

Aquatic Habitat Flooded With Pathogenic Microbes Showing Diverse Antibiotic Resistance

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Abstract: Microorganisms flourish in diverse habitats, including aquatic environments, where they play vital roles but can also pose health risks. Contaminated water—linked to nearly 88% of global illnesses—often harbors antibiotic-resistant bacteria (ARB), fuelled by pollution from human activities like livestock farming, industrial discharge, and wastewater treatment. Urban wastewater reflects community antibiotic use, with bacteria such as *E. coli*, *Acinetobacter*, etc showing multidrug resistance. The spread of resistance genes, aided by plasmids and biofilms, is worsened by co-contaminants like heavy metals and seasonal changes. Despite diagnostic advances, the ecological impact of ARB remains underexplored. Addressing this issue requires strict antibiotic regulation, improved wastewater management, and robust public health policies to protect water quality and limit the spread of resistance.

Key words: Microbial communities, Environmental microbiology, Antibiotics, resistance, Waterborne pathogen.

INTRODUCTION

Water is fundamental to all life forms. Both marine and freshwater ecosystems are home to a diverse range of microorganisms, some of which can cause diseases. According to the World Health Organization, approximately 88% of illnesses globally are linked to contaminated water sources. Human exposure to these pathogens can occur during daily routines such as drinking, bathing, or washing. Certain bacterial species found in freshwater are responsible for waterborne diseases. Moreover, pollution levels are steadily increasing in numerous aquatic environments, posing significant health risks.

Microorganisms are found everywhere in nature and establish communities in various environments, including soil, water, and air. Even the most extreme conditions, such as deep-sea vents, rocky terrains, hot springs, deserts, and polar regions, serve as habitats for these organisms. The presence and activity of microbial communities are vital not only for maintaining ecological balance but also for influencing human health [1].

Recent investigations focusing on environments shaped by human activities—such as food processing units, livestock farming, and wastewater treatment facilities—have revealed new perspectives on how antibiotic-resistant bacteria spread and persist [31-34]. Urban wastewater treatment plants are critical components of the water cycle and their microbial populations offer insight into two key aspects: they reflect the gut microbiota of the surrounding human population and indicate the broader effects of human activities on microbial communities in water systems. In fact, detecting water bodies completely free of antibiotic traces is rare, except in remote mountain streams before these waters flow through agricultural or urban areas [35].

Antibiotics are extensively used to treat illnesses in humans and animals, as well as to promote growth in livestock. However, a large proportion of these antibiotics enter the environment in an active form through excretion. This has led to growing concern about the long-term effects of antibiotic residues in aquatic ecosystems [36,37,38]. Earlier research primarily examined antibiotic resistance in bacteria originating from faecal matter or in those exposed to heavy metals [19,20]. Studies like those by Walter

and Vennes (1985) indicated that hospital effluents contain elevated levels of antibiotic-resistant bacteria compared to other waste sources. Yet, the overall impact of such discharges on native microbial populations remains underexplored. Additionally, pharmaceutical wastewater has been linked to a higher occurrence of both single and multi-drug resistant strains, particularly among *Acinetobacter* species [21]. Although antibiotic resistance is commonly observed in areas with high antibiotic use, resistant strains are now also emerging in natural aquatic environments [22,23].

Escherichia coli, a natural resident of the intestines of warm-blooded animals [3-5], is widely used as a marker for fecal contamination [3,6,7]. However, its effectiveness as an indicator in food and water safety has been debated [8,9], largely because it is also found in non-intestinal environments [6,10,11]. To determine the source of contamination, researchers have employed multiple antibiotic resistance (MAR) indexing, which helps differentiate *E. coli* from high-risk origins, such as those associated with humans, poultry, or swine [12].

Grabow and colleagues have emphasized the need to revisit current water quality guidelines, warning that even seemingly harmless bacteria may carry resistance factors (R factors) that make them dangerous to health [2]. Numerous studies have

linked the use of antibiotics in food production with a rise in drug-resistant infections in both animals and humans [13-17], with animal gut bacteria serving as a significant reservoir of resistance genes carried on plasmids [18].

The extensive use of antibiotics in both healthcare and industrial farming illustrates the selection pressure that promotes resistance development [23]. Vancomycin-resistant enterococci (VRE), for example, have been detected in sewage, in healthy farm animals, in animal-derived food products, and in surface water sources [24,25].

Bacteria have evolved various defence strategies to withstand antibiotic exposure. The genetic instructions for these mechanisms are often found on chromosomes or on plasmids and can be passed down through generations (vertical gene transfer). Additionally, these resistance genes can be transferred between different bacterial species through horizontal gene transfer, often mediated by plasmids [26]. This type of gene transfer, particularly through conjugation, is common in densely populated bacterial environments, such as wastewater systems [27,28]. High microbial density and diversity are typical in biofilms—complex communities of microorganisms found in activated sludge of sewage plants, surface waters, and drinking water pipelines [29,30].

Table 1: Showing Systematic Review of Aquatic habitat and Microbial relation.

S.No.	YEAR	SCIENTIST NAME	OBSERVATION
1.	1984	JON J. CALOMIRIS et al.	The study examined the relationship between metal tolerance and antibiotic resistance in bacteria from raw and distributed water in an Oregon drinking water system. Results showed a correlation between tolerance to Cu^{2+} , Pb^{2+} , and Zn^{2+} and antibiotic resistance in distribution water but not in raw water. Higher tolerance to Al^{3+} and Sn^{2+} was observed in raw water, with no link to resistance [39].
2.	1986	J.G. JONES et al.	The study found that antibiotic resistance in aquatic bacteria varied by species, origin site, and testing methods. Shoreline areas showed higher resistance, and primary isolation with antibiotics increased detected resistance. Sensitivity testing was influenced by inoculum size, temperature, and medium, with Iso-Sensitest Agar unsuitable for many aquatic bacteria [40].
3.	1990	CHARLES W. KASPAR et al.	The study compared antibiotic resistance in <i>E. coli</i> from urban and rural waters using 11 antibiotics. Urban waters showed higher resistance, highlighting <i>E. coli</i> as a potential water quality indicator [41].
4.	1998	LUCA GUARDABASSI et al.	The study found hospital wastewater increased oxytetracycline resistance, while pharmaceutical plant discharge boosted single- and multi-antibiotic resistance in <i>Acinetobacter</i> [42].

5.	2002	Nwadiuto Esiobu et.al.	The study screened bacteria for antibiotic resistance and R plasmids across seven locations. Low-level resistance was common, especially near dairy farms, while high-level resistance was rare and species-specific (<i>Pseudomonas</i> , <i>Enterobacter</i>). Tetracycline resistance was most prevalent (47–89%), with plasmids in 29% of resistant bacteria, underscoring environmental reservoirs as public health concerns [43].
6.	2003	Thomas Schwartz et. al.	The study explored the role of wastewater in spreading antibiotic resistance to drinking water systems. The <i>vanA</i> gene (vancomycin resistance) was found in wastewater and drinking water biofilms, suggesting possible transfer. The <i>mecA</i> gene (methicillin resistance) was confined to hospital wastewater, while <i>ampC</i> genes (β -lactamase) were detected in wastewater, surface water, and drinking water biofilm [44].
7.	2004	Laura F. Webstera et. al.	The study used antibiotic resistance testing to identify human and animal sources of <i>E. coli</i> contamination in South Carolina waters. Higher resistance was found in <i>E. coli</i> from Broad Creek (47%) compared to Okatee River (20%), with patterns linking to WWTPs, suggesting human contamination. AR testing shows promise but needs further validation [45].
8.	(2005)	Miguel Ferreira da Silva et al.	The study examined enterococci in raw and treated wastewater, finding treatment reduced <i>E. hirae</i> but increased <i>E. faecium</i> , with no change in <i>E. faecalis</i> . No vancomycin resistance was detected, but resistance to ciprofloxacin (33%), erythromycin (40%), and tetracycline (57%) persisted. Treatment failed to eliminate resistant strains and increased ciprofloxacin resistance [46].
9.	2008	Fernando Baquero et al.	The study highlighted water environments as hubs for antibiotic resistance spread through gene exchange among bacteria, driven by antibiotics, disinfectants, and heavy metals. Strategies to reduce resistance include optimizing wastewater and manure management, improving disinfection, and preventing the mixing of human, animal, and environmental bacteria. Enhanced methods are needed to track resistance genes, detect antibiotics, and eliminate resistant bacteria [47].
10.	2009	Klaus Kümmerer et al.	The review found limited data on the environmental impact of antibiotic-resistant bacteria, with antibiotics from hospitals not being a major source of resistance in drinking water. The main concern is the spread of resistant bacteria. To reduce resistance, it is essential to use antibiotics prudently, control their spread, and improve drug development and wastewater management to minimize environmental contamination [48].
11.	2009	Xu-Xiang Zhang et. al.	The review found antibiotic resistance genes (ARGs) in various water environments, including hospital and animal wastewater, sewage, treatment plants, and drinking water. Molecular methods like PCR, real-time PCR, DNA sequencing, and hybridization are used to detect and study ARGs in these water sources [49].
12.	2010	A. queckiewicz et. al.	The study found that wastewater treatment reduced fecal indicators, but effluent still contained $>10^4$ CFU/100ml, with 90% showing antimicrobial resistance (AMR). <i>Enterococcus faecium</i> and <i>Enterococcus faecalis</i> were resistant to multiple antibiotics, while <i>E. coli</i> showed resistance to ampicillin, piperacillin, and tetracycline, with one case of ESBL production. Multidrug-resistant strains were found in 9% of <i>E. coli</i> and 29% of enterococci in treated effluent [50].
13.	2012	Agnese Lupo et. al.	The study highlights that antibiotic resistance is driven by clinical overuse and polluted freshwater environments, with rivers spreading resistant bacteria and accelerating resistance through interactions with native microbes. The lack of environmental data complicates risk prediction, while

			pollution worsens the issue, disrupting ecosystems. Improving water quality and monitoring can help reduce health risks and slow the spread of resistance [51].
14.	2012	E. Amaya et. al.	The study found high antibiotic resistance in <i>E. coli</i> isolates from hospital sewage and well-water samples in León, Nicaragua. Hospital sewage showed resistance to ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid, and trimethoprim-sulphamethoxazole. 19% of well isolates were resistant to multiple antibiotics, including third-generation cephalosporins. ESBL-producing <i>E. coli</i> with <i>blaCTX-M</i> genes were present in hospital sewage and 26% of resistant well isolates, with <i>blaCTX-M-9</i> dominant in hospital sewage and <i>blaCTX-M-1</i> in well water [52].
15.	2012	Vangelis Economou et. al.	The study found 28 <i>Salmonella</i> serovars in river and coastal waters of northwestern Greece, with 34.8% of <i>S. enteritidis</i> and all <i>S. virchow</i> showing multi-drug resistance. <i>Listeria</i> spp. were fully antibiotic-susceptible, while <i>Campylobacter</i> spp., <i>E. coli</i> O157, <i>Cryptosporidium</i> , and <i>Giardia</i> were absent. Indicator bacteria counts showed weak correlations with pathogens, highlighting the need for monitoring resistant bacteria in aquatic environments [53].
16.	2014	Anita et. al.	The study assessed water resources in Mathura, India, focusing on <i>E. coli</i> prevalence due to high pilgrimage influx and the impact on the shrinking Yamuna River. Of 100 water samples, 40 tested positive for <i>E. coli</i> , including 14 from the river. Total coliform counts ranged from 1.08 to 6.34 log ₁₀ CFU/mL. Antibiotic sensitivity testing revealed significant resistance in <i>E. coli</i> isolates, emphasizing the need to protect water resources for public health [54].
17.	2014	Asma Akhter et al.	The study found varying levels of antibiotic resistance among coliform populations in Gomti River water, with lower resistance at site III (treated wastewater) compared to more polluted sites I and II. <i>E. coli</i> showed higher resistance than <i>Enterobacter</i> spp., particularly to ampicillin, chloramphenicol, sulfonamides, and tetracycline. Most isolates had MICs of 2–5 µg/mL for ciprofloxacin, tetracycline, and amoxicillin, indicating concerning antibiotic tolerance [55].
18.	2014	Tandra Mohanta et. al.	The study assessed the impact of urbanization and seasonal variations on antibiotic-resistant bacteria in water sources in West Bengal, India. MAR bacteria were most prevalent in the River Hooghly (71.01%), followed by the River Kangsabati (15.38%) and groundwater (8.33%). The highest MAR prevalence and ARI values were observed post-monsoon, suggesting increased antibiotic exposure due to surface runoff during this season [56].
19.	2015	Frederick Adzitey et. al.	The study found that 37.90% of <i>E. coli</i> isolates from drinking water in Tamale Metropolis, Ghana, were resistant. High resistance was observed to vancomycin (94.64%) and erythromycin (85.71%), while high susceptibility was noted for ciprofloxacin (94.64%) and gentamicin (91.07%). MAR indices ranged from 0.11 to 0.56, indicating significant multi-drug resistance [57].
20.	2015	Biswas et. al.	The study assessed bacterial load, fecal contamination indicators, and antibiotic resistance in the Ganga River water at Serampore, West Bengal. A total of 110 bacterial isolates were identified, including 25 fecal coliforms, 50 <i>E. coli</i> , and <i>Enterobacter</i> spp. The presence of coliform bacteria indicated significant fecal contamination, highlighting the need for conventional water treatment [58].

21.	2015	SHIVI BHASIN et. al.	The study found <i>Salmonella typhi</i> contamination in the Kshipra River ranged from 2-158 x 10 ³ CFU/100 ml, exceeding WHO limits. Higher contamination levels were observed in summer and during rainfall, particularly at sites like Ramghat, Mangalnath, and Triveni. Mass pilgrimages significantly increased <i>S. typhi</i> levels, highlighting the need for improved sanitation and water pollution control [59].
22.	2017	Stanley C. Onuoha et. al.	The study evaluated the bacterial quality of surface water in Afikpo, finding heterotrophic bacteria counts of 209.5 CFU/100 mL in the Okpu stream, 162.5 CFU/100 mL in Ohino Ngodo, and 162.0 CFU/100 mL in Ngwogo stream. <i>E. coli</i> and <i>Staphylococcus</i> species were the most common isolates (23.1% each). Resistance was highest against sulphamethoxazole, cephalothin, and tetracycline, while azithromycin and imipenem were most effective. The presence of multi-drug resistant strains emphasizes the need for proper water treatment in the area [60].
23.	2017	Zhaojun Chen et. al.	The study assessed antibiotic-resistant <i>E. coli</i> in drinking water sources (Qiantang River and Dongtiao Stream) in Hangzhou, China, finding high resistance to tetracycline, ampicillin, and other antibiotics, with resistance decreasing downstream in the Qiantang River. Water quality parameters, especially nutrient concentration, were linked to resistance. All tetracycline-resistant isolates carried tet genes, often with multiple gene combinations. These drinking water sources may serve as reservoirs for antibiotic resistance, posing a public health risk [61].
24.	2018	Stephen T. Odonkor et. al.	The study assessed the microbiological quality and antibiotic resistance of <i>E. coli</i> from six water sources, finding poor water quality and 49.48% multidrug resistance. High resistance was observed against penicillin, cefuroxime, erythromycin, and tetracycline, while <i>E. coli</i> was more susceptible to nitrofurantoin, cefotaxime, amikacin, gentamicin, and ciprofloxacin. 63% of multidrug-resistant strains had a MAR index >0.2. Nitrofurantoin is recommended for treating waterborne bacterial diseases [62].
25.	2018	Vishal Diwan et. al.	The study characterized seasonal variation in water quality, antibiotic residues, resistance genes, and <i>E. coli</i> resistance patterns in the Kshipra River over one year. Significant changes were observed in water quality and antibiotic residue levels. <i>E. coli</i> isolates showed resistance to ampicillin, cefepime, and meropenem, with most being ESBL producers from phylogenetic groups A or B1. Seasonal variations in antibiotic residues and resistance genes highlighted the need for regulations to control antibiotic pollution in the river [63].
26.	2020	Ashish Kumar Singh et. al.	The study assessed the water quality of spring water in Sikkim, India, finding high levels of <i>E. coli</i> and other <i>Enterobacteriaceae</i> . Antibiotic resistance was observed, with strains resistant to ampicillin, amoxicillin, ceftazidime, and carbapenem. <i>E. coli</i> isolates carried both virulence and antibiotic-resistance genes, indicating significant fecal contamination and the presence of multi-drug-resistant bacteria in the water [64].
27.	2020	Manish Kumar et.al.	The study evaluated antibiotic-resistant bacteria, resistance genes, and metal concentrations in the Kelani River, Sri Lanka, highlighting seasonal impacts. <i>E. coli</i> (10–27 CFU/ml) exhibited multidrug resistance, particularly to older antibiotics like tetracycline and sulfamethoxazole. Resistance genes for tetracyclines, sulfonamides, β -lactams, and fluoroquinolones were detected, with significant seasonal variations influencing fluoroquinolone resistance [65].

28.	2020	Manju Purohit et. al.	Mass bathing in the Kshipra River caused a decrease in pH, turbidity, and dissolved oxygen while increasing total dissolved solids and free CO ₂ . TSS, BOD, and COD were highest post-bathing. MDR <i>E. coli</i> and ESBL producers ranged from 9–44% and 6–24%, respectively. Coliform and <i>E. coli</i> counts, along with antibiotic resistance, were significantly higher during and after bathing compared to pre-bathing. The study emphasizes the need for guidelines and regulatory measures to limit the environmental spread of resistant bacteria [66].
29.	2020	Cho , C.R. Jackson et.al.	S. Cho's study assessed the prevalence and antimicrobial resistance (AR) of <i>Salmonella</i> , <i>E. coli</i> , and <i>Enterococcus</i> in surface water, highlighting their role in spreading AR. These bacteria, often linked to human and animal waste, are found globally and pose health risks. However, limited data on their prevalence and sources underscores the need for better monitoring to address AR concerns [67].
30.	(2020)	Bhasin Shivi et al.	The study investigated bacterial diversity in the Kshipra River, finding pathogenic bacteria (e.g., <i>Salmonella</i> , <i>Pseudomonas</i> , <i>Shigella</i> , <i>Vibrio</i>) and fecal contamination, indicating pollution from domestic sewage and excreta. Waterborne diseases like typhoid, cholera, and gastrointestinal infections were prevalent. Bacterial density correlated with physicochemical parameters (temperature, DO, BOD, COD). The study emphasizes the need for immediate action to reduce anthropogenic pollution and restore river health [68].
31.	2020	Tamanna Zerín et.al.	The study assessed water quality from various sources, revealing the Buriganga River had the highest contamination (HPC: 7.7×10^4 cfu/ml, TCC: 2.3×10^4 cfu/100 ml). Predominant bacteria included <i>E. coli</i> and <i>Staphylococcus aureus</i> , showing significant resistance to amoxicillin and tetracycline. The study calls for urgent action to improve water quality and regulate antibiotic use [69].
32.	2023	Prabhjot Singh Jassal et.al.	The study reviewed antibiotic presence in major South Asian rivers, finding contamination in rivers across India, Sri Lanka, Nepal, and Bangladesh due to increased antibiotic use and inadequate sewage treatment. Antibiotics like ciprofloxacin, kanamycin, and trimethoprim were most prevalent in rivers such as the Ganga, Kelani, and Brahmaputra. This contamination threatens aquatic ecosystems, promotes microbial resistance, and poses health risks. The study calls for strict monitoring and mitigation measures to address the environmental impact of antibiotic residue [70].
33.	2024	Bukola Margaret Popoola et.al.	The study evaluated the water quality of five rivers in Oyo town, Nigeria, revealing that Sogidi River had the highest microbial load (6.36 log CFU/mL). Among 32 bacterial species identified, 81.8% were multidrug-resistant, with <i>Corynebacterium kutscheri</i> and <i>Aeromonas spp.</i> showing 90.9% resistance. The rivers are unsafe for consumption due to the presence of multidrug-resistant bacteria [71].
34.	2024	Omprakash A. Kadam et al.	The study identified multiple antibiotic resistance (MAR) in coliform bacteria from the Penganga River at Hadgaon town, noting seasonal variations in coliform counts. The highest counts occurred during the monsoon, with PRHS-4 at 1800 CFU/100 ml and PRHS-2 at 220 CFU/100 ml. Among 39 bacterial isolates (<i>Citrobacter</i> , <i>Enterobacter</i> , <i>K. pneumoniae</i> , <i>E. coli</i>), 80% were resistant to penicillin, while 70% were sensitive to streptomycin [72].
35.	2024	M. Ulfata, b et. al.	The Lahore Canal water is contaminated with harmful bacteria such as <i>E. coli</i> , <i>Salmonella</i> , and <i>Vibrio</i> . <i>Staphylococcus</i> showed the highest resistance to penicillin and maximum sensitivity to teicoplanin, making the water

			unsafe for drinking, bathing, and irrigation. This poses serious health risks and necessitates urgent treatment to meet WHO standards [73].
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CONCLUSION

Studies from various regions reveal that water bodies—especially those near urban areas, hospitals, and agricultural zones—are becoming significant reservoirs of antibiotic-resistant bacteria and resistance genes. Common pathogens like *E. coli*, *Enterococcus*, and *Salmonella* show resistance to widely used antibiotics, with many strains being multidrug-resistant. Factors such as industrial discharge, untreated sewage, heavy metals, and seasonal runoff contribute to this growing problem. Despite some reduction through wastewater treatment, many resistant bacteria persist and even reach drinking water systems. Seasonal events like mass bathing and monsoon rains further intensify contamination levels. The presence of resistance genes (e.g., *blaCTX-M*, *tet*, *vanA*) in environmental waters highlights the risk of gene transfer and the urgent need for better water management, antibiotic regulation, and resistance monitoring strategies.

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