Distribution of ESBL producing *E. coli* and resistance pattern in riverine and wastewater system of Kathmandu Valley

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Summary

The environmental waster is recognized as a critical but underexplored reservoir for resistant bacteria. Urban rivers in rapidly growing cities are increasingly shaped by human activity, receiving continuous inputs of domestic sewage, hospital effluents, animal farm drainage, and urban runoff (1, 2). In order to assess the prevalence, antimicrobial sensitivity, and ESBL-producing patterns of *E. coli* in environmental water, a longitudinal study was conducted in the major three river basins (Bagmati, Manohara, and Nakkhu) of the Kathmandu Valley (KV). This study reported that *E. coli* was detected consistently across all sources of environmental water (river water, and wastewater from animal farm and hospital), in all seasons, all gradient of river, and all types of rivers (Bagmati, Manohara and Nakkhu). Comparatively, prevalence of *E. coli* has been reported higher in river water, but multidrug resistance (MDR) *E. coli* and ESBL-producing *E. coli*(ESBLEC) reported higher in hospital and animal farm, respectively. Similarly, prevalence rate of ESBLEC and MDR was found moderate but increasing trend of their prevalence was reported higher in downstream of river gradient. Ceftriaxone and ciprofloxacin exhibited the highest resistance, while carbapenem and colistin resistance were either absent or rare. These findings indicate widespread environmental dissemination of AMR *E. coli* in KV in a One Health interface.

Methods

From November 2024 to October 2025, a longitudinal study was conducted in river basin of the KV, Nepal. Over four seasons, a total of 276 water samples were collected from hospitals (n = 36), animal farms (n = 95), and rivers (n = 145) across KV, covering 33.5 km of the Bagmati river, 22.7 km of the Manohara river, and 16.2 km of the Nakkhu river basin. Sampling sites were chosen based on the intensity of human–animal interaction, accessibility, wastewater discharge points, and river basin mapping. Water samples were collected aseptically, transported under cold chain conditions, and processed within 24 hours. The isolation and confirmation of *E. coli* were accomplished using conventional microbiological techniques, such as enrichment, membrane filtration, culture, biochemical testing, and MALDI-TOF MS. The Vitek Compact 2 system was used to perform antimicrobial susceptibility testing (AST), and combination disk diffusion test were used to evaluate ESBL production. STATA 14 was used to conduct descriptive statistics and chi-square tests, with a significance level of p-value < 0.05. The Central Veterinary Laboratory's Institutional Biosafety Committee (IBC 03-082/83) has reviewed and approved the study protocol.

Results

Prevalence of E. coli across, river, source, season, location

Among 276 environmental water samples, prevalence of *E. coli* was 86.9% (240). *E. coli* was highly prevalent in both river water (86.9%) and hospital effluent (86.1%), whereas a substantially lower prevalence was observed in animal farm wastewater (52.6%). *E. coli* showed consistently high prevalence across all seasons, ranging from 84.1% in summer to 91.3% in winter, indicating year-round fecal contamination and persistent with a slight increase during winter. *E. coli*was highly prevalent in all rivers (85.3–90.6%), with the highest level in Manohara (90.6%). *E. coli* prevalence varied along the river stream. *E. coli* was consistently high across different stream (86.0-88.0%).

Prevalence of ESBLEC

Among the *E. coli* isolates (n = 240), 28.3% (68/240) were identified as ESBL producers. Source-wise, the highest ESBL prevalence was observed in animal farm wastewater (36.1%), followed by hospital wastewater (25.8%) and river water (23.8%). Seasonal variation was evident, with the highest proportion of ESBL-producing E. coli detected in spring (36.7%) and the lowest in summer (17.2%). Among rivers, the Bagmati River had the highest ESBL prevalence (32.1%), followed by the Manohara River (29.3%) and the Nakkhu River (17.6%). By

river section, ESBL prevalence was highest in downstream sites (33.7%), compared with upstream (27.3%; 24/88) and midstream (22.7%) sites.

Antimicrobial resistance in E. coli

The MIC value of different 17 types of antibiotics for *E. coli* isolates from water sample was calculated. Resistance among *E. coli* isolates (N = 240) was highest to ciprofloxacin (19.2%) and ceftriaxone (21.3%), followed by trimethoprim–sulfamethoxazole (15.0%), and cefuroxime (12.1%). Moderate resistance was observed to amoxicillin/clavulanate and piperacillin/tazobactam (each 8.3%) and cefepime (7.5%). Lower resistance rates were detected for amikacin (3.8%), tigecycline (3.3%), and fosfomycin (7.1%). Resistance to carbapenems was absent for ertapenem and meropenem, while imipenem resistance was observed in 10.0% of isolates. Colistin resistance was rare (0.4%).

Antimicrobial resistance in E. coli by river

The Bagmati river showed the highest and most diverse resistance, including ceftriaxone (22.9%), ciprofloxacin (18.3%), and trimethoprim–sulfamethoxazole (15.3%), with rare resistance to colistin (0.8%). The Manahara river exhibited moderate resistance but the highest ciprofloxacin resistance (29.3%), with ceftriaxone at 22.4%. In contrast, the Nakkhu river showed a narrower profile but very high trimethoprim–sulfamethoxazole resistance (36.1%), alongside ceftriaxone (22.2%) and ciprofloxacin (13.9%).

Antimicrobial resistance in E. coli by river gradient

Upstream sites showed comparatively lower resistance, notably to ceftriaxone (13.6%), ciprofloxacin (18.2%), and trimethoprim–sulfamethoxazole (11.4%), indicating limited anthropogenic impact. Midstream sites demonstrated higher resistance, particularly to ceftriaxone (22.7%), ciprofloxacin (22.7%), imipenem (15.2%), and trimethoprim–sulfamethoxazole (16.7%). Downstream sites exhibited the highest resistance overall, especially to ceftriaxone (27.9%), cefuroxime (16.3%), and trimethoprim–sulfamethoxazole (17.4%).

Antimicrobial resistance in E. coli by season

Seasonally, ceftriaxone resistance peaking in autumn (35.6%) and remaining lower in summer (8.6%). The cefuroxime showed relatively stable resistance across seasons (8–14%). Resistance to β-lactam/β-lactamase inhibitor combinations was generally low, except for piperacillin/tazobactam, which showed a marked increase in spring (25.0%). Among fluoroquinolones, ciprofloxacin resistance was consistently high, particularly in spring (26.7%) and autumn (22.0%), with a noticeable decline during summer (10.3%). Carbapenem resistance was rare, with no resistance detected to ertapenem or meropenem in any season and low, seasonally variable resistance to imipenem.

Antimicrobial resistance in E. coli by sample source

Overall, resistance was most pronounced in hospital source, followed by animal, with the environment acting as a reservoir linking both sectors. Hospital wastewater isolates showed the highest resistance to ceftriaxone (29.0%), cefuroxime (25.8%), and trimethoprim–sulfamethoxazole (32.3%). Animal farm wastewater isolates had the greatest resistance to ciprofloxacin (26.5%), ceftriaxone (24.1%), and trimethoprim–sulfamethoxazole (18.1%). River water isolates exhibited moderate but widespread resistance, particularly to ceftriaxone (17.5%) and ciprofloxacin (14.3%). Carbapenem resistance was absent across all sources.

Prevalence of MDR bacteria according to source and river location

Of 240 *E. coli* isolates, 50 (20.8%) were multidrug resistant. MDR prevalence was highest among hospital wastewater isolates (32.3%), followed by animal farm wastewater (22.9%) and river water isolates (16.7%). Seasonal variation was evident, with higher prevalence in autumn (25.4%) and lower prevalence in summer (12.1%). Among river systems, the Bagmati had the highest proportion of MDR isolates (32.1%), followed by the Manohara (29.3%) and Nakkhu (17.6%). A gradual increase in MDR prevalence was observed from upstream (18.2%) to downstream (23.3%) river sites.

Discussion

This study demonstrates a high prevalence of *E. coli* (86.9%) in river and wastewater sources of KV, indicating widespread fecal contamination. Previous investigations in the Bagmati River reported *E. coli* detection in 76.9% of water samples, confirming persistent contamination of river systems due to anthropogenic activities (3). The slightly higher prevalence might be due to limited sample size and sampling point. The prevalence of ESBLEC (28.3%) in this study was lower than that reported in recent studies from the Bagmati river, where prevalences of 91.0% and 33.3% were recorded (4). Earlier studies were limited to river water samples, had relatively small sample sizes, and were conducted only during the winter season, while the present study incorporated a longe river stretch and more diverse sample set, including multiple environmental sources and more upstream sites. Despite the lower overall prevalence, the downstream increase in ESBLEC identified in this study supports

previous evidence that AMR bacteria accumulate along river stretches receiving untreated or poorly treated wastewater inputs (3, 4).

The observed MDR prevalence (20.8%) is comparatively lower than earlier reports from Nepal, where MDR among riverine *E. coli* isolates reached up to 80% (3). This variation may reflect differences in antimicrobial selection pressure, temporal changes, and methodological approaches. Nonetheless, the higher MDR burden in hospital wastewater compared with animal and river sources in this study is consistent with previous findings highlighting hospital effluents as major reservoirs of resistant bacteria (5). Similarly, the gradient increase in resistance from upstream to downstream sites supports the concept of rivers acting as conduits for AMR dissemination (3).

The AMR patterns observed in this study, particularly high resistance to third-generation cephalosporins and fluoroquinolones, are in line with national and regional reports from Nepal, which indicate widespread resistance to these commonly used antibiotics, while carbapenem resistance remains low or absent (5). Resistance to β-lactam/β-lactamase inhibitor combinations was generally low and stable across seasons, although the spring increase in piperacillin/tazobactam and persistently high ciprofloxacin resistance, particularly in spring and autumn, suggest seasonal influences of wastewater discharge, and reduced dilution during drier periods (6). In contrast, carbapenem resistance remained rare, with no resistance to ertapenem or meropenem and only occasional imipenem resistance, indicating limited circulation of carbapenem-resistant E. coli in the environment, though even sporadic detection remains a public health concern (7).

Conclusion

This study revealed widespread contamination of rivers and wastewater sources in KV with E. coli, including ESBL-producing and multidrug-resistant strains. Resistance was highest in hospital and animal farm wastewater and increased from upstream to downstream river sites, indicating the role of wastewater discharge in environmental AMR dissemination. High resistance to cephalosporins and fluoroquinolones, despite low carbapenem and colistin resistance, raises important One Health concerns. These findings underscore the need for integrated AMR surveillance, effective wastewater treatment, and strengthened antimicrobial stewardship to mitigate environmental transmission of resistant and ESBLEC in urban city, Kathmandu.

Keywords: Antibiotic resistance, E. coli, environmental wastewater, ESBL, Kathmandu Valley.

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