

Spatial Variation of Antibiotic-resistant *Escherichia coli* along Bagmati River, Kathmandu Valley

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ABSTRACT

Background: Antimicrobial resistant *Escherichia coli* has been reported in Bagmati river of Kathmandu Valley; however, studies on its spatial distribution are lacking, despite being essential for effective water management. Therefore, this cross-sectional study was conducted to determine the spatial distribution of antibiotic-resistant *E. coli* along Bagmati river from March-September 2025.

Methods: This work was conducted at Department of Microbiology Tri-Chandra Multiple Campus. Water samples collected in duplicate from fifteen sites of Bagmati river were processed for most probable number count and isolation of *E. coli*. BOD and DO were calculated by Winkler's titrimetric method. Antibiotic susceptibility test was done by disk diffusion technique. Chi square test, correlation assessment, cluster and factor analysis were conducted.

Results: The coliform count, *E. coli* isolates, Biochemical oxygen demand and dissolved oxygen levels ranged from 5–26,500 MPN/100 mL, 0–42, 1.2–418.75 mg/L, and 8.05–0.15 mg/L, respectively, from Baghdwar to Chobar. No *E. coli* isolates were recovered from Site 1. Cluster analysis based on these variables classified river into four distinct segments, with Sites 1 and 15 forming separate clusters. Significant differences were observed in antibiotic resistance pattern of *E. coli* isolates across the sampling sites. Factor analysis further grouped the river sites into five clusters, indicating spatial variation in antimicrobial resistant *E. coli* along the river continuum.

Conclusions: Conventional water quality indicators are insufficient to capture spatial complexity of river health. Integrating fecal contamination and antibiotic resistance patterns provides a more comprehensive assessment of pollution hotspots, supporting targeted management strategies under Nepal's Nationally Determined Contribution (NDC) 3.0.

Keywords: Antimicrobial resistance; bagmati river; *E. coli*; spatial analysis.

INTRODUCTION

Antibiotic-resistant bacteria (ARB) are found in the environment, where they can spread through water, soil, land, and living organisms. Polluted rivers, serve as important hotspots that support the growth and dissemination of these resistant bacteria.¹ The Bagmati river and its tributaries flowing through the Kathmandu valley have become important reservoirs of antibiotic-resistant bacteria (ARB).² Resistant bacteria are reported from multiple locations along the river³, yet the spatial connections between these sites and the factors driving their distribution remain unclear. Hence, this study was conducted to determine the presence of antimicrobial resistance *Escherichia coli* and its spatial relationship with dissolved oxygen level (DO) and biochemical oxygen

demand (BOD) physicochemical parameters in Bagmati river. These findings would support the development of site-specific, evidence-based control strategies to limit the spread of antibiotic-resistant organisms within the river system.

METHODS

A cross-sectional study was conducted along the Bagmati river, extending from Baghdwar to Chobar and covering a stretch of approximately 22.48 km from April to August 2025. A total of 15 sampling sites were stratified at equal intervals along the river using Google Maps to determine precise collection points. Ethical approval prior to sample collection was obtained from the Research Ethical Review Committee of the Institute

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The sample size was calculated using the formula $n = Z^2 p(1 - p) / e^2$, where n represents the total sample size. Taking the prevalence as 40% ($p = 0.4$), with a total population of 1, a 95% confidence interval, and a 5% margin of error, the calculated sample size was $n = 369$.³

Water samples were collected from locations near the predetermined stratified sites using sterile BOD bottles. Duplicate samples were obtained from each of the 15 sampling sites. Each sample was carefully labeled with the date, time, collection site, and the geographic coordinates of every location were recorded.

Microbiological and physicochemical analyses were carried out at the microbiology laboratory of Tri-Chandra Multiple Campus. Coliform counts were determined using the five-tube Most Probable Number (MPN) method. For each sample, a total of 15 tubes were used, including five tubes for double dilution and ten tubes for single dilution, inoculated with 10 mL, 1 mL, and 0.1 mL of water samples, respectively. *E. coli* was isolated using Eosin Methylene Blue (EMB) agar and incubated at 37°C and 44.5°C. Identification was based on colony morphology and biochemical characteristics.⁴

The isolated *E. coli* strains were tested against six categories of antibiotics: penicillin (ampicillin, 10 µg), macrolides (erythromycin, 15 µg), aminoglycosides (amikacin, 30 µg), fluoroquinolones (ciprofloxacin, 5 µg), cephalosporins (cefotaxime, 30 µg), and phenicols (chloramphenicol, 30 µg). Antimicrobial susceptibility testing (AST) was carried out using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. Standard *E. coli* ATCC 25922 was used for quality control throughout the analysis.⁵ The zones of inhibition were measured and interpreted following the Clinical and Laboratory Standards Institute (2020) guidelines.⁶

Additionally, Biological Oxygen Demand (BOD) and Dissolved Oxygen (DO) levels of the water samples were determined using the Winkler's titrimetric method.⁷

The data collected were first recorded in a laboratory logbook and later entered into Microsoft Excel for organization. The dataset was then coded and analyzed using SPSS version 25. A chi-square test was performed to assess the association between the antibiotic resistance patterns of *E. coli* isolated from different sampling sites. Correlation analysis was also carried out to examine the relationship between antibiotic-resistant *E. coli* and the

BOD and DO levels of the water. In addition, hierarchical cluster analysis for the categorization of sampling sites of Bagmati River on the basis of four variables, MPN count, *Escherichia coli*, biochemical oxygen demand (BOD) and dissolved oxygen (DO). These variables were employed to determine a Euclidean distance matrix, which measures similarity within sampling sites. Locations with minimal Euclidean distances were grouped collectively, while sites with more substantial Euclidean distances were distributed to different clusters. The cluster size was determined automatically using the hierarchical clustering algorithm, with no manual scoring or weighting of the variables. Factor analysis was used to illustrate the antimicrobial resistance (AMR) profile associated with six antibiotics: erythromycin, ampicillin, cefotaxime, ciprofloxacin, amikacin, and chloramphenicol. Cluster analysis, factor analysis biplot, and correlation analyses were performed using Exploratory software version 6.10.3.

RESULTS

The upstream section had relatively low coliform counts (<100 MPN/100 mL) compared to downstream section, reaching up to 26,500 MPN/100 mL. The fecal indicator bacterium *E. coli* was not detected at Site 1. Biological Oxygen Demand (BOD) values were low in the upstream region (1.2-2.1 mg/L) but increased markedly toward the downstream section, where values reached 418.75 mg/L. In contrast, dissolved oxygen (DO) levels gradually declined from 8.05 mg/L at the upper upstream to only 0.15 mg/L downstream (Table 1).

Clustering of the sampling sites based on MPN counts, presence of *E. coli*, and BOD and DO values revealed that the spatial distribution of Bagmati river could be categorized into four distinct clusters. The water sampling point at Baghdwar, the uppermost upstream site and downstream site 15 formed a distinct single cluster, separate from all other sampling points (Figure 1).

A total of 396 *E. coli* isolates from Bagmati river water were tested against six different classes of antibiotics. More than 90% of the isolates were resistant to ampicillin, erythromycin and cefotaxime. A significant difference in antibiotic resistance pattern of *E. coli* isolated from different sampling sites in Bagmati river was observed (Table 2).

The factor analysis biplot showed that the sampling sites formed five distinct clusters based on the antibiotic resistance pattern of *E. coli* isolates against the tested antibiotics. The principal components elucidated 68.5% of cumulative variations in the antibiotics resistance of

E. coli isolated from 14 sites of Bagmati river (Baghdwar to Chobar). The colored dots represent sampling sites (Figure 2).

Table 1. MPN count, BOD and DO measure in Bagmati river water samples.

Sites	Latitude	Longitude	MPN/100 mL	<i>E. coli</i>	BOD (mg/L)	DO (mg/L)
1	27° 49'26.76" N	85° 22'42.52" E	5	0	1.2	8.05
2	27° 48'39.25"N	85° 23'21.84"E	11	6	1.45	7.85
3	27° 46'53.19"N	85° 23'12.16"E	48	9	1.65	7.3
4	27° 46'9.27"N	85° 25'21.62"E	51	15	2.05	7.05
5	27° 45'26.92"N	85° 25'13.71"E	94	22	2.1	6.75
6	27° 45'17.12"N	85° 25'17.86"E	1150	20	51.8	2.35
7	27° 44'4.80"N	85° 24'1.69"E	1900	23	88.5	2.2
8	27° 42'58.70"N	85° 22'30.35"E	2150	32	151	1.9
9	27° 42'17.70"N	85° 20'58.79"E	2950	34	188.15	1.7
10	27° 40'54.30"N	85° 20'28.33"E	3000	35	208.5	1.55
11	27° 40'49.15"N	85° 19'48.88"E	3000	38	303.85	1.1
12	27° 41'5.35"N	85° 17'59.69"E	3950	38	335.35	0.85
13	27° 40'31.55"N	85° 17'43.56"E	5450	40	371.25	0.55
14	27° 39'47.24"N	85° 17'47.99"E	10200	42	401.35	0.3
15	27° 39'24.62"N	85° 17'36.14"E	26500	42	418.75	0.15

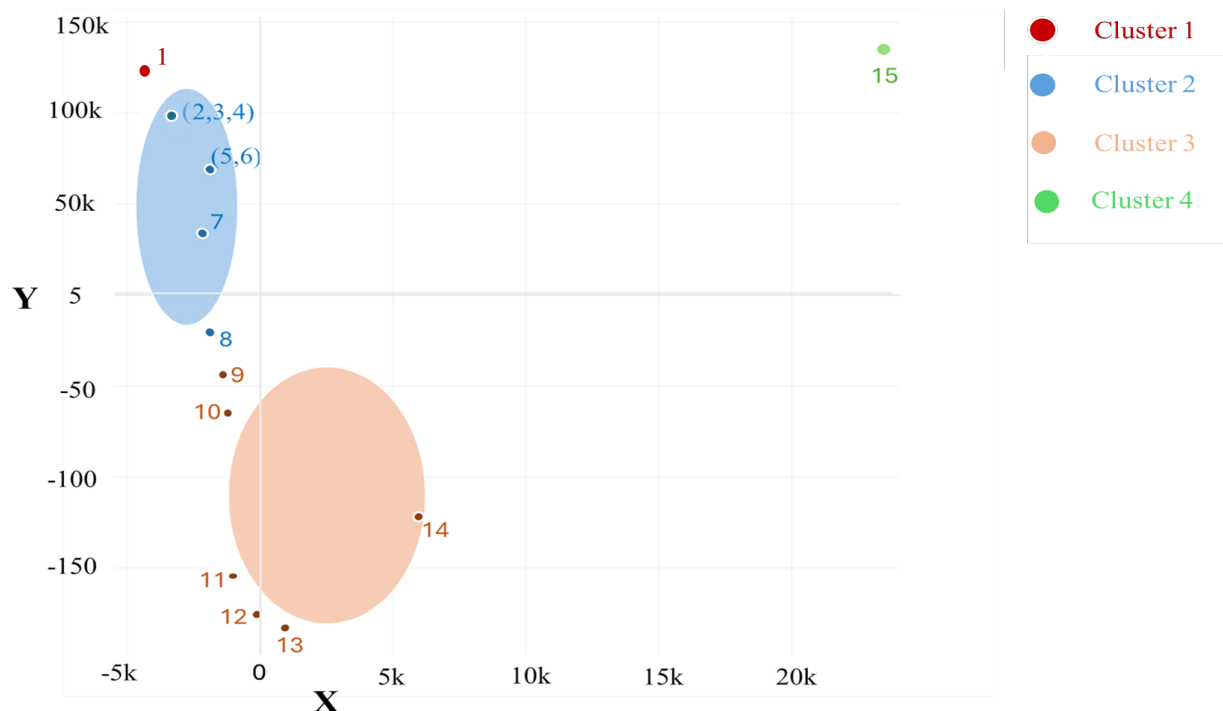
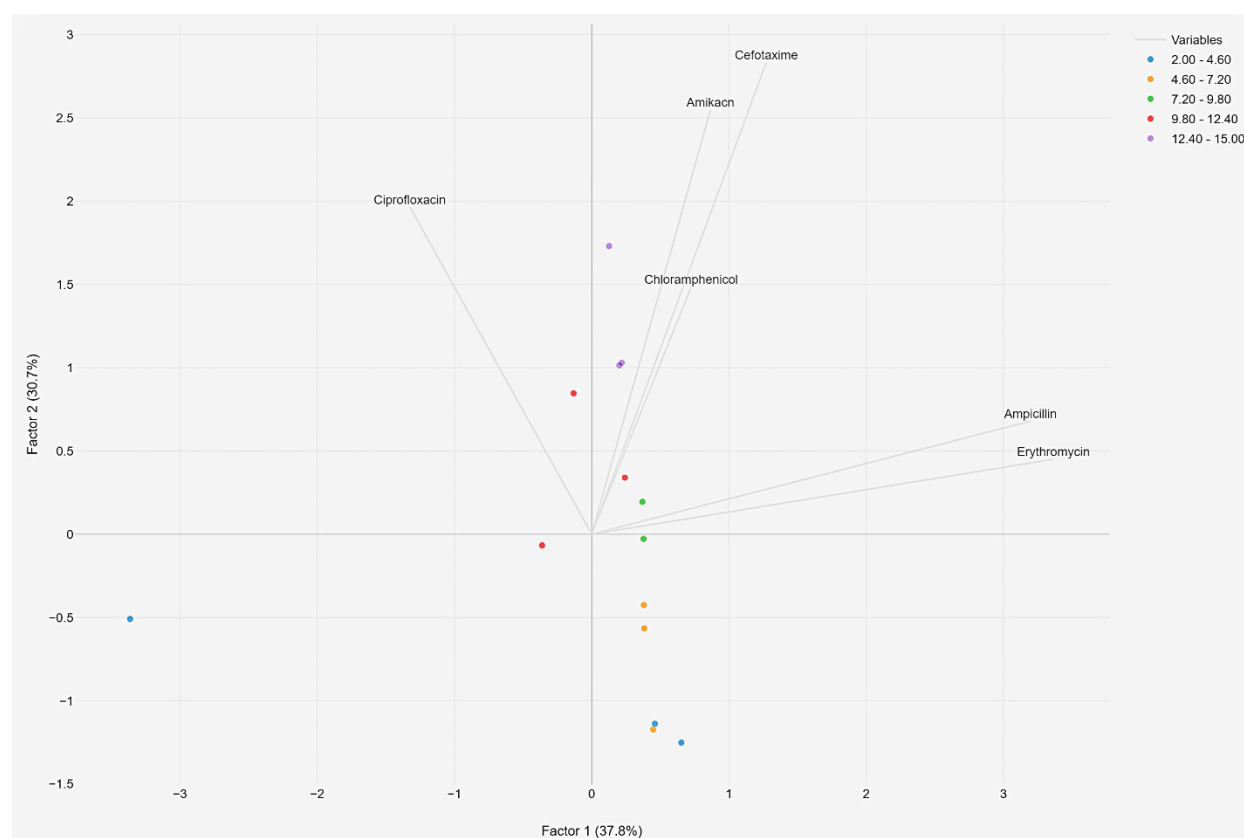


Figure 1. Cluster analysis of Bagmati river sampling locations based on MPN count, *E.coli* occurrence, BOD and DO.

Table 2. Antibiotic resistant status of *E. coli* in different sampling points.

Site	N	AK	AMP	E	C	CIP	CTX
% (n)							
2	4	25 (1)	25 (1)	25 (1)	0	75 (3)	4 (100)
3	9	33.3 (3)	88.9 (8)	100 (9)	0	22.2 (2)	9 (100)
4	17	29.4 (5)	100 (17)	100 (17)	11.8 (2)	23.5 (4)	16 (94.1)
5	22	40.9 (9)	100 (22)	100 (22)	4.5 (1)	40.9 (9)	21 (95.5)
6	20	35 (7)	100 (20)	100 (20)	60 (12)	30 (6)	18 (90)
7	23	39.1 (9)	100 (23)	100 (23)	30.4 (7)	30.4 (7)	23 (100)
8	32	40.6 (13)	100 (32)	100 (32)	22 (7)	71.9 (7)	26 (81.3)
9	34	41.2 (14)	100 (34)	100 (34)	32.4 (11)	85.3 (29)	29 (85.3)
10	35	68.6 (24)	100 (35)	100 (35)	40.1 (14)	60 (21)	26 (74.3)
11	30	60 (18)	100 (30)	90 (27)	70 (21)	80 (24)	24 (80)
12	38	60.5 (23)	100 (38)	94.7 (36)	68.4 (26)	73.7 (28)	38 (100)
13	40	60 (24)	100 (40)	100 (40)	62.5 (25)	75 (30)	40 (100)
14	42	64.3 (27)	100 (42)	100 (42)	52.4 (22)	69 (29)	38 (90.5)
15	50	62 (31)	100 (50)	100 (50)	32 (64)	54 (27)	49 (98)
Total	396	52.5 (208)	99 (392)	98 (388)	45.5 (180)	61.1 (242)	91.2 (361)
p value		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Figure 2. Factor analysis biplot showing antimicrobial resistance patterns among *E. coli* across different sampling sites.

Significant correlations were observed between antibiotic resistance patterns and water quality parameters. A strong positive correlation was found between *E. coli* counts and BOD values. Positive correlations were also observed between coliform counts and BOD, as well as between *E. coli* and coliform counts (Table 3).

Table 3. Correlation analysis of the variables (Amikacin, Ciprofloxacin, Chloramphenicol, Coliform count, *E. coli*, BOD, DO).

Variables		Correlation	p value
Amikacin	DO	-0.63	0.0117
Ciprofloxacin	DO	-0.63	0.0101
Chloramphenicol	DO	-0.86	0.3 e ⁻⁴
Chloramphenicol	BOD	0.66	0.0066
Amikacin	BOD	0.665	0.0067
Ciprofloxacin	BOD	0.63	0.0104
<i>E. coli</i>	BOD	0.9058	0.334 e ⁻⁵
Coliform count	BOD	0.716	0.026
<i>E. coli</i>	Coliform count	0.58	0.022
Coliform count	DO	-0.552	0.032
BOD	DO	-0.87	0.2 e ⁻⁵

DISCUSSION

Surface water bodies are continuously impacted by multiple sources of contamination, such as fecal matter, domestic and industrial waste, sewage discharge, pharmaceuticals, and hospital effluents.⁸ These polluted environments create favorable conditions for the emergence and spread of antimicrobial-resistant (AMR) bacteria.⁹ In addition, the nature and level of contamination at different sites strongly influence the variety of resistance patterns found within microbial communities.¹⁰ Conducting spatial characterization is therefore crucial for accurate risk assessment, designing targeted mitigation strategies, formulating evidence-based management policies, and identifying potential pathways of AMR transmission beyond clinical settings.¹¹

This study shows a distinct spatial decline in river water quality from upstream to downstream, as indicated by a substantial reduction in dissolved oxygen (DO) and gradually increases in MPN, *E. coli*, and BOD. With low fecal contamination (MPN 5-94/100 mL; *E. coli* 0-22/100 mL), low BOD (1.2-2.1 mg/L), and high DO (6.75-8.05 mg/L), upstream sites had comparatively good water quality, suggesting little anthropogenic influence and conditions conducive to aquatic life. On the contrary side, midstream sites displayed a substantial rise in BOD (51.8-208.5 mg/L) and microbial indicators (MPN

up to 3,000/100 mL; *E. coli* up to 35/100 mL), with a corresponding decrease in DO (1.55-2.35 mg/L). This abrupt change points to considerable inputs of organic waste and untreated or insufficiently treated domestic sewage, which increases the microbial load and oxygen demand.¹² Sewage releases substantial quantities of biodegradable organic matter, which contributes to elevated levels of fecal coliforms and biochemical oxygen demand (BOD).¹³ Having exceedingly high MPN values of up to 26,500 per 100 mL, elevated *E. coli* counts of 38 to 42 per 100 mL, excessive BOD levels (303.85-418.75 mg/L), and extremely depleted dissolved oxygen concentrations (0.15-1.1 mg/L), the downstream sites displayed the most extreme level of pollution. The declining water quality of the river can be attributed to the gradual build up of pollutants along its course, along with slower water flow, reduced turbulence, and a weakened natural ability to cleanse itself.¹⁴ As the Bagmati river flows within a relatively confined basin, there is limited scope for dilution or recovery, making natural restoration of the river increasingly difficult under these conditions.¹⁵

The cluster analysis grouped the study area into four distinct clusters, showing that human activities influence river water quality differently at different locations. This uneven distribution of pollution highlights the need to reassess how river sections are categorized in future studies. The isolated cluster represents the relatively clean upstream region, whereas the downstream clusters reflect the gradual accumulation of pollutants along the river course.

Factor analysis grouped the sampling sites into five clusters, indicating spatial variation in the antibiotic resistance patterns of *E. coli* along the river. The strong clustering of resistance to ampicillin and erythromycin suggests co-selection under long-term selective pressure, likely driven by widespread community use and similar environmental exposure.¹⁶ In contrast, resistance patterns associated with cefotaxime and ciprofloxacin point toward plasmid-mediated resistance and may be linked to the discharge of hospital and pharmaceutical waste into the river.¹⁷ The persistence of these resistance patterns also suggests possible contributions from diffuse environmental and agricultural sources, the horizontal transfer of resistance genes, and environmental reservoirs that support the survival and spread of resistant bacteria.¹⁸

Overall, the results suggest that antibiotic resistance in *E. coli* is only weakly affected by environmental conditions in the upstream section of the river, except

for commonly used antibiotics such as ampicillin and erythromycin. In contrast, the substantial variation in resistance observed in the midstream and downstream sections is mainly associated with increasing human activities and their uneven impacts along the river, making anthropogenic pressure the dominant factor shaping resistance patterns throughout the river system.¹⁹

The correlation analysis showed a strong relationship between organic pollution, microbial biomass, and antibiotic-resistant organisms in the Bagmati River. This close association likely arises from their common fecal sources and their similar response to increasing pollution along the river. As organic pollutants accumulate, they create favorable conditions for microbial growth and the selection of resistant bacteria.²⁰ These environmental conditions can influence both the abundance and spatial distribution of these microorganisms, contributing to the observed differences in their occurrence across various sections of the river. The negative correlation of resistance with DO to amikacin, ciprofloxacin and chloramphenicol indicates that antibiotic resistant *E. coli* was more often identified in river sections with lower DO levels. Low DO is a commonly used indicator of organic pollution, often caused by the discharge of untreated sewage and other anthropogenic wastes. Such conditions are associated with increased fecal contamination, the presence of antibiotic residues and other pollutants, which may lead to the persistence and spread of antibiotic-resistant bacteria. The results highlight an increased burden of antimicrobial resistance in the Bagmati River which is associated with declining water quality.

The pronounced spatial variation in antibiotic resistance along the river indicates that resistance patterns are shaped by local environmental conditions and the intensity of human activities.²¹ Inputs of antibiotic residues, resistant bacteria, and resistance genes from sources such as domestic sewage, hospital effluents, and urban discharges occur unevenly along the river, leading to distinct resistance profiles in different sections.²² Areas with higher pollution loads, characterized by elevated BOD, reduced dissolved oxygen, and increased fecal contamination, provide favorable conditions for the survival and spread of resistant microorganisms and the transfer of resistance genes.²³ The differences in resistance levels observed between the different water sections demonstrate that antimicrobial resistance is not uniformly distributed across the river system. These findings emphasize spatial investigations in guiding effective water management strategies aimed

at achieving the targets of NDC 3.0. By identifying antimicrobial resistance hotspots and providing insights into the environmental and human-related factors that contribute to resistance, such investigations can help inform targeted actions to limit the spread of antimicrobial resistance and improve the health of river ecosystems.

More precise generation of spatial distribution could have been accomplished by the direct detection of the antibiotic components, heavy metals and organic pollutants in the river and its role in development and persistence of the antibiotic-resistant bacteria.

CONCLUSIONS

The stretch of the Bagmati river from Baghdwar to Chobar can be broadly divided into upstream, midstream, and downstream sections based on physicochemical characteristics. However, when fecal coliform data were considered, the sampling sites formed four distinct clusters, and analysis of antimicrobial-resistant *E. coli* further divided them into five clusters. These findings show that physicochemical parameters alone do not fully capture the complexity of spatial variation in the river. Including microbiological indicators and antimicrobial resistance patterns provides a more comprehensive understanding of river health. Therefore, integrating spatial analysis of fecal contamination and resistance profiles is important for identifying impacted zones and developing targeted, site-specific strategies to improve water quality across the river system.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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