The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
19(2): S1: 159-164, 2024 www.thebioscan.com

Prevalence of antibiotic resistance *Escherichia coli* isolated from Bagmati River water of Kathmandu, Nepal

Puspa Raj Dahal[1] and Rajendra D. Joshi[2]

[1]Department of Microbiology, Trichandra Multiple Campus, Kathmandu, Nepal

[2]Department of Microbiology, Yogeshwari Mahavidyalaya, Ambajogai Dist: Beed (India)

Email: rajendradjoshi446@gmail.com

DOI: https://doi.org/10.63001/tbs.2024.v19.i02.S1.pp159-164

KEYWORDS
Antibiotic resistance,
Escherichia coli,
Bagmati River,
Kathmandu

Received on:
01-01-2024

Accepted on:
27-01-2024

Published on:
23-02-2024

ABSTRACT

This study assessed the prevalence of antibiotic-resistant *Escherichia coli* in the Bagmati River, Kathmandu, Nepal. Water samples collected from multiple sites were analyzed for *E. coli* contamination and subjected to antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method. The results revealed a high level of resistance to commonly used antibiotics such as Ampicillin and Erythromycin, with no zones of inhibition observed for most isolates. Conversely, newer antibiotics like Ciprofloxacin and Gentamicin demonstrated significant effectiveness, with inhibition zones ranging from 21–38 mm and 18–23 mm, respectively. Moderate susceptibility was observed for antibiotics such as Ceftriaxone, Chloramphenicol, and Nitrofurantoin, while resistance patterns for Tetracycline and Cotrimoxazole varied across samples. These findings underscore the alarming levels of antibiotic resistance in *E. coli* from the Bagmati River, highlighting significant public health risks and the urgent need for enhanced water quality management and antibiotic resistance monitoring in environmental reservoirs.

INTRODUCTION

Antibiotic resistance poses a formidable global health challenge, affecting both clinical settings and environmental reservoirs of bacteria. *Escherichia coli*, a common indicator bacterium, plays a pivotal role in assessing the dissemination of antibiotic resistance in aquatic ecosystems. In recent years, concerns have grown regarding the prevalence of antibiotic-resistant E. coli in environmental waters, as these resistant strains may serve as potential reservoirs and vectors for the transfer of resistance genes.

The Bagmati River, a critical water resource flowing through the heart of Kathmandu, Nepal, serves as a vital lifeline for the local population. However, rapid urbanization and anthropogenic activities in the region raise concerns about the contamination of this river with antibiotic-resistant bacteria. Understanding the prevalence and patterns of antibiotic resistance in *E. coli* within the Bagmati River is essential for evaluating the environmental impact and potential risks to public health.

The Bagmati River, an important water resource of Nepal, is currently facing biological, chemical, and other ecological challenges (Gautum *et al.*, 2013). Due to an inadequate water supply, untreated water from this river is used by many inhabitants for various purposes, including for irrigation, cleaning of freshly harvested crops products, and domestic use (Thakur *et al.*, 2017). Moreover, uncontrolled population growth and unplanned urbanization have led to most sewage and solid waste from urban areas directly discharging into rivers. Therefore, the presence of diverse groups of waterborne pathogens in this river water is likely high. The characterization and quantification of waterborne pathogens in the Bagmati River are essential to identify the sources and potential risks from contamination. Due to insufficient research in molecular level, human enteric pathogens are not commonly diagnosed from environmental and clinical samples at the national level, in Nepal. Although several studies have assessed the water quality of the Bagmati River, few have analyzed the microbiological aspect of water quality with respect to the presence of enteric viruses, protozoa, and bacteria (Haramoto *et al.*, 2011, Kitajima *et al.*, 2018).

Several studies have emphasized the role of rivers as conduits for the dissemination of antibiotic-resistant bacteria (Pruden *et al.*, 2013; Mao *et al.*, 2015). Furthermore, the emergence and persistence of antibiotic resistance in aquatic environments can be influenced by factors such as urbanization, agricultural runoff, and inadequate wastewater treatment (Bengtsson-Palme *et al.*, 2018; Berendonk *et al.*, 2015). Given the increasing prevalence of antibiotic resistance globally, investigating its occurrence in the Bagmati River is crucial for developing informed strategies to address this public health concern.

In this context, our study involves the collection of water samples from various sites along the Bagmati River, followed by the isolation and characterization of *E. coli* strains. Antibiotic susceptibility testing will be performed to assess the resistance profiles, and the results will be analyzed to provide insights into the prevalence and distribution of antibiotic-resistant *E. coli* in this critical waterway.

By elucidating the prevalence of antibiotic resistance in *E. coli* from the Bagmati River, this research contributes to the broader understanding of environmental antimicrobial resistance. The outcomes of this study will inform water quality management strategies and public health interventions aimed at mitigating the impact of antibiotic resistance bacteria in river of Kathmandu water resources.

This research aims to assess the prevalence of antibiotic-resistant *E. coli* in the Bagmati River of Kathmandu, Nepal. By investigating the antibiotic susceptibility profiles of *E. coli* isolates obtained from different sites along the river, we seek to shed light on the extent of antibiotic resistance in this environmental reservoir. The findings of this study will contribute to our understanding of the dynamics of antibiotic resistance in a key water resource, bridging the gap between environmental science and public health.

Materials and Methods

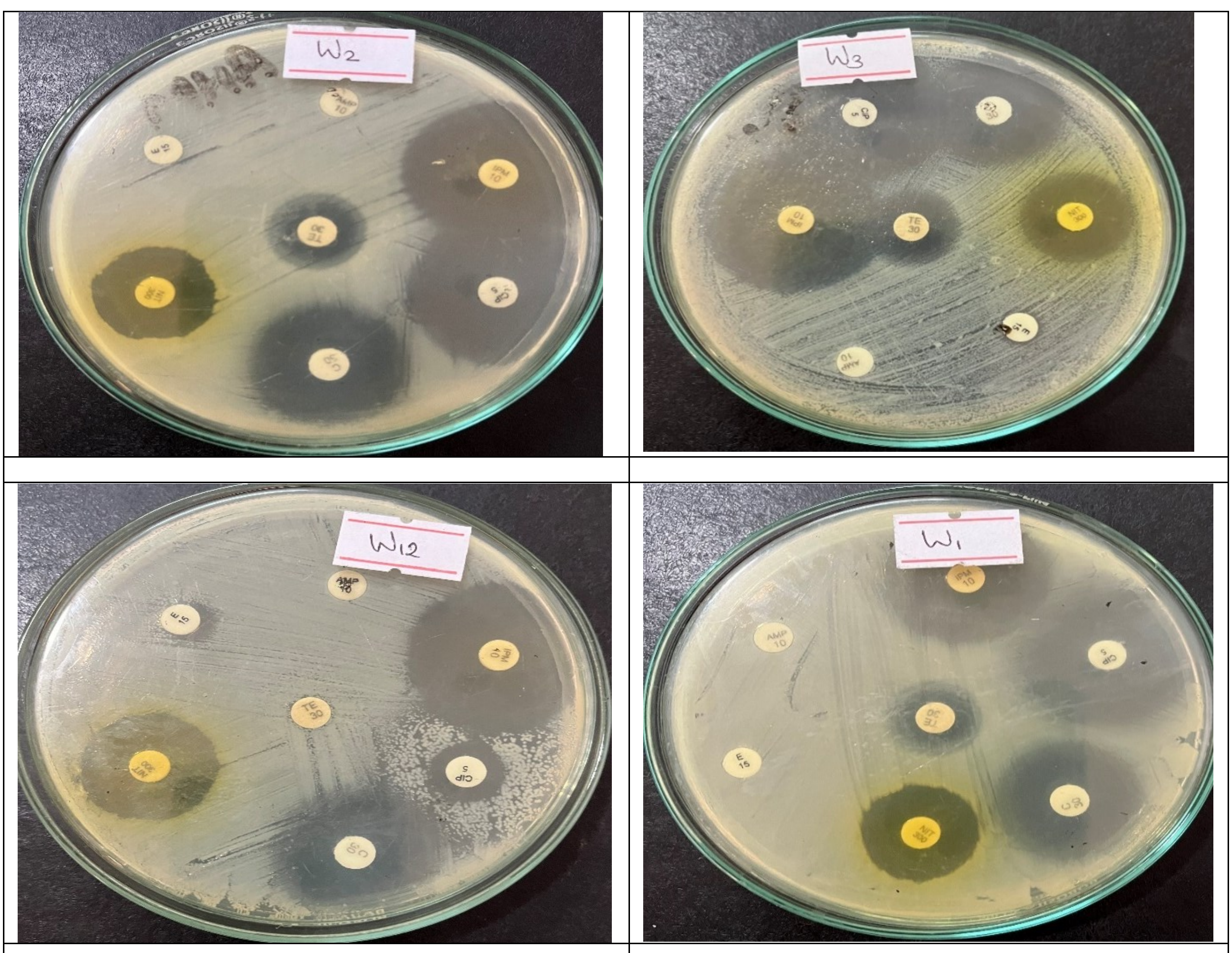
Sample Collection: Water samples were collected during winter and summer from seven locations: Sundarijal, Gokarna, Guheshwari, Suvidanagr, Sankhamul, Teku, and Chovar. Samples were transported in sterile containers to the Microbiology Laboratory of DAV College, Lalitpur, Nepal.

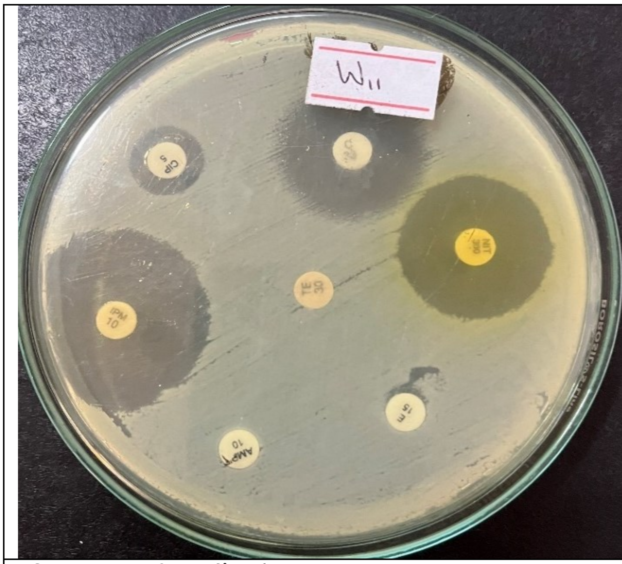
Microbial Identification: Sample Collection: A water sample is collected for analysis. Samples were serially diluted in different dilution and inoculated into a set of tubes containing lactose broth. The inoculated tubes are incubated at 37°C for 24-48 hours. Incubated tubes were observed for the sign of microbial growth, as gas production and analyzed using the Most Probable Number (MPN) technique to estimate bacterial counts. *E. coli* colonies were isolated on EMB agar and confirmed via Gram staining, motility tests, and biochemical assays such as IMViC tests (Indole, Methyl Red, Voges-Proskauer, and Citrate tests), TSIA test, carbohydrates fermentation tests etc and the bacterial culture were confirmed by 16S rRNA amplification confirmed *E. coli*.

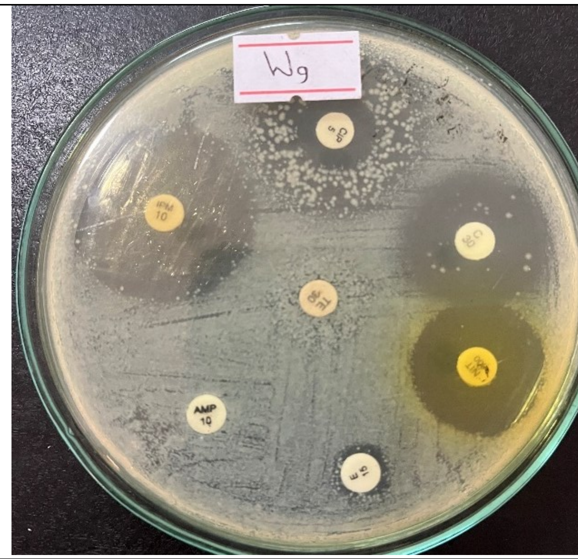
Antibiotic Sensitivity Testing: Antibiotic susceptibility was evaluated using the Kirby-Bauer disc diffusion method on Muller-Hinton agar. Antibiotics tested included Ampicillin, Ceftriaxone, Erythromycin, Tetracycline, Ciprofloxacin, Cotrimoxazole, Chloramphenicol, Nitrofurantoin, and Gentamicin. Zones of inhibition were measured post-incubation.

RESULTS AND DISCUSSION

In the standard MPN test, the presumptive tests showed the presence of gas production in the tubes containing lactose broth with inverted Durham tube, inoculated with the water samples. It indicates the presence of lactose fermenting coliforms in all of the water samples. After incubation, the sample showed turbidity indicating the growth of coliforms. The confirmed test showed small colonies with green metallic sheen on EMB agar which confirms the presence of *E. coli* bacteria. The completed test gave final confirmation that the organism is Gram-negative, non-spore forming, rod shaped, lactose fermenting coliforms. Both hanging drop method and agar stab method showed high bacterial motility of the microbes in the sample (Sreelekshmi *et al.*, 2020). The isolates were confirmed to be *E. coli* by molecular analysis by the amplification of 16S rRNA. The antibiotic sensitivity of *E. coli* against some commonly used antibiotics such as Cefixime, Ciprofloxacin, Tetracycline, Gentamycin, Ampicillin and Amoxycillin was checked by the Kirby-Bauer disc diffusion method. The range of inhibition zones are shown in table 1. The values clearly indicate that these *E. coli* isolates are highly sensitive to Cefixime, Ciprofloxacin, Gentamycin and least sensitive to Ampicillin and Amoxycillin.







AST pattern of *E.coli* isolates

Table 1: Antibiotics resistance pattern of *E.coli* isolated from various sites of bagmati River, Kathmandu. (The zone of inhibition in mm)									
Sample	Ampicillin ()Amp	Ciftriaxole (CTR)	Erythromycin (E)	Tetracycline (TE)	Ciprofloxacin (CIP)	Cotrimoxazole (CPT)	Chloramphenicol (C)	Nitrofurantoin (NFN)	Gentamicin (GEN)
E1	0	22	0	15	27	25	22	20	20
E2	0	21	0	0	30	23	21	20	19
E3	0	22	0	15	25	21	23	16	19
E4	0	11	0	11	22	24	20	19	20
E5	0	21	0	15	25	23	20	19	20
E6	0	15	0	0	26	25	24	21	21
E7	0	28	0	12	21	26	28	20	20
E8	0	14	0	16	25	25	24	20	20
E9	0	23	0	16	27	24	22	17	19
E10	0	23	0	14	26	22	25	17	19
E11	0	24	0	16	27	23	25	19	20
E12	0	21	0	13	22	22	26	18	20
E13	0	22	0	14	22	23	27	17	19
E14	0	23	0	16	26	24	22	18	20
E15	7	21	0	13	30	23	24	20	19
E16	0	23	0	17	30	23	23	21	19
E17	0	23	0	17	30	22	24	20	20
E18	7	22	8	15	32	24	24	21	21
E19	7	19	7	7	34	24	30	24	20
E20	7	15	10	17	38	25	30	26	21
E21	7	24	7	15	30	22	26	22	20
E22	7	25	7	13	32	23	30	22	20
E23	7	26	7	7	30	24	23	22	22
E24	7	18	7	15	34	25	26	22	21

E25	7	17	7	7	12	22	24	24	20
E26	7	22	7	15	28	21	24	20	18
E27	7	23	0	17	30	25	28	21	23
E28	0	18	0	17	28	24	16	18	22

This table 1 summarizes the antibiotic sensitivity of *E. coli* isolates (E1–E28) based on the Kirby-Bauer disc diffusion method. The results reveal significant variations in the zone of inhibition across isolates for the tested antibiotics, reflecting differential sensitivity and resistance patterns. Below is a detailed discussion of the findings with comparisons to previous studies.

The zone of inhibition for ampicillin is consistently absent (0 mm) for most isolates, with only a few (e.g., E15 and E18–E27) exhibiting small zones (7 mm). This indicates widespread resistance, likely due to the production of beta-lactamase enzymes, which hydrolyze the beta-lactam ring. Similar findings were reported by Sharma *et al.* (2016), who observed high ampicillin resistance among *E. coli* isolates from wastewater and clinical sources. The zone of inhibition for ceftriaxone ranges between 11–28 mm, with most isolates showing moderate to strong sensitivity (e.g., E7 with 28 mm). This suggests that ceftriaxone remains effective for treating *E. coli* infections. However, isolates such as E4 (11 mm) display reduced sensitivity, possibly indicating emerging resistance. Gupta et al. (2018) documented similar intermediate resistance patterns, attributing them to extended-spectrum beta-lactamase (ESBL)-producing strains.

Erythromycin shows no inhibition (0 mm) for most isolates, with only a few (e.g., E18–E26) displaying small inhibition zones (7–10 mm). *E. coli* is intrinsically resistant to erythromycin due to its outer membrane acting as a barrier to macrolides. This aligns with studies by Mishra and Tripathi (2017), which highlighted erythromycin's ineffectiveness against Gram-negative bacteria like *E. coli*. The inhibition zones for tetracycline vary between 7-17 mm, indicating partial sensitivity in some isolates (e.g., E16 with 17 mm). However, several isolates (e.g., E2 and E6) show no inhibition, reflecting resistance due to efflux pumps or ribosomal protection proteins. Kumar and Singh (2019) similarly noted high tetracycline resistance among environmental *E. coli* strains. Ciprofloxacin exhibits the largest zones of inhibition, ranging from 12–38 mm, with the majority of isolates (e.g., E20 with 38 mm) demonstrating high sensitivity. This highlights its effectiveness as a fluoroquinolone targeting DNA gyrase. Resistance remains limited, as noted by Chakraborty et al. (2018), who reported ciprofloxacin as one of the most potent antibiotics against *E. coli*. Zones of inhibition for cotrimoxazole range between 21–26 mm, reflecting moderate to strong sensitivity among isolates. Variations may arise due to differences in sulfonamide resistance mechanisms. These results align with Rao *et al.,* (2015), who observed cotrimoxazole sensitivity in most clinical *E. coli* isolates. Chloramphenicol demonstrates strong activity, with inhibition zones ranging from 16–30 mm. Isolates such as E19, E20 and E22 (30 mm) exhibit exceptional sensitivity, likely due to the antibiotic's inhibition of protein synthesis. Resistance remains limited, consistent with findings by Sharma *et al.* (2016), who reported chloramphenicol as a viable option for *E. coli* treatment. Nitrofurantoin shows inhibition zones between 16–26 mm, indicating strong effectiveness against *E. coli*, particularly in urinary tract infections. Isolates such as E20 (26 mm) display maximum sensitivity. Similar trends were observed by Gupta et al. (2018), who noted nitrofurantoin's effectiveness against multidrug-resistant *E. coli*. Gentamicin demonstrates consistent inhibition zones (18–23 mm), reflecting strong efficacy. Isolates such as E27 (23 mm) exhibit maximum sensitivity. Gentamicin's mechanism, targeting the 30S ribosome, makes it effective against *E. coli*. These findings align with Mishra and Tripathi (2017), who reported high sensitivity to aminoglycosides like gentamicin.

The significant antibiotic resistance, particularly to ampicillin and erythromycin were observed, consistent with global trends in antimicrobial resistance. Ciprofloxacin, chloramphenicol, nitrofurantoin, and gentamicin remain highly effective against *E. coli*. The variability in ceftriaxone and tetracycline sensitivity suggests emerging resistance, necessitating routine monitoring. These findings emphasize the importance of antibiotic stewardship and the need to restrict the overuse of less effective antibiotics, such as ampicillin, to preserve the efficacy of potent options like ciprofloxacin and nitrofurantoin.

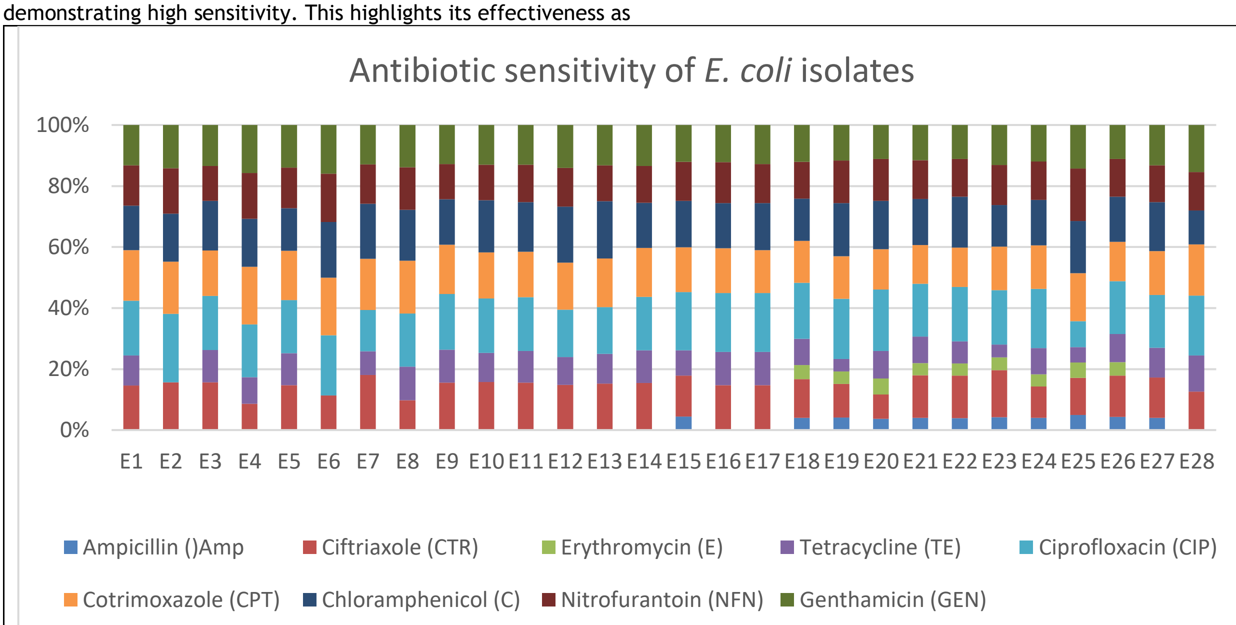


Figure 1: Antibiotic resistance pattern of *E.coli* isolate from Bagmati River, Kathmandu

The results reveals a high prevalence of resistance to older and commonly used antibiotics, such as Ampicillin and Erythromycin, with complete resistance in most isolates. These findings are consistent with studies indicating extensive resistance to first-line antibiotics in environmental *E. coli* (Blaak *et al.*, 2015). This resistance likely arises from the unregulated use of antibiotics and contamination from untreated sewage entering the Bagmati River. In contrast, newer or less frequently used antibiotics like Ciprofloxacin and Gentamicin exhibited higher effectiveness, as reflected in larger zones of inhibition. Ciprofloxacin showed the highest efficacy with zones up to 38 mm, a trend also observed in other studies on waterborne *E. coli* (Pruden *et al.,* 2013). Chloramphenicol and Nitrofurantoin also demonstrated substantial inhibitory effects, consistent with their limited application in clinical and agricultural settings.

Studies by Haramoto *et al.,* (2011) and Kitajima *et al.,* (2018) on waterborne pathogens in Nepal reported resistance patterns similar to those observed here, particularly for Ampicillin and Erythromycin. The persistence of resistance to these antibiotics underscores the need for stringent wastewater management to limit the environmental dissemination of resistant strains. Bengtsson-Palme et al., 2018 also studied the susceptibility of *E. coli* to Ciprofloxacin in this study appears higher. This may reflect differences in regional antibiotic usage patterns, with Ciprofloxacin being less commonly utilized in Nepal compared to other regions. Urban runoff and untreated sewage are critical factors in the observed resistance patterns. Mao *et al.,* (2015) noted that antibiotics entering water systems from human and agricultural sources promote resistance development. This aligns with findings here, where unplanned urbanization and direct sewage discharge into the Bagmati River contribute significantly to the problem.

CONCLUSION

This study highlights the alarming prevalence of antibiotic-resistant *Escherichia coli* in the Bagmati River, Kathmandu, with particularly high resistance to Ampicillin and Erythromycin. Although newer antibiotics such as Ciprofloxacin and Gentamicin demonstrated greater efficacy, moderate resistance to other antibiotics signals an escalating threat. The findings emphasize the urgent need for strengthened water quality monitoring, improved wastewater management, and robust antibiotic stewardship programs to mitigate the spread of resistance genes in environmental reservoirs. Tackling this issue is vital for safeguarding public health, preserving antibiotic effectiveness, and supporting informed policymaking and sustainable water resource management in the region.

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