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SHORT COMMUNICATION

Assessment of Oxygen Demand and Antibiotic Resistant *Citrobacter* spp in the Bagmati River

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AMR *Citrobacter* in Bagmati River

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ABSTRACT

Objectives: To correlate the Oxygen demand with antibiotic resistant *Citrobacter* species in Bagmati river.

Methods: A Cross-sectional study was conducted from May to August 2025 at the Department of Microbiology, Tri-Chandra Multiple Campus, Kathmandu. Fifteen water samples collected in duplicate were examined for biological oxygen demand (BOD), dissolved oxygen (DO), most probable number (MPN) counts and presence of antibiotic resistant *Citrobacter* species. The correlation analysis was conducted using Exploratory software version 6.10.3.1.

Results: The MPN count of total coliforms was lowest at the upstream site (4 MPN/100 mL), and peaked downstream (35,000 MPN/100 mL). This was accompanied by a decline in DO (8.7, 4.2, and 1.2 mg/L) and an increase in BOD (0.1, 250.8, and 425.4 mg/L). *Citrobacter* appeared as pink, non-metallic colonies on EMB agar, and 394 isolates were confirmed biochemically. Among these, 100% were resistant to ampicillin (penicillin) and 99 % (390/394) toward erythromycin (macrolide), while resistance to cefotaxime (68%), amikacin (53%,aminoglycoside), ciprofloxacin (49%, fluoroquinolone), and chloramphenicol (44%, phenicol) was also recorded. Positive correlation ($r = 0.835$, $p < 0.05$) was observed for presence of *Citrobacter* and BOD and whereas negative correlation ($r = -0.85$, $p < 0.05$) was observed for presence of *Citrobacter* and level of DO.

Conclusion: The increase in BOD and decrease of DO in the downstream region and its correlation with the presence of antibiotic resistant *Citrobacter* species signifies polluted water as contributor to antibiotic resistant coliform.

Key words: Bagmati River, *Citrobacter* spp, antimicrobial resistance, correlation

INTRODUCTION

The Bagmati River, traversing the Kathmandu Valley, represents a vital component of Nepal's cultural, religious, and ecological landscape (Yonzon & K.c, 2019). Despite its significance, the river has experienced substantial deterioration in water quality due to rapid urbanization, uncontrolled sewage discharge, and inadequate regulatory oversight, particularly in downstream segments (Shultana & Khan, 2022). The dissolved oxygen and biochemical oxygen demand of water signifies the pollution level in surface water (Tamrakar & Parajuli, 2019). Polluted aquatic environments serve as reservoirs for diverse microbial communities, including opportunistic pathogens capable of acquiring and disseminating antimicrobial resistance (AMR) determinants (Meradji et al., 2025).

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Citrobacter species, Gram-negative coliform bacteria widely distributed in soil, water, and the intestinal tracts of humans and animals, exemplify such pathogens (Jabeen et al., 2023). This study examines the presence, antibiotic susceptibility patterns of *Citrobacter* spp and its correlation with the physicochemical parameters of the isolates from different stretches of the Bagmati River, providing critical insight into the contribution of polluted water to the dissemination of antibiotic-resistant bacteria.

METHODS

Study design

A cross-sectional study was carried out from May to August 2025, at the Department of Microbiology, Tri-Chandra Multiple Campus, Kathmandu.

Study area

Bagmati river flowing from Baghdwar to Chobar inside Kathmandu valley.

Sampling sites

Fifteen sampling sites were stratified through google maps with equal distance between all the sampling points.

Sample collection and transportation

Fifteen water samples were collected in duplicate from three major segments of the Bagmati River-upstream (relatively pristine areas), midstream (urbanized sections), and downstream (heavily polluted stretches). Grab samples were obtained from subsurface water using sterile 300 mL BOD bottles, transported in an ice box to the laboratory.

Sample processing

Physicochemical parameters, including dissolved oxygen (DO) and biochemical oxygen demand (BOD) were analyzed by using the titration method (Ma et al., 2020), and total coliform counts were determined using five tubes Most Probable Number (MPN) technique, with positive tubes confirmed in Brilliant Green Lactose Bile Broth (BGLB) following APHA (2017) standards. Samples showing gas production and color change were further cultured on Eosin Methylene Blue (EMB) agar, and presumptive isolates were subculture on nutrient agar and characterized using Gram staining, catalase and oxidase tests, and a panel of biochemical tests including IMViC, Triple Sugar Iron (TSI) agar reactions, oxidative-fermentative tests, and urease activity (Jabeen et al., 2023b). *Citrobacter* spp were subjected to antimicrobial susceptibility testing (AST) using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar for ampicillin (10 µg), erythromycin (15 µg), amikacin (30 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), and chloramphenicol (30 µg), and zones of inhibition were measured based on CLSI (2023). Quality control was maintained using standard *E. coli* ATCC 25922 (Ghimire et al., 2023).

Ethical consideration

Ethical approval was obtained from the Institutional Review Committee, IOST, TU (IRC Ref. No.: 26-081/82) and sampling permission was granted by the Department of National Parks and Wildlife Reserve, Kathmandu (Ref no.: 3065).

RESULTS

Correlation between *Citrobacter* isolates and Oxygen demand value of Bagmati river

The correlation analysis revealed a strong positive relationship between BOD and *Citrobacter* spp The MPN per 100 mL showed a moderate positive correlation with BOD and a moderate, yet significant, positive correlation with *Citrobacter* spp (r = 0.59, p = 0.00057, t = 3.8). In contrast, a negative correlation was observed between MPN/100 mL and dissolved oxygen (DO) levels. BOD and DO were strongly inversely correlated and a similarly strong negative association were found between *Citrobacter* spp and DO (Table 1).

Table 1: Correlation analysis of different physiological and microbial parameters

SN	Parameters	r	p	t
1	BOD & *Citrobacter* spp	0.8345	9.9 e $^{-9}$	8.01
2	MPN/100 mL & BOD	0.683	0.0003	4.95
3	MPN/100 mL & *Citrobacter* spp	0.59	0.00057	3.8
4	MPN/100 mL & DO	-0.513	0.036	-3.16
5	BOD & DO	-0.84	5.6 $^{e-9}$	-8.24
6	*Citrobacter* spp & DO	-0.85	2.38 -9	-8.6

Chi square test of significance for antimicrobial resistance pattern in *Citrobacter* spp along river streams

Among 394 isolates, all were resistant to ampicillin (penicillin class) and 99% (392/394) towards erythromycin (macrolide class), while resistance to cefotaxime (68%, cephalosporin class), amikacin (53%, aminoglycoside class), ciprofloxacin (49%, fluoroquinolones class), and chloramphenicol (44%, phenicol class) was also recorded. The chi-square test showed statistically significant differences in the distribution of antibiotic resistance among isolates across the river sections. Specifically, the p-values for AK (amikacin, 0.015), E (erythromycin, 0.033), C (chloramphenicol, <0.001), CIP (ciprofloxacin, 0.005), and CTX (cefotaxime, 0.001) indicate that the presence of antibiotic resistant *Citrobacter* spp to these antibiotics varies significantly along the river.

DISCUSSION

The isolation of antibiotic-resistant and multidrug-resistant *Citrobacter* spp from the Bagmati River highlights the impact of unchecked wastewater discharge on river sources. The progressive deterioration of river water quality downstream is a direct consequence of accumulating anthropogenic influence (Castro et al., 2021). As the river receives increasing loads of domestic sewage, industrial effluent, and organic waste, its biochemical oxygen demand (BOD) rises substantially. This surge in organic matter fuels microbial metabolism, a process that consumes dissolved oxygen (DO) and leads to a marked decline in oxygen available for aquatic life (Yuliati et al., 2023). Our correlation analysis reveals a tightly coupled relationship between organic pollution and microbial contamination in the river system. We observed a strong positive association between BOD and *Citrobacter* spp ($r = 0.8345$), indicating that increasing organic load directly promotes the proliferation of these enteric bacteria. The results show a strong inverse link between dissolved oxygen and *Citrobacter* bacteria ($r = -0.85$), suggesting *Citrobacter* species flourish in oxygen-poor, organically rich conditions. Consequently, the resulting hypoxic conditions selectively favor the growth of facultative anaerobic bacteria like *Citrobacter*. This pattern of contamination is systemic, as shown by the positive correlations between MPN (a measure of fecal bacteria) and both BOD ($r = 0.683$) and *Citrobacter* spp ($r = 0.59$) (Holcomb & Stewart, 2020). The corresponding negative relationship between MPN and DO reinforces that fecal pollution intensifies as oxygen levels drop. Collectively, these results demonstrate that the decline in water quality driven by high organic load and oxygen depletion creates a favorable environment for the survival and growth of fecal indicator bacteria and specific pathogens, directly reflecting the cumulative impact of sewage inputs downstream (Bisimwa et al., 2022).

As anticipated, BOD exerted a strong positive effect on bacterial counts, underscoring the role of organic pollution in promoting *Citrobacter* proliferation. Conversely, DO levels showed a pronounced negative relationship, where higher oxygen concentrations corresponded with a substantial reduction in bacteria (Mutai et al., 2024). The statistical significance of both variables confirms that the interplay between organic nutrient availability and oxygen concentration are central determinants shaping the spatial distribution of *Citrobacter* spp in the river (Li et al., 2025).

The analysis of antibiotic resistance along the river showed a clear and statistically significant pattern for all five antibiotics tested. The consistently low p-values ($p < 0.000$ to $p = 0.033$) across the upstream, midstream, and downstream sections indicate that the differences in resistance levels are unlikely to have occurred by chance. These findings suggest that the position along the river is an important factor shaping the distribution of antibiotic-resistant bacteria. As the river flows downstream, the prevalence of resistance changes in a consistent and notable way. This pattern reflects the cumulative impact of human activities such as untreated wastewater discharge and agricultural runoff on the river environment (Sidrach-Cardona et al., 2014). Increasing pollutant input downstream likely creates greater selective pressure, allowing resistant bacterial strains to become more dominant across multiple antibiotic classes (Tello et al., 2012).

Our findings serve as a warning to people residing near the downstream and midstream areas of Bagmati river. Curbing the spread of antibiotic-resistant bacteria in this river system requires immediate and targeted interventions. Specifically, mitigating organic pollution through improved wastewater treatment and strict control of sewage discharge is not just beneficial but critical for public health.

CONCLUSION

This study demonstrates that the Bagmati River harbors antibiotic-resistant *Citrobacter* spp with resistance patterns intensifying in downstream stretches characterized by deteriorating Oxygen demand quality. The combination of elevated coliform loads, reduced dissolved oxygen, high biochemical oxygen demand are prominent marker for elevated drug resistant *Citrobacter* in Bagmati river.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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