs even in the absence of antibiotic exposure. This occurs because resistance determinants for antibiotics and metals frequently coexist within the same microbial cells or are linked through shared physiological and genetic mechanisms. In contaminated river ecosystems, microorganisms are simultaneously exposed to multiple stressors, including antibiotics, heavy metals, disinfectants, and organic pollutants. Such conditions create strong selective pressures favoring resistant microbial populations. Since heavy metals remain environmentally persistent for prolonged periods, they can continuously enrich microbial communities carrying both MRGs and ARGs, thereby sustaining environmental resistomes long after antibiotic concentrations decline[3], [4].Similar patterns have been reported in Bangladeshi rivers, where ARG abundance positively correlated with metal resistance determinants associated with arsenic, copper, mercury, cadmium, and zinc resistance [33].

7.2. Mechanisms of Co-selection

Several mechanisms contribute to the co-selection of antibiotic resistance by heavy metals. These mechanisms include cross-resistance, co-resistance, and co-regulation, each involving distinct biological processes that facilitate the persistence and dissemination of resistance determinants.

7.2.1. Cross-resistance

Cross-resistance occurs when a single physiological mechanism confers resistance to both antibiotics and heavy metals. In many bacteria, multidrug efflux pumps can transport a wide range of toxic compounds, including antimicrobial agents and metal ions. These membrane-associated transport systems reduce intracellular accumulation of toxic substances and enhance survival under stressful environmental conditions. Microbial communities inhabiting contaminated stretches of the Ganga River possess numerous genes associated with efflux-mediated resistance and environmental adaptation [4]. Exposure to heavy metals may therefore select for microorganisms possessing broadspectrum resistance mechanisms that simultaneously confer tolerance to antibiotics. This phenomenon allows bacterial populations to maintain antibiotic resistance even in environments where antibiotic concentrations are relatively low.Metagenomic investigations of river sediments have identified numerous transporter-associated genes linked to both antibiotic and metal resistance. Efflux systems belonging to the resistance-nodulation-cell division (RND), ATP-binding cassette (ABC), and cation diffusion facilitator (CDF) families are frequently detected in microbial communities inhabiting contaminated river environments [10], [18]. Cross-resistance may also arise through oxidative stress response systems. Heavy metals induce the formation of reactive oxygen species (ROS), which damage cellular components and activate protective stress-response pathways. Many of these defense systems contribute to tolerance against both metal toxicity and antimicrobial exposure, thereby enhancing microbial survival under multiple environmental stressors.

7.2.2. Co-resistance

Co-resistance represents one of the most important mechanisms linking metal contamination and antibiotic resistance. This process occurs when ARGs and MRGs are physically linked on the same genetic element, such as plasmids, transposons, integrons, or genomic islands. Selection for metal resistance consequently promotes the maintenance of associated ARGs because both resistance determinants are inherited together. Metagenomic investigations of the Ganga River have demonstrated extensive distributions of MRGs, ARGs, and MGEs within riverine microbial communities [3], [4]. Particularly abundant metal resistance determinants include genes associated with arsenic and copper resistance, which frequently coexist with multidrug resistance pathways. The presence of MGEs facilitates the horizontal transfer of linked resistance determinants among bacterial populations, accelerating dissemination throughout aquatic ecosystems.Multiple studies have demonstrated the co-occurrence of ARGs and MRGs within mobile genetic elements recovered from river ecosystems. Metagenomic analyses of the Ganga River identified multidrug resistance genes co-localized with arsenic, copper, mercury, and zinc resistance determinants [4]. Similarly, Faruk et al (2025)[33], reported that ARGs and MRGs frequently occurred within the same genomic contexts in Bangladeshi rivers, supporting the role of co-resistance in environmental resistance dissemination. Sediment environments appear especially important in co-resistance dynamics because they contain high microbial densities, elevated contaminant concentrations, and abundant opportunities for genetic exchange. Comparative studies of the Ganga River revealed higher abundance of both ARGs and MRGs in sediments than in overlying waters, supporting the role of sediments as hotspots for resistance evolution and dissemination [5], [8].

7.2.3. Co-regulation

Co-regulation occurs when resistance to antibiotics and heavy metals is controlled by shared regulatory networks. Exposure to one stressor may activate global regulatory pathways that simultaneously induce expression of multiple resistance determinants. In such cases, metal exposure can stimulate antibiotic resistance gene expression even when antibiotics are absent. Microorganisms inhabiting contaminated river systems frequently possess complex stress-response networks that coordinate adaptation to diverse environmental challenges. Heavy metal exposure can activate transcriptional regulators associated with efflux pumps, oxidative stress defenses, membrane modifications, and DNA repair systems. Because many of these responses contribute to both metal tolerance and antibiotic resistance, co-regulation represents an effective mechanism for maintaining multidrug-resistant phenotypes in contaminated environments [3].Metal-induced activation of oxidative stress pathways, membrane transport systems, and global transcriptional regulators can increase expression of both metal resistance and antibiotic resistance determinants. Studies examining river microbial communities have identified stress-response genes closely associated with ARGs and MRGs, suggesting that shared regulatory mechanisms contribute significantly to co-selection [10], [18].

7.3. Shared Resistance Pathways

The existence of shared resistance pathways provides a mechanistic basis for co-selection. Several physiological systems involved in metal resistance also contribute directly or indirectly to antimicrobial resistance. Among the most important are multidrug efflux pumps, biofilm formation mechanisms, oxidative stress response pathways, membrane permeability modifications, and detoxification enzymes. Studies conducted in the Ganga River have identified bacterial taxa such as *Pseudomonas*, *Acinetobacter*, and *Flavobacterium* that possess substantial capacities for both antibiotic and metal resistance [5], [12]. These microorganisms frequently harbor multiple resistance determinants that enable adaptation to polluted environments. Biofilm formation further strengthens shared resistance pathways by enhancing protection against antibiotics, heavy metals, and other environmental stressors.Oxidative stress defense systems constitute another shared pathway. Exposure to heavy metals generates reactive oxygen species (ROS), while many antibiotics induce oxidative damage as part of their antimicrobial activity. Enzymes such as catalase, superoxide dismutase, glutathione reductase, and peroxidases therefore contribute to resistance against both antibiotics and heavy metals [14], [20].Metagenomic analyses have revealed strong correlations among ARGs, MRGs, and MGEs, suggesting that shared resistance mechanisms contribute significantly to resistance persistence within riverine ecosystems [4]. These interactions highlight the interconnected nature of environmental resistance evolution.

7.4. Evidence from Freshwater Ecosystems

Freshwater ecosystems worldwide increasingly demonstrate links between heavy metal contamination and antimicrobial resistance. River systems receiving industrial discharges, municipal wastewater, and agricultural runoff often exhibit elevated abundances of both ARGs and MRGs. Such observations support the hypothesis that heavy metals contribute to environmental resistome expansion through co-selection processes. In the lower Ganga River, metagenomic analyses identified strong correlations between multidrug resistance genes and resistance determinants for arsenic, copper, mercury, and zinc [4]. Similarly, studies conducted along anthropogenically impacted stretches of the Ganga revealed temporal and spatial variations in ARGs that closely mirrored changes in metal resistance gene abundance [3]. The Ganga River provides compelling evidence of these interactions. Ghosh et al (2023) [4], reported widespread occurrence of ARGs and MRGs throughout the lower stretch of the river, with arsenic and copper resistance genes among the most abundant metal resistance determinants. Similarly, Samson et al (2023) [3], documented spatiotemporal variations in microbiome composition, resistome structure, and metal resistance pathways along anthropogenically impacted sections of the river. These studies consistently demonstrated co-occurrence of ARGs, MRGs, and MGEs, suggesting strong interactions between metal contamination and antibiotic resistance dissemination.Investigations in Bangladeshi rivers demonstrated that ARG-rich microbial communities frequently co-occurred with abundant MRGs and mobile genetic elements. Resistance determinants associated with arsenic, cadmium, copper, and mercury were particularly prevalent, suggesting that metal contamination contributes substantially to environmental resistome maintenance [33].Studies of carbapenem-resistant *Klebsiella pneumoniae* isolated from river systems in Odisha further demonstrated significant positive correlations between heavy metal concentrations and clinically important β-lactam resistance genes, including *blaOXA48*, *blaTEM*, and *blaSHV*[38].Additional support comes from investigations of arsenic-resistant bacterial populations isolated from the Ganga River. Verma et al (2024)[20], identified indigenous bacteria capable of tolerating elevated arsenic concentrations while exhibiting adaptive physiological responses to metal stress. Such findings indicate that chronic metal exposure can select for specialized microbial populations possessing multiple resistance traits.

7.5. Experimental Studies Demonstrating Co-selection

Experimental evidence supporting co-selection has emerged from both culture-based and metagenomic investigations.

Isolation of heavy metal-tolerant bacterial populations from the Ganga River demonstrated the ability of microorganisms to survive under elevated metal concentrations while maintaining diverse resistance characteristics. Seasonal assessments revealed significant populations of bacteria capable of tolerating multiple heavy metals, indicating long-term adaptation to contaminant exposure.[14]. Metagenomic studies provide additional evidence by demonstrating statistical associations among ARGs, MRGs, and MGEs. Correlation analyses conducted in the Ganga River consistently revealed positive relationships between metal resistance determinants and antibiotic resistance pathways [3], [4]. Similarly, arsenic-resistant bacteria isolated from the Ganga River possessed multiple stress-response mechanisms, biofilm-forming capabilities, and resistance traits that improved survival under both metal and antibiotic exposure conditions [13]. Metagenomic analyses further reveal that microbial communities inhabiting metal-polluted environments contain significantly greater abundances of ARGs, MRGs, and mobile genetic elements than communities from less contaminated sites [18], [31].These findings support the hypothesis that metal contamination can indirectly enrich antimicrobial resistance through shared physiological and genetic mechanisms.Although direct experimental validation remains limited in environmental settings, these observations strongly support the role of co-selection in shaping riverine resistomes.

7.6. Environmental Factors Influencing Co-selection

Several environmental factors influence the magnitude and effectiveness of co-selection processes in freshwater ecosystems. Heavy metal concentration is a primary determinant because elevated contamination levels increase selective pressure for resistant microbial populations. Persistent contaminants such as arsenic, copper, cadmium, chromium, and lead are particularly important because they remain biologically available for extended periods [4]. Sediment characteristics also play a crucial role. High organic matter content, elevated microbial density, abundant extracellular DNA, and accumulation of contaminants make sediments favorable environments for horizontal gene transfer and resistance maintenance [5], [8]. Additional factors including pH, dissolved oxygen, nutrient availability, temperature, hydrological fluctuations, and wastewater inputs further influence microbial community structure and resistance dynamics.Anthropogenic activities represent another critical factor. Urbanization, industrialization, agricultural runoff, and untreated wastewater discharge collectively introduce both metals and antibiotics into river ecosystems, intensifying opportunities for co-selection and resistance dissemination [1], [6].Hydrological factors also play important roles. Seasonal flooding, monsoonal runoff, and wastewater discharge alter contaminant distributions and microbial community structure. Studies from the Ganga and Brahmaputra rivers demonstrated significant spatiotemporal variation in ARG and MRG abundance associated with changing environmental conditions [3], [18].Furthermore, the presence of mobile genetic elements, bacteriophages, and biofilms enhances resistance dissemination and increases the likelihood of coselection events.

7.7. Ecological Consequences of Co-selection

The ecological consequences of co-selection extend beyond microbial resistance and encompass broader ecosystem-level impacts. Persistent selection for resistant microorganisms can alter microbial community composition, reduce biodiversity, and modify ecosystem functioning. Sensitive taxa may decline while resistant populations become increasingly dominant, potentially affecting nutrient cycling, organic matter decomposition, and biogeochemical processes. Co-selection also contributes to the expansion of environmental resistomes by promoting maintenance and dissemination of ARGs within natural microbial communities. Increased abundance of ARGs enhances opportunities for horizontal gene transfer and raises the likelihood that clinically relevant resistance determinants will be transferred to pathogenic bacteria. The coexistence of ARGs, MRGs, virulence factors, and MGEs within riverine ecosystems therefore represents a significant environmental and public health concern [3], [8]. Metagenomic studies of the Saryu, Brahmaputra, and Bangladeshi river systems consistently demonstrate that environments with elevated metal contamination harbor more complex resistomes characterized by ARGs, MRGs, virulence factors, and mobile genetic elements [4], [31], [33]. These findings suggest that heavy metal pollution contributes not only to ecological degradation but also to the emergence and dissemination of antimicrobial resistance on a regional and global scale.

Table 4: Evidence demonstrating co-selection of antibiotic resistance genes (ARGs) and metal resistance genes (MRGs) in the Ganga River

Study	Study Area	Resistance Elements Identified	Major Findings	Implications for Co-selection	Evidence of Co-selection
[4]	Lower Ganga	ARGs, MRGs, mobile genetic elements (MGEs), multidrug resistance genes	Metagenomic analysis revealed simultaneous occurrence of ARGs, MRGs, and MGEs within the same microbial communities. Arsenic, copper, mercury, and zinc resistance genes co-occurred with multidrug resistance determinants.	Demonstrates potential genetic linkage and co-selection of antibiotic and metal resistance under environmental contamination.	Strong evidence
[3]	Anthropogenically impacted stretch of the Ganga River, India	Resistome, mobilome, bacteriophages, metal resistance determinants	Spatio-temporal variation in microbial communities showed significant association between ARG abundance and metal resistance genes.	Indicates environmental pollutants, including metals, contribute to maintenance and dissemination of ARGs.	Strong evidence

[5]	Ganga River water and sediment	ARGs, biofilm-forming bacteria, multidrug-resistant isolates	Sediments contained higher ARG abundance and bacterial diversity than water samples, with multiple resistance pathways detected.	Suggests sediments function as long-term reservoirs where heavy metals and ARGs may accumulate together.	Moderate (ndirect evidence)
[8]	Ganga River sediments	ARGs, virulence factors, plasmid-associated genes	Metagenomic analysis identified numerous ARGs, virulence factors, and stress-response pathways in sediment microbiomes.	Indicates contaminated sediments provide conditions favorable for persistence and transfer of resistance determinants.	Moderate (Indirect evidence
[20]	Ganga River (Varanasi), India	Arsenic resistance genes, arsenite oxidase, biofilm-associated traits	Isolation of arsenic-resistant *Kurthiagibsonii* capable of arsenite oxidation and biofilm formation under metal stress	Supports the concept that metal contamination selects for highly adapted microbial populations capable of maintaining resistance traits.	Supports metal-driven selection
[53]	Seasonal bacterial diversity study	Heavy metal-tolerant bacteria, metal resistance traits	Seasonal surveys revealed widespread occurrence of bacteria tolerant to arsenic, cadmium, chromium, copper, nickel, and iron.	Suggests persistent heavy metal contamination may enrich microbial populations carrying resistance determinants.	Supports co-selection hypothesis

Abbreviations: ARGs = Antibiotic Resistance Genes; MRGs = Metal Resistance Genes; MGEs = Mobile Genetic Elements; MDR = Multidrug Resistance.

8. MOBILE GENETIC ELEMENTS LINKING ARGs AND METAL RESISTANCE

The environmental dissemination of antimicrobial resistance is largely driven by the mobility of genetic determinants among microbial populations. While antibiotic resistance genes (ARGs) and metal resistance genes (MRGs) may originate in specific bacterial taxa, their widespread occurrence in aquatic ecosystems is primarily facilitated by mobile genetic elements (MGEs). These elements mediate horizontal gene transfer (HGT), enabling resistance determinants to move between phylogenetically diverse microorganisms and across environmental compartments. Consequently, MGEs play a central role in the evolution, maintenance, and dissemination of environmental resistomes[3], [4], [18], [33]. The Ganga River represents an important model for understanding the role of MGEs in resistance dissemination because it contains abundant ARGs, MRGs, and diverse microbial communities exposed to multiple anthropogenic stressors. Metagenomic studies conducted along different stretches of the river have consistently demonstrated the coexistence of ARGs, MRGs, and MGEs, suggesting active genetic exchange and environmental dissemination of resistance determinants [3], [4]. The accumulation of contaminants in river sediments, coupled with high microbial density and extensive biofilm formation, creates favorable conditions for horizontal gene transfer and MGE-mediated resistance spread.

8.1. Plasmids

Plasmids are extrachromosomal, self-replicating DNA molecules that serve as major vehicles for horizontal gene transfer in bacterial populations. They frequently carry genes conferring resistance to antibiotics, heavy metals, disinfectants, and other environmental stressors. Because plasmids can move between unrelated bacterial species through conjugation, they play a critical role in the dissemination of multidrug resistance within aquatic ecosystems.

In river environments, plasmidmediated resistance is particularly important because microorganisms are continuously exposed to selective pressures arising from wastewater discharge, industrial contamination, and heavy metal pollution. Metagenomic investigations of the Ganga River have revealed extensive distributions of ARGs and MRGs associated with bacterial communities inhabiting both water and sediment environments [4], [5]. Another metagenomic analyses of the Ganga River identified numerous plasmid-associated sequences carrying multidrug resistance genes together with arsenic, copper, mercury, and zinc resistance determinants [4]. Similarly, studies of the Brahmaputra River sediment revealed extensive plasmid-mediated resistance networks involving both antibiotic and metal resistance genes [18]. Plasmids frequently carry multiple resistance determinants simultaneously, allowing selection for one trait to indirectly maintain others. Consequently, heavy metal contamination can promote persistence of antibiotic resistance through plasmid-mediated coselection processes. This phenomenon is particularly relevant in the Ganga River, where copper-, arsenic-, and multidrug-resistance determinants frequently occur together within microbial communities [4].

8.2. Integrons

Integrons are genetic platforms capable of capturing, integrating, and expressing gene cassettes, including those encoding antimicrobial resistance. Unlike plasmids, integrons are not self-mobile; however, they are often associated with plasmids and transposons, which facilitate their dissemination among bacterial populations.Integrons play a critical role in the evolution of environmental resistomes because they enable rapid acquisition and accumulation of multiple resistance determinants. River ecosystems contaminated with wastewater and industrial effluents provide ideal conditions for integron-mediated adaptation due to high microbial diversity and continuous exposure to selective agents.

The Ganga River exhibits widespread occurrence of ARGs associated with multidrug resistance pathways, indicating the presence of genetic mechanisms capable of accumulating diverse resistance determinants [3], [5]. The co-occurrence of ARGs, MRGs, and mobile genetic elements reported in metagenomic analyses suggests that integrons may contribute significantly to resistance dissemination within riverine microbial communities [4]. Their ecological importance lies in their ability to facilitate rapid microbial adaptation under changing environmental conditions. Another metagenomic investigation in Bangladeshi river systems identified integron-associated ARGs among dominant resistance determinants, highlighting their contribution to environmental resistance dissemination [4], [33]. The frequent co-occurrence of integrons with MRGs further supports their role in co-selection processes.

8.3. Transposons

Transposons, commonly known as “jumping genes,” are mobile DNA elements capable of moving between different locations within genomes or between chromosomes and plasmids. These elements often carry ARGs, MRGs, virulence factors, and regulatory genes, thereby contributing substantially to genetic diversity and microbial adaptation.Transposons contribute significantly to resistance dissemination because they can integrate into chromosomes, plasmids, and other mobile elements. This mobility promotes the assembly of complex resistance regions containing multiple adaptive genes. In freshwater ecosystems, transposons facilitate the movement of resistance determinants among bacterial populations inhabiting water columns, sediments, and biofilms. Their association with plasmids enhances mobility and increases opportunities for horizontal transfer. Studies conducted in the Ganga River have identified strong associations among ARGs, MRGs, and MGEs, indicating that transposon-mediated gene transfer likely contributes to resistance dissemination [4], [8]. Another several metagenomic studies of river sediments have identified transposon-associated ARGs and MRGs within microbial communities exposed to anthropogenic contamination [10], [18]. In metal-contaminated environments, transposons frequently contain both antibiotic and metal resistance determinants, facilitating co-selection and long-term maintenance of resistance traits.

8.4. Insertion Sequences

Insertion sequences (ISs) are the simplest class of transposable elements and consist primarily of genes encoding transposition enzymes flanked by short terminal repeat sequences.

Although they do not typically carry resistance genes themselves, insertion sequences influence microbial evolution by promoting genomic rearrangements, activating neighboring genes, and facilitating mobilization of ARGs and MRGs.

The importance of insertion sequences in environmental resistance dissemination arises from their ability to enhance genetic plasticity. By inserting near resistance genes, IS elements may alter gene expression patterns and increase resistance phenotypes. Furthermore, insertion sequences contribute to the formation of composite transposons that mobilize larger genomic regions containing multiple resistance determinants. The extensive diversity of microbial communities inhabiting the Ganga River provides opportunities for insertion sequence-mediated genetic diversification. Environmental pressures imposed by antibiotics, heavy metals, and other pollutants may favor microorganisms possessing highly adaptable genomes capable of responding rapidly to changing conditions[3], [18].

8.5. Bacteriophages as Vectors of Resistance Genes

Bacteriophages, or bacterial viruses, are increasingly recognized as important vectors for the dissemination of resistance genes in aquatic ecosystems. Through transduction, bacteriophages transfer genetic material between bacterial hosts, contributing to horizontal gene transfer independently of plasmids and conjugation mechanisms. Recent metagenomic studies of the Ganga River have revealed diverse phage communities associated with riverine bacterial populations[30], [34].These findings suggest that phages may contribute to environmental dissemination of resistance determinants across different ecological niches. Because bacteriophages can infect multiple bacterial hosts and persist under various environmental conditions, they represent highly effective vehicles for genetic exchange[3]. The ecological significance of phage-mediated transfer is amplified in environments characterized by dense microbial populations and extensive biofilm formation. Sediments and biofilms provide favorable habitats for interactions among bacteria and phages, increasing opportunities for transduction-mediated resistance dissemination. Consequently, bacteriophages are increasingly regarded as important components of environmental resistomes and should be considered alongside plasmids, integrons, and transposons in resistance monitoring programs.

8.6. Environmental Dissemination Through Mobile Elements

The dissemination of ARGs and MRGs within river ecosystems results from the combined activities of multiple mobile genetic elements operating simultaneously. Wastewater discharge, industrial pollution, agricultural runoff, and urbanization introduce resistant microorganisms and resistance determinants into aquatic environments, where MGEs facilitate their transfer among indigenous microbial populations.

The Ganga River provides compelling evidence for the environmental importance of MGE-mediated resistance dissemination.

Metagenomic investigations have consistently demonstrated strong associations among ARGs, MRGs, MGEs, and microbial community composition across different river compartments [3], [4]. Sediments, in particular, function as hotspots for genetic exchange because they contain high microbial densities, elevated concentrations of contaminants, abundant extracellular DNA, and extensive biofilm communities [5], [8]. Anthropogenic pressures further enhance dissemination processes by increasing selective pressure for resistant microorganisms. Urbanized sections of the Ganga River generally exhibit greater abundance of resistance determinants than upstream regions, reflecting the influence of wastewater inputs and human activities on environmental resistomes[6]. The persistence of heavy metals in sediments also contributes to maintenance of MGE-associated resistance genes through co-selection mechanisms. Collectively, plasmids, integrons, transposons, insertion sequences, and bacteriophages form an interconnected network of genetic exchange pathways that drive resistance evolution in river ecosystems. Their ability to mobilize ARGs and MRGs across diverse microbial populations highlights the dynamic nature of environmental resistomes and emphasizes the importance of integrating mobile genetic elements into freshwater antimicrobial resistance surveillance programs. Understanding the role of MGEs in the Ganga River is particularly important because this ecosystem serves as a major freshwater resource supporting millions of people. Continued monitoring of plasmid-mediated resistance, integron abundance, phage communities, and other mobile elements will be essential for assessing environmental AMR risks and developing effective strategies to limit the spread of resistance determinants in freshwater environments.

9. ENVIRONMENTAL SIGNIFICANCE OF ARB, ARGs, AND METAL RESISTANCE

The increasing occurrence of antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), and metal resistance genes (MRGs) in freshwater ecosystems has emerged as a major environmental concern. Traditionally, antimicrobial resistance was regarded primarily as a clinical problem; however, recent advances in environmental microbiology and metagenomics have revealed that natural ecosystems play a crucial role in the persistence, evolution, and dissemination of resistance determinants. River ecosystems are particularly important because they receive continuous inputs of antibiotics, resistant microorganisms, heavy metals, wastewater, and other contaminants from anthropogenic sources. These pollutants collectively influence microbial community structure, ecological processes, and resistance dynamics, thereby transforming rivers into environmental reservoirs of antimicrobial resistance [3], [4], [22]. The Ganga River exemplifies this phenomenon because of its extensive anthropogenic pressures, diverse microbial communities, and documented occurrence of ARGs, MRGs, and mobile genetic elements (MGEs). Metagenomic investigations have demonstrated widespread distributions of resistance determinants throughout both water and sediment compartments of the river, highlighting their ecological significance and long-term environmental implications [4], [8]. Understanding the environmental consequences of resistance dissemination is therefore essential for evaluating ecosystem health and developing sustainable river management strategies.Recent metagenomic investigations conducted in the Brahmaputra, Saryu, Teesta, Odisha river systems, and Bangladeshi rivers have revealed extensive distributions of ARGs, MRGs, virulence factors, and mobile genetic elements (MGEs), highlighting the ecological significance of resistance dissemination in freshwater ecosystems [17], [18], [21], [54]. Beyond their public health implications, these resistance determinants influence microbial diversity, ecosystem functioning, nutrient cycling, and ecological stability.

9.1. Alteration of Microbial Diversity

Microbial diversity is a fundamental determinant of ecosystem stability and functioning. The introduction of antibiotics, heavy metals, and resistant microorganisms into river ecosystems exerts selective pressure that can significantly alter microbial community composition. Sensitive microbial taxa may decline or disappear under contaminant stress, whereas resistant populations become increasingly dominant. Studies conducted in the Ganga River have revealed distinct shifts in microbial community structure associated with pollution gradients and anthropogenic influences. Metagenomic analyses demonstrated dominance of bacterial groups such as Proteobacteria, Firmicutes, Acidobacteria, Chloroflexi, and Bacteroidota in contaminated river environments, reflecting adaptation to polluted conditions [8], [16]. Urbanized stretches of the river often exhibit increased abundance of Gram-negative bacteria and chemo-organo-heterotrophic microorganisms capable of tolerating environmental stressors [6].Similar observations were reported from the Brahmaputra and Saryu rivers, where environmental stressors altered microbial community structures and favored bacteria possessing ARGs, MRGs, and stress-response genes [18], [31].The widespread occurrence of ARGs and MRGs further contributes to microbial community restructuring by providing selective advantages to resistant organisms. Consequently, microbial assemblages become increasingly dominated by populations possessing resistance determinants, reducing ecological heterogeneity and potentially affecting ecosystem resilience [3]. Heavy metal contamination further contributes to biodiversity loss by selecting for metal-tolerant microorganisms capable of surviving under toxic conditions. Studies of heavy metal-tolerant bacterial populations from the Ganga River identified specialized microbial assemblages adapted to elevated concentrations of arsenic, cadmium, chromium, copper, nickel, and iron [14].

Such community shifts may reduce functional redundancy and increase ecosystem vulnerability to future environmental disturbances.

9.2. Changes in Ecosystem Functioning

Microorganisms perform essential ecological functions including organic matter decomposition, nutrient transformation, pollutant degradation, and biogeochemical cycling. Alterations in microbial diversity resulting from resistance selection can therefore have significant consequences for ecosystem functioning. In the Ganga River, microbial communities contribute extensively to degradation of xenobiotic compounds, carbon turnover, and nutrient cycling processes [16]. However, contamination by antibiotics and heavy metals can modify microbial metabolic activities and alter functional gene distributions. Metagenomic investigations have identified substantial changes in functional pathways associated with environmental adaptation, stress response, resistance mechanisms, and pollutant degradation in contaminated sections of the river [4], [8]. The enrichment of resistant microorganisms may modify metabolic capabilities and alter interactions among microbial taxa. Metagenomic analyses of river sediments have demonstrated that pollutioninduced microbial shifts affect genes involved in carbon metabolism, energy production, stress response, and nutrient transformation pathways [10], [18].

9.3. Impact on Nutrient Cycling

Nutrient cycling represents one of the most important ecological functions performed by riverine microorganisms. Microbial communities regulate transformations of carbon, nitrogen, phosphorus, sulfur, and other essential elements, thereby maintaining ecosystem productivity and biogeochemical equilibrium. The presence of ARB, ARGs, and MRGs can influence nutrient cycling processes through changes in microbial composition and metabolic activity. Studies of the Ganga River have identified diverse microbial populations involved in nitrification, denitrification, ammonification, phosphorus turnover, and degradation of organic compounds [8], [16]. However, environmental stressors such as heavy metals and antibiotics may selectively inhibit sensitive microorganisms while favoring resistant taxa possessing different metabolic capabilities. Experimental studies involving metal-tolerant bacteria from the Ganga River suggest that prolonged metal exposure alters microbial metabolic pathways and influences nutrient processing capabilities [14].Heavy metal contamination is particularly important because toxic metals can interfere with enzymatic activities involved in nutrient transformations. Elevated concentrations of arsenic, copper, cadmium, and chromium may disrupt microbial metabolism and reduce efficiency of nutrient cycling pathways [2]. Metagenomic investigation of the Brahmaputra River revealed substantial shifts in functional genes involved in nitrogen metabolism, amino acid biosynthesis, and carbon degradation pathways under contaminated conditions [18]. At the same time, resistant microorganisms capable of tolerating these contaminants may become dominant, altering nutrient turnover rates and ecosystem functioning. Therefore, resistance evolution and contaminant exposure have the potential to affect biogeochemical processes at ecosystem scales.

9.4. Persistence of Resistance Reservoirs

One of the most significant environmental consequences of ARB, ARGs, and MRGs is the formation of long-term resistance reservoirs. River ecosystems receive continuous inputs of resistance determinants from wastewater treatment plants, municipal sewage, hospital discharges, industrial effluents, and agricultural runoff. Once introduced into aquatic environments, resistance genes may persist for extended periods through association with microbial communities, biofilms, sediments, and mobile genetic elements. Unlike many organic pollutants, heavy metals are non-degradable and remain in aquatic environments indefinitely. Their persistence maintains continuous selective pressure on microbial populations, promoting the retention of ARGs and MRGs even in the absence of antibiotics [4], [33]. This phenomenon contributes to the long-term stability of environmental resistomes.The Ganga River has been repeatedly identified as an important environmental reservoir of ARGs and MRGs [4], [5]. Comparative analyses revealed that sediment environments contain significantly higher abundances of resistance determinants than water columns, indicating their role as long-term repositories for environmental resistomes[5], [8]. Sediments accumulate contaminants, extracellular DNA, microbial biomass, and mobile genetic elements, creating conditions favorable for resistance persistence and horizontal gene transfer.Metagenomic investigations further demonstrated co-occurrence of ARGs, MRGs, virulence factors, and mobile genetic elements throughout the river system [3], [50]. Studies from the Brahmaputra, and Saryu rivers have demonstrated that sediment-associated microbial communities harbor substantially greater ARG diversity and abundance than the overlying water column [18], [21]. Such interactions enhance the stability and dissemination of resistance determinants, increasing the likelihood that clinically relevant resistance genes will persist within environmental microbial communities.

9.5. Ecological Risk Assessment

The widespread occurrence of ARB, ARGs, and MRGs in freshwater ecosystems has important implications for ecological risk assessment. Traditional environmental monitoring programs have primarily focused on chemical pollutants and conventional water quality parameters; however, increasing evidence suggests that biological contaminants such as resistance determinants should also be considered important indicators of ecosystem health.

The Ganga River provides a valuable case study for evaluating ecological risks associated with environmental resistance. Multiple investigations have documented extensive distributions of ARGs, MRGs, MGEs, and resistant microbial populations throughout the river basin [3], [4], [8]. These findings indicate that resistance determinants have become integral components of the riverine microbiome and represent potential risks to both environmental and public health. Ecological risk assessment frameworks should therefore incorporate microbial indicators alongside traditional physicochemical parameters. Factors such as ARG abundance, MRG occurrence, mobile genetic element distribution, microbial diversity, and resistance hotspots can provide valuable information regarding ecosystem integrity and pollution impacts. Sediment compartments deserve particular attention because they function as major reservoirs of contaminants and resistance determinants [5].Furthermore, the coexistence of heavy metal contamination and antimicrobial resistance complicates ecological risk evaluations. Metagenomic studies of the Ganga River have demonstrated strong associations among ARGs, MRGs, and environmental pollutants, suggesting that chemical and biological stressors interact to influence ecosystem health [4]. Consequently, integrated monitoring approaches that simultaneously assess contaminant levels, microbial community composition, and resistance determinants are required for comprehensive environmental risk assessment.Similarly, studies of Bangladeshi rivers identified resistance hotspots characterized by high concentrations of ARGs and MRGs associated with urbanization, wastewater discharge, and industrial activities [33]. The detection of clinically important resistance determinants such as β-lactamase genes, carbapenem resistance genes, and multidrug resistance genes in environmental settings underscores the need for integrated risk assessment frameworks.From an ecological perspective, resistance dissemination may lead to reduced biodiversity, altered ecosystem functioning, disrupted nutrient cycling, and increased dominance of opportunistic pathogens. From a public health perspective, environmental reservoirs of resistance genes increase the likelihood of resistance transfer into human-associated microbial populations through recreational activities, irrigation, aquaculture, and drinking water pathways.

10. CLINICAL AND PUBLIC HEALTH IMPLICATIONS

The increasing occurrence of antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), and metal resistance genes (MRGs) in freshwater ecosystems has significant implications for human health and disease management. Rivers serve as critical interfaces connecting environmental, animal, and human microbiomes, thereby facilitating the transmission of resistant microorganisms and resistance determinants across ecological boundaries. The Ganga River is particularly important in this context because it supports nearly half a billion people through its use for drinking water, domestic activities, agriculture, fisheries, recreation, and religious practices. Simultaneously, the river receives substantial inputs of municipal sewage, hospital wastewater, industrial effluents, agricultural runoff, and urban discharges that introduce resistant microorganisms and environmental contaminants into the aquatic ecosystem [1], [2]. Recent metagenomic investigations have revealed widespread distributions of ARGs, MRGs, virulence factors, multidrug resistance pathways, and mobile genetic elements (MGEs) throughout various stretches of the Ganga River [2], [5], [8]. The persistence of these resistance determinants in both water and sediment environments raises serious concerns regarding human exposure, pathogen evolution, and the emergence of difficult-to-treat infections. Consequently, environmental antimicrobial resistance is increasingly recognized as a major public health challenge requiring integrated management strategies. Recent metagenomic investigations conducted in the Brahmaputra, Saryu, Yamuna, Teesta, and Bangladeshi river systems have revealed extensive distributions of ARGs, multidrug-resistant bacteria, virulence factors, and mobile genetic elements (MGEs), indicating that freshwater ecosystems have become important environmental reservoirs of resistance [3], [10], [21][17], [21]. The public health implications of these findings extend beyond environmental contamination and include the emergence of multidrugresistant pathogens, increased disease burden, and challenges to antimicrobial therapy.

10.1. Waterborne Transmission Pathways

Water serves as one of the most important pathways for the dissemination of resistant microorganisms and resistance genes. Rivers contaminated with untreated wastewater, industrial effluents, and agricultural runoff can transport ARB and ARGs over large geographical distances, facilitating their spread among environmental and human-associated microbial communities. ARGs and ARB can be transmitted through direct exposure to contaminated water, consumption of inadequately treated drinking water, agricultural irrigation, aquaculture systems, recreational activities, and wastewater reuse practices. Resistant bacteria released into river systems may persist in water columns, sediments, and biofilms for prolonged periods, facilitating their spread within aquatic ecosystems [22].The Ganga River receives enormous volumes of municipal sewage and wastewater throughout its course, particularly in densely populated urban regions [1], [6]. These inputs introduce resistant bacteria and resistance genes originating from hospitals, households, livestock facilities, and industrial sources.Metagenomic analyses of the Ganga River have identified numerous clinically relevant resistance determinants, including β-lactam, tetracycline, sulfonamide, macrolide, aminoglycoside, and multidrug resistance genes distributed throughout riverine microbial communities [3], [4].

The widespread occurrence of these determinants highlights the potential for environmental transmission through waterborne pathways.Such findings indicate that river water can function as an environmental reservoir and transmission medium for antimicrobial resistance. The movement of contaminated water downstream further facilitates dissemination of resistance determinants across broad spatial scales, increasing potential exposure risks for human populations.

10.2. Human Exposure Routes

Human exposure to environmental antimicrobial resistance can occur through multiple pathways. Direct contact with contaminated water during bathing, swimming, washing, and religious activities represents a major route of exposure. In the Ganga basin, millions of people rely on river water for domestic and cultural purposes, increasing opportunities for interaction with resistant microorganisms.Consumption of contaminated drinking water is another important exposure pathway. Although water treatment systems reduce microbial contamination, inadequate treatment infrastructure and accidental contamination may allow resistant bacteria and ARGs to enter drinking water supplies. Irrigation of crops using contaminated river water also represents a potential route through which resistance determinants may enter food chains and ultimately reach human populations [2].The Ganga River represents a notable example because millions of individuals depend upon its waters for domestic, agricultural, and religious purposes. Large-scale religious gatherings such as the Kumbh Mela substantially increase human-river interactions and may contribute to the dissemination of resistant microorganisms. Metagenomic studies conducted during mass gathering events have reported elevated microbial loads, increased ARG abundance, and higher prevalence of multidrug-resistant bacteria compared with non-event periods [31]. Fish and other aquatic organisms inhabiting contaminated river systems may accumulate resistant bacteria within their microbiota. Human consumption of such organisms can contribute to transmission of resistance determinants. Furthermore, occupational exposure among fishermen, agricultural workers, wastewater treatment personnel, and individuals involved in river management may increase contact with resistant microorganisms. These diverse exposure pathways highlight the interconnected nature of environmental and public health risks associated with antimicrobial resistance.

10.3. Emergence of Multidrug-Resistant Pathogens

One of the most concerning consequences of environmental antimicrobial resistance is the emergence and dissemination of multidrug-resistant (MDR) pathogens. River ecosystems provide favorable conditions for horizontal gene transfer among environmental and pathogenic bacteria because they contain diverse microbial communities, abundant mobile genetic elements, and multiple selective pressures. Studies conducted in the Ganga River have identified bacterial genera such as *Pseudomonas*, *Acinetobacter*, and other opportunistic pathogens associated with multidrug resistance pathways [5], [12]. Metagenomic investigations further revealed extensive distributions of ARGs related to β-lactam resistance, tetracycline resistance, aminoglycoside resistance, vancomycin resistance, and multidrug resistance mechanisms [3], [4]. Other studies conducted in river systems have identified MDR strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and other members of the ESKAPE pathogen group [12], [53], [54]. These organisms frequently possess resistance determinants against multiple antibiotic classes, including β-lactams, carbapenems, tetracyclines, aminoglycosides, fluoroquinolones, and sulfonamides. Particularly alarming is the detection of carbapenem-resistant *Klebsiella pneumoniae* carrying clinically important genes such as *blaOXA-48*, *blaTEM*, and *blaSHV* within river ecosystems [38]. Carbapenems are often considered last-resort antibiotics for treating severe infections, and resistance to these drugs significantly limits therapeutic options.Metagenomic analyses of the Brahmaputra and Saryu rivers have further identified numerous virulence factors co-occurring with ARGs and mobile genetic elements, suggesting that environmental bacteria may possess both pathogenic potential and multidrug resistance capabilities [18], [21].The coexistence of ARGs, MRGs, virulence factors, and MGEs within environmental microbial communities enhances the likelihood of resistance transfer to pathogenic bacteria. Sediment environments are particularly important because they function as hotspots for microbial interactions and horizontal gene transfer [8]. Consequently, environmental reservoirs may contribute to the emergence of clinically significant MDR pathogens capable of causing infections that are increasingly difficult to treat using conventional antibiotics.

10.4. One Health Perspective

The environmental dissemination of antimicrobial resistance highlights the importance of adopting a One Health approach that recognizes the interconnectedness of human, animal, and environmental health. Resistance determinants circulate continuously among these sectors through wastewater discharge, agricultural activities, livestock production, wildlife interactions, and environmental contamination. The Ganga River exemplifies the complexity of One Health interactions because it receives pollutants and microorganisms from multiple anthropogenic sources while simultaneously supporting human populations, livestock, fisheries, wildlife, and agricultural systems [1]. Resistant microorganisms originating from one sector can be transmitted to others through environmental pathways, creating a continuous cycle of resistance dissemination.Metagenomic studies demonstrating widespread occurrence of ARGs, MRGs, and MGEs in the river environment further support the need for integrated surveillance systems capable of monitoring resistance across environmental, clinical, and agricultural sectors [3], [4]. Effective management of antimicrobial resistance therefore requires coordinated actions that address environmental pollution, wastewater treatment, antibiotic stewardship, and ecosystem health simultaneously.

10.5. Global AMR Burden Linked to Environmental Reservoirs

Environmental reservoirs of antimicrobial resistance are increasingly recognized as important contributors to the global AMR burden. Freshwater ecosystems act not only as repositories of resistance determinants but also as dynamic environments where resistance evolves, persists, and disseminates. Rivers contaminated with antibiotics, heavy metals, resistant bacteria, and mobile genetic elements provide ideal conditions for the maintenance and spread of environmental resistomes. The Ganga River has emerged as one of the most extensively studied examples of environmental antimicrobial resistance in a large river system. Investigations consistently demonstrate widespread occurrence of ARGs, MRGs, virulence factors, and resistant microbial populations throughout the basin [3], [4], [5], [8]. The persistence of these resistance determinants in water, sediments, and biofilms suggests that environmental reservoirs may contribute substantially to long-term AMR dissemination.Metagenomic studies conducted across diverse river systems consistently report extensive environmental resistomes containing ARGs, MRGs, virulence factors, and mobile genetic elements [3], [18], [33]. These findings suggest that environmental reservoirs function as long-term sources of resistance determinants capable of entering human and animal populations. The global public health implications are substantial. Environmental reservoirs increase opportunities for the emergence of novel resistance combinations, facilitate geographic dissemination of resistance determinants, and contribute to the evolution of multidrug-resistant pathogens. In regions with limited wastewater treatment infrastructure and high population densities, these risks may be particularly pronounced.

11. MODERN APPROACHES FOR DETECTION AND MONITORING

The growing recognition of freshwater ecosystems as reservoirs of antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), metal resistance genes (MRGs), and mobile genetic elements (MGEs) has intensified the need for advanced monitoring and surveillance strategies. Traditional microbiological techniques have contributed significantly to our understanding of environmental antimicrobial resistance; however, recent developments in molecular biology, nextgeneration sequencing, bioinformatics, and geospatial technologies have transformed the detection and characterization of environmental resistomes. These approaches provide comprehensive insights into microbial diversity, resistance gene distribution, ecological interactions, and environmental drivers of resistance dissemination. In the Ganga River, the application of metagenomics, highthroughput sequencing, quantitative molecular techniques, and environmental monitoring frameworks has substantially improved understanding of microbial ecology and resistance dynamics across diverse riverine habitats [3], [4], [5], [8].

11.1. Culture-Dependent Methods

Culture-dependent methods remain fundamental tools for the isolation, identification, and characterization of environmental microorganisms. These approaches involve cultivation of bacteria on selective or differential media followed by phenotypic, biochemical, and antimicrobial susceptibility analyses. Despite limitations associated with the inability to culture many environmental microorganisms, culture-based techniques remain valuable for studying viable bacterial populations and assessing phenotypic resistance characteristics. Several investigations conducted in the Ganga River have utilized culture-dependent approaches to isolate antibioticresistant and heavy metal-tolerant bacterial strains. Verma et al (2024)[20], successfully isolated and characterized arsenic-resistant *Kurthiagibsonii* from Ganga water samples, demonstrating the utility of culture-based methods for investigating microbial adaptation to environmental contaminants. Similarly, Srivastava and Verma (2025)[12], employed culturable bacterial isolates to evaluate antibiotic resistance profiles and biofilm-forming capabilities of riverine microorganisms. Such studies provide important information regarding microbial physiology, resistance phenotypes, and ecological adaptation under environmental stress conditions.

11.2. Quantitative PCR (qPCR)

Quantitative polymerase chain reaction (qPCR) has become one of the most widely used molecular tools for environmental monitoring of antimicrobial resistance. This technique enables rapid, sensitive, and quantitative detection of specific ARGs, MRGs, virulence genes, and microbial biomarkers directly from environmental samples. In freshwater ecosystems, qPCR facilitates monitoring of resistance gene abundance across spatial and temporal gradients. The technique is particularly useful for tracking clinically relevant resistance determinants and evaluating the effectiveness of wastewater treatment and pollution control strategies. Although most Ganga River studies have focused on metagenomic approaches, targeted molecular quantification remains an important complementary tool for validating resistance patterns identified through sequencing analyses [3], [4]. The high sensitivity and reproducibility of qPCR make it valuable for routine environmental surveillance programs aimed at detecting emerging resistance threats.

11.3. Metagenomics

Metagenomics has revolutionized environmental microbiology by enabling direct analysis of genetic material recovered from environmental samples without the need for microbial cultivation. This approach provides comprehensive insights into microbial diversity, community structure, functional potential, and resistance gene distribution. The application of metagenomics has significantly advanced understanding of the Ganga River microbiome. Srivastava and Verma (2023a)[5], employed metagenomic analyses to compare bacteriomes and resistomes of water and sediment samples, revealing extensive distributions of ARGs and distinct microbial community structures. Similarly, Ghosh et al (2023)[4], utilized metagenomics to investigate functional microbial diversity and identify ARGs, MRGs, and MGEs within the lower stretch of the river. These studies demonstrated that metagenomic approaches provide unprecedented resolution for characterizing environmental resistomes and identifying microbial taxa associated with resistance dissemination.Metagenomics is particularly advantageous because it captures both culturable and unculturable microorganisms, thereby providing a more complete representation of environmental microbial communities. Furthermore, the technique enables simultaneous assessment of taxonomic composition, functional genes, metabolic pathways, and ecological interactions.

11.4. Shotgun Sequencing

Shotgun metagenomic sequencing represents one of the most powerful approaches for environmental resistance surveillance. Unlike targeted sequencing methods, shotgun sequencing randomly sequences all genetic material present in environmental samples, allowing comprehensive characterization of microbial communities and their functional capabilities. Several investigations of the Ganga River have employed shotgun sequencing to examine microbial diversity and resistance profiles. Rout et al (2024)[8], utilized shotgun metagenomic sequencing to characterize bacterial diversity, functional genes, ARGs, and virulence factors within river sediments. Similarly, Ghosh et al (2023) and Samson et al (2023) [3], [4], employed high-throughput sequencing approaches to investigate resistome composition and spatiotemporal variations in microbial communities. These studies demonstrated the effectiveness of shotgun sequencing in identifying novel resistance determinants, resistance-associated pathways, and ecological drivers of microbial adaptation.The ability of shotgun sequencing to simultaneously detect ARGs, MRGs, virulence factors, bacteriophages, and MGEs makes it an indispensable tool for environmental AMR research.

11.5. Resistome Analysis

Resistome analysis refers to the comprehensive characterization of all resistance genes present within a microbial community.

Environmental resistome studies provide critical information regarding the diversity, abundance, distribution, and potential mobility of resistance determinants. The Ganga River has become an important model system for environmental resistome investigations. Comparative resistome analyses revealed significant differences between water and sediment compartments, with sediments generally harboring greater ARG abundance and diversity [5].

Metagenomic studies further identified resistance pathways associated with β-lactams, tetracyclines, aminoglycosides, macrolides, vancomycin, and multidrug resistance mechanisms [3], [4].Resistome analyses also provide valuable insights into co-selection processes because they enable simultaneous assessment of ARGs, MRGs, and MGEs. Such information is essential for understanding resistance evolution, environmental persistence, and ecological risks associated with antimicrobial resistance.

11.6. Bioinformatics Tools

Advances in sequencing technologies have generated enormous quantities of environmental genomic data, necessitating the development of sophisticated bioinformatics tools for data processing and interpretation. Bioinformatics pipelines enable taxonomic classification, functional annotation, resistance gene identification, pathway reconstruction, and ecological network analyses. Studies conducted on the Ganga River have relied extensively on bioinformatics approaches to characterize microbial communities and environmental resistomes. Metagenomic datasets generated from water and sediment samples have been analyzed using computational tools to identify bacterial taxa, ARGs, MRGs, virulence factors, and metabolic pathways [4], [5], [8]. Correlation and network analyses have further revealed associations among microbial taxa, resistance determinants, environmental variables, and contamination gradients.Bioinformatics tools therefore serve as essential components of modern environmental surveillance programs by transforming raw sequencing data into biologically meaningful information. Their continued development will further enhance our capacity to predict resistance dissemination and identify emerging environmental threats.

11.7. GIS-Based Environmental Surveillance

Geographic Information Systems (GIS) have become increasingly important for environmental monitoring and spatial analysis of pollution, microbial communities, and resistance dissemination. GIS integrates environmental, geographical, hydrological, and microbiological datasets to identify contamination hotspots, evaluate spatial trends, and support decision-making processes. The Ganga River basin encompasses diverse environmental conditions and anthropogenic pressures distributed across thousands of kilometers. Consequently, GIS-based approaches offer valuable opportunities for mapping pollution sources, resistance hotspots, wastewater discharge points, industrial clusters, and microbial diversity patterns. Hydrogeochemical investigations of the Ganga have already demonstrated the utility of spatially explicit environmental assessments for understanding contaminant dynamics across the river basin [1].When combined with metagenomics, remote sensing, and environmental monitoring data, GIS-based surveillance systems can facilitate identification of regions vulnerable to antimicrobial resistance dissemination. Such integrated approaches are particularly useful for developing targeted management strategies and prioritizing remediation efforts in heavily impacted stretches of the river. Modern detection and monitoring approaches have transformed the study of environmental antimicrobial resistance. Culturedependent methods, qPCR, metagenomics, shotgun sequencing, resistome analysis, bioinformatics, and GIS-based surveillance collectively provide powerful tools for investigating microbial ecology and resistance dissemination in freshwater ecosystems. In the Ganga River, these approaches have revealed extensive distributions of ARGs, MRGs, MGEs, and resistant microbial populations, highlighting the importance of advanced monitoring frameworks for environmental AMR surveillance. Continued integration of molecular, computational, and geospatial technologies will be essential for improving resistance risk assessment, environmental management, and long-term monitoring of freshwater ecosystems.

12. THE GANGA RIVER AS A CASE STUDY

The Ganga River represents one of the most important freshwater ecosystems in the world and serves as a unique model for investigating the interactions among microbial diversity, antimicrobial resistance, heavy metal pollution, and environmental health. Originating from the Gangotri Glacier in the Himalayas and flowing approximately 2,525 km before discharging into the Bay of Bengal, the river traverses diverse ecological zones and supports nearly 40% of India's population [30]. The river basin sustains agriculture, industry, fisheries, transportation, drinking water supply, and numerous cultural and religious activities. However, rapid urbanization, industrial expansion, population growth, and inadequate wastewater treatment have resulted in substantial environmental degradation, making the Ganga one of the most intensively studied river systems for environmental antimicrobial resistance and pollution research [2], [6]. Recent advances in metagenomics and environmental microbiology have revealed that the Ganga River harbors diverse microbial communities together with abundant antibiotic resistance genes (ARGs), metal resistance genes (MRGs), virulence factors, and mobile genetic elements (MGEs). Consequently, the river provides an excellent case study for understanding how anthropogenic activities influence microbial ecology and resistance evolution in freshwater ecosystems. Table 5, represents recent reports on antimicrobial resistance and metal resistance in the Ganga River and other major rivers worldwide.

12.1. Overview of the Ganga River Ecosystem

The Ganga River basin encompasses a wide range of environmental conditions, extending from pristine Himalayan headwaters to highly urbanized and industrialized downstream regions. These environmental gradients create diverse ecological niches supporting complex microbial communities. Studies conducted across different stretches of the river have identified bacterial assemblages dominated by Proteobacteria, Firmicutes, Bacteroidota, Acidobacteria, Chloroflexi, Actinobacteria, and Verrucomicrobiota, reflecting substantial microbial diversity and ecological complexity [8], [16].Microbial communities in the Ganga play essential roles in nutrient cycling, organic matter decomposition, pollutant degradation, and ecosystem functioning. Metagenomic analyses have revealed the presence of diverse functional pathways associated with carbon metabolism, nitrogen cycling, xenobiotic degradation, environmental adaptation, and stress responses [8]. However, these ecological processes are increasingly influenced by anthropogenic pollution and resistance selection pressures.

12.2. Sources of Microbial and Metal Contamination

The Ganga River receives contaminants from multiple anthropogenic sources throughout its course. Municipal sewage represents one of the largest contributors to microbial pollution, introducing large quantities of fecal microorganisms, antibiotic-resistant bacteria, pathogens, and resistance genes into the aquatic environment [1], [6]. Urban centers located along the river generate substantial wastewater loads, much of which is discharged untreated or partially treated.Industrial activities constitute another major source of contamination. Tanneries, textile industries, paper mills, pharmaceutical industries, chemical manufacturing facilities, and metal-processing units release effluents containing heavy metals, organic pollutants, and other contaminants into the river system [1], [30]. Agricultural runoff further contributes nutrients, pesticides, veterinary antibiotics, and metal residues originating from fertilizers and livestock production systems.Religious activities and mass gatherings also contribute to microbial contamination. Ritual bathing, disposal of offerings, cremation practices, and temporary population increases during major religious events can elevate microbial loads and influence water quality. Collectively, these contamination sources create a complex environmental matrix that promotes resistance dissemination and ecological disturbance.

12.3. Antibiotic-Resistant Bacteria Reported from the Ganga

Numerous studies have documented the occurrence of antibiotic-resistant bacteria (ARB) throughout the Ganga River. Comparative analyses of water and sediment microbiomes revealed extensive distributions of bacterial taxa associated with resistance pathways and environmental adaptation [5]. Dominant genera include *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Hydrogenophaga*, *Nitrospira*, and several other taxa known to harbor resistance determinants. Metagenomic investigations identified resistance pathways associated with β-lactam antibiotics, tetracyclines, aminoglycosides, macrolides, vancomycin, CAMP resistance pathways, and multidrug resistance mechanisms [4], [5]. Urbanized downstream regions generally exhibit higher abundances of ARGs and resistant bacterial populations than upstream sections, reflecting the influence of anthropogenic activities and wastewater inputs [6]. Recent culture-based studies further demonstrated the occurrence of biofilmforming bacteria exhibiting resistance to multiple antibiotics [12]. These findings indicate that the Ganga River functions as an important environmental reservoir of ARB and resistance determinants.

12.4. Heavy Metal Pollution Status

Heavy metal contamination represents another major environmental concern within the Ganga River basin. Industrial discharges, urban runoff, agricultural inputs, and geological processes contribute metals such as arsenic, cadmium, chromium, copper, zinc, nickel, and lead to river environments [1], [4]. Metagenomic analyses conducted in the lower stretch of the river identified extensive distributions of metal resistance genes associated with arsenic, copper, zinc, cadmium, and other toxic metals [4]. Investigations involving arsenic-resistant bacterial isolates further confirmed the presence of microbial populations adapted to elevated metal concentrations [20]. Seasonal assessments of heavy metal-tolerant bacteria also demonstrated widespread microbial adaptation to metal-contaminated environments throughout the river system [14].The persistence of heavy metals within sediments is particularly important because sediments act as long-term contaminant reservoirs and continuously influence microbial community composition and resistance evolution.

12.5. Evidence of ARGs and Metal Resistance Genes

One of the most significant findings emerging from recent Ganga River research is the extensive occurrence of ARGs and MRGs across both water and sediment environments. Srivastava and Verma (2023a) [5], demonstrated that sediment samples contained significantly higher ARG abundance than water samples, emphasizing the role of sediments as environmental resistance reservoirs. Similarly, Rout et al (2024) [8], identified diverse ARGs, virulence factors, and functional resistance determinants within river sediments. Ghosh et al. (2023) [4], reported widespread distributions of ARGs, MRGs, and mobile genetic elements throughout the lower stretch of the river, while Samson et al (2023) [3], documented spatiotemporal variations in microbiome composition and resistome structure. Commonly detected ARG categories included β-lactam, tetracycline, aminoglycoside, vancomycin, macrolide, and multidrug resistance genes. Metal resistance determinants associated with arsenic, copper, zinc, and cadmium were also abundant.The coexistence of ARGs, MRGs, and MGEs provides strong evidence for co-selection processes and highlights the potential for horizontal gene transfer within riverine microbial communities.

12.6. Implications for Public Health

The occurrence of ARB, ARGs, and MRGs in the Ganga River has important implications for public health because millions of people depend directly on river resources. Human exposure may occur through drinking water, bathing, recreational activities, irrigation, fisheries, and consumption of agricultural products cultivated using river water. The persistence of multidrug resistance genes and opportunistic pathogens within aquatic environments raises concerns regarding transmission of resistance determinants to human-associated microbial communities [5], [12]. Furthermore, the coexistence of virulence factors, ARGs, and mobile genetic elements increases the potential emergence of difficult-to-treat infections. From a One Health perspective, the Ganga River functions as a critical interface linking environmental, animal, and human health. Consequently, resistance dissemination within the river ecosystem may have implications extending beyond environmental boundaries and contribute to broader public health challenges associated with antimicrobial resistance.

12.7. Knowledge Gaps and Research Needs

Despite significant advances in understanding microbial ecology, antimicrobial resistance, and heavy metal contamination in the Ganga River, several critical knowledge gaps remain that limit our ability to accurately assess environmental risks and develop effective mitigation strategies. Current evidence demonstrates widespread occurrence of antibiotic resistance genes (ARGs), metal resistance genes (MRGs), mobile genetic elements (MGEs), and resistant microbial communities throughout the river system; however, most available studies remain observational and descriptive, providing limited mechanistic insight into resistance evolution and dissemination.One of the major limitations of existing research is the strong geographical bias in sampling efforts. Most investigations have focused on selected urban or heavily polluted stretches of the river, while comparatively little information is available from pristine upstream regions, tributaries, floodplain wetlands, groundwater–river interfaces, and estuarine environments. Consequently, a comprehensive basinscale understanding of resistance dissemination across the entire Ganga continuum remains lacking. Future studies should adopt standardized sampling frameworks covering the complete river trajectory from Gomukh to the Bay of Bengal to enable meaningful spatial comparisons and identification of resistance hotspots.Another important gap concerns temporal dynamics.

Current studies are largely based on single-time-point investigations or short-term sampling campaigns. Seasonal hydrological variations associated with monsoon rainfall, flooding, sediment resuspension, pilgrimage events, and wastewater discharge patterns may substantially influence microbial communities and resistance gene abundance. Long-term monitoring programs are therefore required to determine whether observed resistance patterns represent transient fluctuations or persistent ecological trends. Although metagenomic studies have consistently reported co-occurrence of ARGs, MRGs, and MGEs, direct evidence demonstrating causal relationships between heavy metal contamination and antimicrobial resistance remains limited. Existing studies largely infer coselection through correlation analyses. Experimental validation of cross-resistance, co-resistance, and co-regulation mechanisms under environmentally relevant conditions is still lacking. Controlled microcosm and mesocosm experiments investigating the effects of arsenic, copper, chromium, cadmium, zinc, and mixed-metal contamination on resistome evolution would substantially improve mechanistic understanding of co-selection processes in river ecosystems.The role of mobile genetic elements in resistance dissemination also remains insufficiently explored. Although plasmids, integrons, transposons, and bacteriophages have been implicated in horizontal gene transfer, their specific contribution to ARG and MRG mobility within the Ganga microbiome has not been systematically quantified. Future research should employ long-read sequencing technologies, plasmidomics, viromics, and metatranscriptomics to identify actively transferred resistance determinants and characterize their transmission networks. Another significant limitation is the lack of functional validation of resistance genes detected through metagenomic approaches. Most studies identify ARGs and MRGs based on sequence similarity; however, the actual expression, functionality, and transferability of these genes remain uncertain. Integrating metagenomics with metatranscriptomics, proteomics, and culture-based functional assays would help distinguish between dormant genetic reservoirs and actively expressed resistance determinants. Sediments have repeatedly been identified as hotspots of ARG accumulation, yet sediment-associated resistance dynamics remain poorly understood. The interactions among sediment chemistry, heavy metal accumulation, biofilm development, extracellular DNA persistence, and horizontal gene transfer require detailed investigation. Given that sediments serve as long-term repositories of contaminants and resistance determinants, future monitoring programs should place greater emphasis on sediment microbiomes rather than focusing exclusively on water samples. Current knowledge regarding bacteriophage-mediated resistance transfer is also extremely limited. Considering the ecological importance of phages in microbial population dynamics and gene exchange, systematic characterization of the Ganga virome is urgently needed. Viromic studies could reveal previously overlooked pathways of ARG dissemination and provide insights into the feasibility of phage-based interventions for resistance management. From a public health perspective, a critical gap exists in linking environmental resistomes with clinically relevant antimicrobial resistance. Most studies document the environmental presence of ARGs but do not establish direct transmission pathways to human pathogens. Integrated One Health investigations combining environmental, clinical, veterinary, and wastewater datasets are required to determine the extent to which environmental reservoirs contribute to regional and national AMR burdens. Finally, future research should move beyond descriptive biodiversity assessments toward predictive environmental surveillance. The integration of high-resolution metagenomics, machine learning, artificial intelligence, GIS-based spatial modelling, remote sensing, hydrochemical monitoring, and climate data could facilitate early warning systems for AMR emergence. Such multidisciplinary approaches would enable risk prediction, identification of resistance hotspots, and development of targeted intervention strategies. Addressing these research gaps is essential for advancing our understanding of the complex interactions among microbial ecology, heavy metal contamination, and antimicrobial resistance in the Ganga River. Future investigations should prioritize mechanistic studies, long-term basin-scale monitoring, functional validation of resistance determinants, and One Health-based surveillance frameworks to support evidence-based management and mitigation of environmental antimicrobial resistance.

Table 5: Recent reports on antimicrobial resistance and metal resistance in the Ganga River and other major rivers worldwide

River System	Country	Major Findings on AMR and Metal Resistance	Key Resistance Determinants Identified	Ref
Ganga River (Lower Stretch)	India	Nanopore metagenomics revealed widespread ARGs, MRGs, and MGEs; multidrug resistance genes were dominant; arsenic, copper, mercury, zinc resistance genes were abundant	MDR genes, arsenic resistance genes, copper resistance genes, zinc resistance genes, MGEs	[4]
Ganga River (Anthropogenically Dynamic Stretch)	India	Sediment microbiome contained resistance genes against 28 antibiotic classes and 23 metal types; phage-associated resistance dissemination detected	MDR genes, tetracycline resistance, copper and zinc resistance genes, bacteriophage-associated genes	[3]
Ganga River Water and Sediment	India	Sediments harbored higher ARG diversity than water; vancomycin resistance particularly enriched in sediments	β-lactam, vancomycin, tetracycline, aminoglycoside, macrolide resistance genes	[6]

Ganga River (Upstream vs Downstream)	India	Urbanization increased Gram-negative bacteria and ARG abundance in downstream regions; β-lactam resistance dominated	β-lactam, CAMP resistance, multidrug resistance, vancomycin resistance	[5]
Ganga River Sediments	India	Metagenomics revealed abundant ARGs, virulence factors, and functional genes associated with environmental adaptation	ARGs, virulence factors, plasmid-associated genes	[8]
Ganga River (Varanasi)	India	Isolation of arsenic-resistant *Kurthiagibsonii* capable of arsenite oxidation and biofilm formation under arsenic stress	aioA-associated arsenic oxidation pathway, arsenic resistance determinants	[20]
Ganga River	India	High prevalence of antibiotic-resistant bacteria with strong biofilm-forming capacity; Gram-negative bacteria predominated	Resistance to ampicillin, vancomycin, rifampicin, polymyxin, bacitracin	[12]
Ganga and Yamuna Rivers	India	Multiple antibiotic-resistant *Escherichia coli* associated with elevated microbial contamination and poor water quality	Penicillin resistance, vancomycin resistance, MAR-positive *E. coli*	[53]
Brahmaputra River Sediments	India	Shotgun metagenomics identified 50 ARG types, plasmid-related genes, virulence factors, and extensive microbial diversity	Multidrug, β-lactam, tetracycline, sulfonamide, aminoglycoside resistance genes	[18]
Teesta River	India	Heavy metal-tolerant bacterial communities exhibited dual tolerance to antibiotics and metals across altitudinal gradients	Heavy metal tolerance traits linked with antibiotic resistance	[17]
Odisha River System	India	Carbapenem-resistant *Klebsiella pneumoniae* carrying blaOXA-48, blaTEM, and blaSHV genes correlated with heavy metal contamination	Emergence of clinically relevant MDR pathogens	[15]
Bindal and Rispana Rivers	India	Untreated wastewater identified as primary driver of ARG dissemination; limited evidence for heavy metal co-selection	sul1, sul2, ermF, tetW, intI1	[15]
Padma and Jamuna River Network (including Bongshai and Kumar Rivers)	Bangladesh	Shotgun metagenomics revealed coexistence of ARGs and MRGs; Bongshai showed greater resistome diversity	Carbapenem, colistin, β-lactam, aminoglycoside, mercury, arsenic, lead resistance genes	[33]
Arsenic-Contaminated Groundwater Systems	Bangladesh	Arsenotrophic bacteria harboredarsB, acr3, arsD, arsH, arsR genes associated with arsenic detoxification and resistance	arsB, arsD, arsH, arsR, acr3	[19]
Surface and Reclaimed Water Systems	USA	Enterococcus populations exhibited antimicrobial resistance, demonstrating freshwater environments as reservoirs of AMR determinants	Enterococcus-associated antimicrobial resistance profiles	[25]

Abbreviations: AMR = Antimicrobial Resistance; ARGs = Antibiotic Resistance Genes; MRGs = Metal Resistance Genes; MDR = Multidrug Resistance; MGEs = Mobile Genetic Elements; MAR = Multiple Antibiotic Resistance; ARB = Antibiotic-Resistant Bacteria.

13. MITIGATION STRATEGIES AND FUTURE DIRECTIONS

The increasing occurrence of antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), metal resistance genes (MRGs), and mobile genetic elements (MGEs) in the Ganga River highlights the urgent need for integrated mitigation strategies and long-term management interventions. The persistence of antimicrobial resistance (AMR) within freshwater ecosystems is driven by multiple anthropogenic factors, including untreated municipal wastewater, industrial discharges, agricultural runoff, heavy metal contamination, and rapid urbanization. Recent metagenomic investigations have demonstrated that the Ganga River functions as an important environmental reservoir of ARGs and MRGs, with sediments acting as long-term repositories for resistance determinants[4], [5], [8]. Consequently, effective mitigation requires coordinated actions encompassing pollution control, environmental surveillance, wastewater treatment improvements, policy implementation, and One Health-based monitoring systems.

13.1. Wastewater Treatment Improvements

Municipal wastewater represents one of the largest sources of resistant microorganisms, ARGs, pharmaceuticals, and nutrients entering the Ganga River. Several studies have demonstrated that downstream urban regions receiving sewage discharges exhibit significantly higher abundance of ARGs and resistant microbial populations compared with upstream stretches [6]. Therefore, improving wastewater treatment infrastructure should be considered a primary strategy for reducing environmental AMR dissemination. Conventional wastewater treatment plants are often designed to remove organic pollutants and suspended solids but may not effectively eliminate ARGs, resistant bacteria, heavy metals, or emerging contaminants. Future wastewater management strategies should incorporate advanced treatment technologies such as membrane filtration, advanced oxidation processes, ultraviolet disinfection, ozonation, activated carbon adsorption, and tertiary polishing systems. Decentralized wastewater treatment facilities may also be beneficial for rapidly growing urban areas where centralized treatment infrastructure remains inadequate. Continuous monitoring of treated effluents for ARGs, MRGs, and microbial contaminants should become an integral component of wastewater management programs.

13.2. Industrial Discharge Management

Industrial activities contribute substantially to heavy metal contamination and environmental stress within the Ganga basin. Tanneries, textile industries, chemical manufacturing units, paper mills, pharmaceutical industries, and metal-processing facilities release effluents containing chromium, copper, cadmium, lead, nickel, zinc, and other contaminants [1], [4]. These pollutants not only affect ecosystem health but may also promote co-selection of antibiotic resistance. Effective industrial discharge management requires strict enforcement of effluent quality standards and adoption of cleaner production technologies. Pre-treatment of industrial wastewater prior to discharge should be mandatory, particularly for industries generating metal-rich effluents.

Installation of real-time monitoring systems, regular environmental audits, and implementation of zero-liquid-discharge technologies can substantially reduce contaminant loading. Additionally, remediation of historically contaminated sediments should be prioritized in heavily polluted stretches of the river where accumulated metals continue to exert selective pressure on microbial communities.

13.3. Environmental Monitoring Programs

Long-term environmental monitoring is essential for understanding resistance dynamics and evaluating the effectiveness of mitigation strategies. Existing studies of the Ganga River have generated valuable information regarding microbial diversity, ARGs, MRGs, and environmental contamination; however, most investigations remain spatially restricted and temporally limited [3], [8]. Future monitoring programs should adopt standardized methodologies and include routine assessment of microbial communities, resistance determinants, heavy metals, emerging contaminants, and water quality parameters. Monitoring should encompass both water and sediment compartments because sediments frequently contain higher abundances of ARGs and MRGs than overlying waters [5]. Integration of metagenomics, quantitative PCR, bioinformatics, and GIS-based surveillance systems would provide comprehensive datasets capable of identifying resistance hotspots and contamination sources. Such programs would facilitate evidence-based decisionmaking and improve environmental risk assessment.

13.4. One Health Surveillance Systems

The interconnected nature of environmental, animal, and human health necessitates adoption of a One Health approach for AMR management. The Ganga River functions as a critical interface linking wastewater systems, agricultural activities, livestock production, wildlife, fisheries, and human populations. Consequently, resistance determinants circulating within the river ecosystem can potentially influence multiple sectors simultaneously [3], [4]. Future surveillance systems should integrate environmental monitoring with clinical, veterinary, and agricultural AMR surveillance programs. Coordinated data sharing among environmental scientists, microbiologists, healthcare professionals, veterinarians, policymakers, and public health agencies would improve understanding of resistance transmission pathways. One Health surveillance frameworks should specifically target ARGs, MRGs, MGEs, and emerging resistant pathogens capable of moving across environmental and clinical settings. Such integrated approaches are essential for identifying early warning signals and preventing large-scale dissemination of resistance determinants.

13.5. Policy Interventions

Effective management of environmental antimicrobial resistance requires strong policy support and regulatory enforcement. Although several initiatives have been implemented to improve water quality in the Ganga River, environmental AMR remains insufficiently incorporated into existing river management frameworks. Policy interventions should include stricter regulation of wastewater discharge, mandatory monitoring of resistance determinants in treated effluents, improved antibiotic stewardship programs, and stronger controls on industrial pollution. Environmental risk assessment guidelines should be expanded to incorporate ARGs, MRGs, and resistant microorganisms as indicators of ecosystem health. Furthermore, regulations governing agricultural use of antibiotics, disposal of pharmaceutical waste, and industrial effluent management should be strengthened. Public awareness campaigns are equally important because community participation plays a significant role in reducing pollution and improving environmental stewardship. Educational programs emphasizing responsible antibiotic use, proper waste disposal, and river conservation can support broader mitigation efforts.

13.6. Role of Bacteriophages in Resistance Management

Bacteriophages are increasingly recognized as promising biological tools for combating antimicrobial resistance. As natural predators of bacteria, phages can selectively target resistant bacterial populations while minimizing impacts on beneficial microorganisms. Recent investigations of the Ganga River have documented diverse bacteriophage communities associated with environmental microbial populations Samson et al (2023)[3], highlighting their ecological significance within freshwater ecosystems. Phage-based approaches offer several potential advantages for resistance management. Targeted phage applications could reduce populations of antibiotic-resistant bacteria in wastewater treatment systems, contaminated water bodies, and environmental reservoirs. Phages may also contribute to biological control strategies aimed at limiting dissemination of multidrugresistant pathogens. Additionally, studies exploring interactions among bacteriophages, ARGs, and microbial communities could provide valuable insights into resistance dynamics and environmental gene transfer processes[30].

13.7. Future Research Priorities

Despite significant advances in understanding environmental antimicrobial resistance in the Ganga River, numerous research gaps remain. Future investigations should prioritize basin-wide monitoring programs capable of capturing spatial and temporal variations in microbial communities, ARGs, MRGs, and environmental contaminants. Longitudinal studies are particularly important for understanding seasonal fluctuations and long-term resistance trends. Further research is needed to elucidate the mechanisms underlying co-selection of antibiotic resistance by heavy metals and the role of mobile genetic elements in resistance dissemination. High-resolution metagenomic and transcriptomic studies could provide deeper insights into microbial adaptation and resistance evolution under complex environmental conditions. Additional attention should be directed toward sediment-associated resistomes, biofilm communities, bacteriophage-mediated gene transfer, and interactions between emerging contaminants and resistance determinants. Integration of artificial intelligence, machine learning, remote sensing, and GIS-based environmental surveillance systems may enhance predictive modeling and identification of resistance hotspots. Finally, interdisciplinary collaborations involving microbiologists, environmental scientists, hydrologists, clinicians, public health experts, and policymakers are essential for translating scientific findings into effective management strategies. Such collaborative efforts will be critical for safeguarding the ecological integrity of the Ganga River while reducing environmental contributions to the global antimicrobial resistance crisis.

In conclusion, mitigation of antimicrobial resistance in the Ganga River requires a multifaceted approach that combines wastewater treatment improvements, industrial pollution control, environmental monitoring, One Health surveillance, policy interventions, and innovative biological strategies such as bacteriophage applications. Continued research and coordinated management efforts are essential for reducing resistance dissemination, protecting freshwater ecosystems, and safeguarding public health in the future.

14. Conclusion

Antimicrobial resistance (AMR) has emerged as one of the most pressing environmental and public health challenges of the twenty-first century. The evidence synthesized throughout this review demonstrates that freshwater ecosystems, particularly large river systems, play a critical role in the persistence, evolution, and dissemination of antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), metal resistance genes (MRGs), and mobile genetic elements (MGEs). The Ganga River represents a globally important case study because it receives continuous inputs of municipal sewage, industrial effluents, hospital wastewater, agricultural runoff, and other anthropogenic contaminants that collectively shape microbial community dynamics and environmental resistomes. Metagenomic investigations conducted across different stretches of the river consistently reveal widespread distributions of ARGs, MRGs, virulence factors, and multidrug resistance determinants in both water and sediment environments, highlighting the river's role as a significant environmental reservoir of antimicrobial resistance. A major finding emerging from recent research is the close association between heavy metal contamination and antimicrobial resistance. Heavy metals such as arsenic, cadmium, chromium, copper, nickel, lead, and zinc persist in aquatic environments for extended periods and exert continuous selective pressure on microbial communities. This review highlights how co-selection mechanisms, including cross-resistance, co-resistance, and coregulation, facilitate the maintenance and dissemination of ARGs even in the absence of antibiotic exposure. The frequent co-occurrence of ARGs, MRGs, and MGEs within riverine microbial communities underscores the importance of considering environmental contaminants beyond antibiotics when addressing the AMR crisis. Understanding these co-selection processes is therefore essential for predicting resistance evolution, identifying environmental hotspots, and developing effective mitigation strategies. The environmental and public health implications of these findings are substantial. Environmental reservoirs of resistance contribute to the emergence and spread of multidrug-resistant pathogens, increase opportunities for horizontal gene transfer, and create potential exposure pathways for human and animal populations through drinking water, agriculture, fisheries, recreational activities, and cultural practices. From a One Health perspective, the Ganga River serves as a critical interface linking environmental, animal, and human health. Consequently, effective management of environmental AMR requires integrated approaches combining wastewater treatment improvements, industrial pollution control, long-term environmental surveillance, responsible antibiotic stewardship, and coordinated One Health monitoring programs. In conclusion, the Ganga River illustrates the complex interactions among microbial ecology, heavy metal contamination, and antimicrobial resistance in freshwater ecosystems. Addressing these interconnected challenges will require multidisciplinary research, advanced monitoring technologies, and evidence-based policy interventions aimed at reducing pollutant inputs and limiting the environmental dissemination of resistance determinants. Such efforts are essential for protecting ecosystem integrity, preserving freshwater resources, and mitigating the growing global threat of antimicrobial resistance.

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