Prevalence of antibiotic-resistant *Escherichia coli* exceeds that of antibiotic-resistant coliforms in an urban river in Japan.

Kazuaki Matsui[1),2),3)*], Rahman MD Mizanur[2)], Tuguri Inui[1)], Mizuki Oda[1)], and Takeshi Miki[4),5)]

[1)] *Department of Civil and Environmental Engineering, Kindai University, 3-4-1 Kowakae, Higashiosaka, Osaka 577-8502, Japan.*

[2)] *Graduate School of Science and Engineering, Kindai University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502 Japan*

[3)] *Research Institute for Science and Technology, Kindai University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502 Japan*

[4)] *Faculty of Advanced Science and Technology, Ryukoku University, Otsu, Shiga, Japan*

[5)] *Center for Biodiversity Science, Ryukoku University, Shiga, Japan*

*Corresponding author: Kazuaki Matsui

e-mail: kmatsui@civileng.kindai.ac.jp

Abstract

Coliforms and *Escherichia coli* are widely used as fecal indicator bacteria (FIB) in water-quality assessments. However, the potential of these bacteria to act as reservoirs of antimicrobial resistance (AMR) in urban rivers remains insufficiently characterized. This study investigated the dynamics of coliforms and *E. coli* in the Hirano River, an urban river in Osaka, Japan. Sampling was conducted over a 1-month period, including during a combined sewer overflow (CSO) event, and samples were analyzed using CHROMagar™ ECC medium for enumeration and to test resistance to ampicillin and tetracycline. Both coliforms and *E. coli* counts increased by more than two orders of magnitude following CSO discharge, indicating that untreated sewage substantially contributed to fecal contamination. Despite the higher abundance of coliforms, *E. coli* exhibited markedly greater resistance ratios, emphasizing its key role in the dissemination of environmental AMR. These findings highlight the importance of *E. coli* as both an FIB and a reservoir of antibiotic resistance in urban river systems.

Keywords

Antimicrobial resistance, combined sewer overflow, *E. coli*, coliform, CHROMagar™ ECC,

Introduction

Waterborne pathogens are introduced into aquatic environments primarily through fecal contamination (Cui et al. 2019; Rochelle-Newall et al. 2015). To evaluate fecal contamination and assess water safety, culture-based detection of fecal indicator bacteria (FIB) has widely been used (Lange et al. 2013; McConn et al. 2024). Historically, the abundance of coliforms in aquatic environments has been considered an indicator of fecal contamination, but their reliability as proxies for pathogenic microorganisms is limited by environmental factors and non-fecal sources (Chavarria et al. 2024). Because some coliforms can be found naturally in soil and water, they are considered less-specific indicators of fecal pollution (Baudišová 1997). *Escherichia coli* is a coliform species whose only habitat is the intestine, and it survives only a short time outside the host. With the development of *E. coli*-specific media for aquatic samples, enumeration of *E. coli* has become the standard method for assessing fecal contamination, offering greater specificity and reliability than total coliform counts (Kemper et al. 2023).

It is important to detect and quantify *E. coli* and other fecal coliforms not only as indicators of human health risks posed by co-occurring enteric pathogens, including virulent coliform strains but also because of their role as carriers of antibiotic resistance genes (Nnadozie and Odume 2019). Pathogenic bacteria that have acquired antimicrobial resistance (AMR) continue to pose a significant threat to public health (Castaneda-Barba et al. 2024). Recognizing this, the World Health Organization (WHO) has emphasized the need to understand the environmental dynamics of AMR within the "One Health" framework. However, the behavior and distribution of antibiotic-resistant bacteria in aquatic environments remain poorly characterized (WHO 2021a).

Clinical surveillance data from Japan spanning 2014 to 2023 indicate that the annual prevalence of ampicillin resistance in *E. coli*, a β-lactam antibiotic is 49.2–52.6% (The AMR One Health Surveillance Committee 2024; JANIS 2024). Comparable resistance levels have been observed for other coliforms, with rates of 77.4–80.5% in *Klebsiella pneumoniae* and 79.0–82.9% in *Enterobacter cloacae*. These findings imply that environmental coliforms may serve as reservoirs of antibiotic resistance. The notably high resistance rates in clinical *K. pneumoniae* and *E. cloacae* underscore the importance of monitoring not only *E. coli* but also other coliforms as potential AMR reservoirs.

Although AMR is a growing global concern, research in aquatic environments has disproportionately focused on *E. coli*, with far fewer studies examining antibiotic-resistant coliforms more broadly. Characterizing resistance patterns among coliforms in river systems is essential to link clinical observations with environmental distributions, in line with the One Health framework.

In this study, we investigated the dynamics of *E. coli* and other coliforms in an urban river in Osaka, Japan. Using chromogenic agar media, we differentiated colonies of *E. coli* and coliforms while simultaneously assessing their AMR profiles. To establish baseline coliform counts, sampling was conducted continuously over a 1-month period. Additional sampling during a combined sewer overflow (CSO) event enabled evaluation of the impact of sewage discharge on both the abundance and resistance levels of *E. coli* and coliform populations.

Methods and Materials

Sampling was conducted in October and November 2023 at a bridge over the Hirano River (34.63530°N, 135.54944°E), an urban waterway that flows through a densely populated urban area of Osaka City, Japan. A CSO discharge outlet is located 100 m upstream of the sampling point. In 2018, approximately 8.08×10^6 m^3 of untreated wastewater was discharged from the outlet during 65 CSO events (Osaka Prefectural Government 2022). Meteorological data, including total precipitation, maximum intensity, and duration, were obtained from the Japan Meteorological Agency (https://www.jma.go.jp/jma/). On-site measurements of water temperature and pH were conducted using a pH meter (D-71, Horiba, Japan), while conductivity and dissolved oxygen (DO) levels were measured using a multiparameter meter (Multi 3420, WTW, Germany). River water samples were collected from a depth of 10–30 cm below the surface using a bucket attached to a rope. Sampling was performed at 11:00 a.m., and the collected samples were immediately stored in a cooler box with ice packs and transported to the laboratory. All experimental procedures were initiated within 6 h of sampling.

The abundance of *Escherichia coli* and total coliforms was quantified as colony-forming units (CFUs) using CHROMagar ECC™ chromogenic agar medium (Kanto Chemical, Japan), following ISO 9308–1:2014 guidelines (Anonymous 2014). Bacteria were captured on 0.45-μm pore-size mixed cellulose ester filters (Advantec, Japan), which then were placed directly onto agar plates and incubated at 37°C for 24 h. Colonies of *E. coli* and coliforms were distinguished by color (blue and mauve, respectively) according to the manufacturer's specifications (Fig. 1). This differentiation is based on enzymatic activity: β-glucuronidase in *E. coli* cleaves a chromogenic substrate to produce a blue/blue-green color, while β-galactosidase in coliforms cleaves a separate substrate to yield a pink/red color (Lange et al. 2013).

To ensure that colony counts fell within the quantifiable range of 20–200 CFUs per plate, a series of filtered volumes (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 250, and 500 mL) was used for each sample. For volumes of less than 10 mL, 10 mL phosphate-buffered saline (PBS) was added to the filtration funnel and mixed prior to filtration. Each sample was analyzed in triplicate, and the results were expressed as CFUs per 100 mL. Mean values from the three replicates were used to calculate antibiotic resistance ratios.

Statistical analyses were performed using R version 4.5.1. Differences in *E. coli* and coliform counts were assessed using a generalized linear mixed-effects model (GLMM) implemented via the lmer() function in the lme4 package, with bacterial type (*E. coli* or coliform) treated as a fixed effect and sampling date as a random intercept.

Results and Discussion

Figure 2 presents precipitation data and water quality parameters measured at the Hirano River during the sampling period. It rained on four separate occasions. Overall, water quality parameters remained relatively stable, except for a pronounced decline in conductivity and DO on November 11. Based on threshold values established in a previous study (Matsui and Miki 2023), the rainfall events on November 6 and 10 were classified as strong and likely triggered CSOs. Sampling on November 8 was conducted 33 h after rainfall, while the November 11 sampling occurred just 15 h post-rainfall. The conductivity and DO values observed on November 8 may reflect attenuation of CSO effects, whereas the lower values on November 11 likely indicate the inflow of water with distinct quality characteristics.

On November 11, *E. coli* and coliform counts in the river increased by more than 100-fold compared to November 8 (Fig. 3), implying a substantial influx of FIB associated with CSO discharge under low conductivity and DO conditions. Similar patterns have been reported elsewhere: in the Seine River, *E. coli* and intestinal enterococci concentrations increased by two orders of magnitude following CSO events (Passerat et al. 2011), and McGinnis et al. (2018) found significant correlations between human *Bacteroides*, total coliforms, and recent CSO and rainfall events.

During the initial phase of CSO events, total suspended solids, which contribute to electrical conductivity, typically increase due to sewage influx. However, as stormwater dilutes the sewage fraction, conductivity tends to decrease (Al Aukidy and Verlicchi 2017). The decline in conductivity observed on November 11 may therefore reflect this dilution process. Despite the reduced conductivity, *E. coli* and coliform counts were two orders of magnitude higher than during dry-weather conditions. As noted in previous studies, conductivity may not serve as a consistent indicator of FIB abundance during CSO events (Lenaker et al. 2023; Passerat et al. 2011).

Spearman correlation analysis revealed a negative correlation between conductivity and DO and *E. coli* abundance, while correlations with coliform abundance were weaker (Fig. 4). These results imply that the environmental drivers influencing *E. coli* and coliform dynamics differ, although their temporal patterns appeared similar (Fig. 3).

Both coliforms and *E. coli* were consistently detected in the river, even during dry weather. Except during the November 11 CSO event, coliform counts ranged between 3.0×10^3 and 2.0×10^4 CFU/100 mL, while *E. coli* counts ranged between 1.0×10^2 and 5.0×10^2 CFU/100 mL (Fig. 3). A GLMM applied to $\log_{10}$-transformed CFU data indicated that *E. coli* counts were statistically different from coliform counts (estimate = -1.58, 95% CI: -1.69 to -1.48, $p < 0.001$), being approximately 30 to 50 times lower on average. Thus, *E. coli* accounted for approximately 2–3% of the total coliforms in the Hirano River. Geomorphologically, the river belongs to the Yamato River system. Using the same CHROMagar ECC™, Uranishi et al. (2022) detected coliform levels ranging from 1.0×10^2 and 1.0×10^5 MPN/100 mL and *E. coli* levels from 1.0×10^1 and 2.0×10^3 MPN/100 mL at the Yamato River. The detection of similar coliforms and *E. coli* levels in our study implies that these bacteria may be indigenous to the Yamato River system.

Coliforms include various environmental species such as *Klebsiella*, *Enterobacter*, and *Citrobacter* (Leclerc et al. 2001), so their detection in river water is unsurprising. Although *E. coli* is emphasized in WHO guidelines on recreational water quality for its high specificity as an indicator (WHO 2021b), recent studies have documented its persistence and even naturalization in the environment independent of warm-blooded hosts (Jang et al. 2017;

Rumball et al. 2023). Reviews by Devane et al. (2020) and Korajkic et al. (2019) further explored the mechanisms underlying *E. coli* survival and adaptation in aquatic environments, although this aspect is beyond the scope of the present study.

Coliforms, particularly *E. coli*, play a central role in the acquisition, maintenance, and dissemination of antibiotic resistance genes (Anjum et al. 2021). High resistance ratios among other coliforms observed in clinical surveillance, such as *K. pneumoniae* and *E. cloacae*, imply that these species may also be key players in environmental AMR (JANIS 2024). Under the One Health framework, identifying major AMR carriers underscores the interconnectedness of human, animal, and environmental reservoirs. Therefore, to evaluate their contribution to environmental AMR, we assessed the prevalence of ampicillin and tetracycline resistance in coliforms and *E. coli* (Table 1). Three findings are noteworthy. First, ampicillin resistance was more prevalent than tetracycline resistance in both groups. A previous study in the Yasu and Hino rivers (Japan) similarly reported higher rates of ampicillin-resistant *E. coli* (average 25%) compared to tetracycline resistance (0–12%) (Ma et al. 2022), mirroring clinical trends in Japan (The AMR One Health Surveillance Committee 2024). In contrast, *E. coli* isolates from river and drinking water sources in Hangzhou, China, showed higher tetracycline resistance (42%) than ampicillin resistance (29%) (Chen et al. 2017), highlighting regional variation driven by antibiotic usage and environmental conditions.

Second, resistance ratios remained unchanged after the CSO discharge on November 11, despite bacterial counts increasing by more than two orders of magnitude. This implies that the resistance profile of the river water was similar to that of the CSO discharge. Untreated sewage typically contains large numbers of *E. coli* and serves as a major source of antibiotic-resistant strains in receiving waters (Lee et al. 2022; Sidrach-Cardona et al. 2014). While elevated levels of antibiotic resistance genes may reflect the influx of resistant bacteria following CSO input, our data did not indicate an unusually high prevalence of resistance in the CSO itself.

Third, *E. coli* exhibited significantly higher resistance ratios than other coliforms. Based on clinical surveillance, we anticipated high ampicillin and/or tetracycline resistance among environmental coliforms such as *K. pneumoniae* and *E. cloacae*, but this was not supported by our findings. Although *E. coli* accounted for only 2–3% of the coliform population in the Hirano River, its elevated resistance levels emphasize its importance in AMR monitoring. The observed ampicillin resistance ratio in *E. coli* (16.2–32.4%) was slightly lower than hospital-associated levels (50.8% in 2023) (JANIS 2024), but consistent with other environmental reports (Ma et al. 2022). These results imply that *E. coli* is a key contributor to environmental AMR and that increases in its abundance may facilitate further AMR in aquatic ecosystems.

Conclusion

CSO events increased both coliform and *E. coli* counts by more than 100-fold, indicating that untreated sewage inflow is a major source of fecal contamination in the Hirano River. The consistent detection of *E. coli* under dry-weather conditions implies chronic contamination. Although *E. coli* represented only 2–3% of total coliforms, it exhibited significantly higher antibiotic resistance ratios than other coliforms. This underscores its role not only as an indicator of fecal pollution but also as a key carrier of antibiotic resistance in the Hirano River. Environmental parameters influencing *E. coli* dynamics may differ from those affecting coliforms. While the route of chronic contamination remains unclear, identifying its sources and understanding *E. coli* behavior in urban rivers is critical for mitigating the spread of environmental AMR under the One Health framework.

Funding

This study was supported by JSPS KAKENHI Grant Numbers JP20H04348, JP24K03094, and the River Fund of The River Foundation, Japan.

Acknowledgments

We thank S. Morikawa, M. Komeda, and the staff of the Laboratory of Urban Microbial Ecology for assistance with field and laboratory work.

Conflicts of interest

There are no conflicts of interest to declare.

References

Al Aukidy M, Verlicchi P (2017) Contributions of combined sewer overflows and treated effluents to the bacterial load released into a coastal area. Sci Total Environ 607-608:483-496. https://doi.org/10.1016/j.scitotenv.2017.07.050

Anjum MF, Schmitt H, Börjesson S, Berendonk TU, Stehling EG, Boerlin P, Topp E, Jardine C, Li XW, Li B, Dolejska M, Madec JY, Dagot C, Guenther S, Walsh F, Villa L, Veldman K, Sunde M, Krzeminski P, Wasyl D, Popowska M, Järhult J, Örn S, Mahjoub O, Mansour W, Thái DN, Elving J, Pedersen K, Network W (2021) The potential of using *E. coli* as an indicator for the surveillance of antimicrobial resistance (AMR) in the environment. Curr Opin Microbiol 64:152-158. https://doi.org/10.1016/j.mib.2021.09.011

Anonymous (2014) ISO 9308-1:2014 Water quality – Enumeration of *Escherichia coli* and coliform bacteria – Part 1: Membrane filtration method for waters with low bacterial background flora.

Baudišová D (1997) Evaluation of *Escherichia coli* as the main indicator of faecal pollution. Water Sci Technol 35:333-336. https://doi.org/10.2166/wst.1997.0755

Castaneda-Barba S, Top EM, Stalder T (2024) Plasmids, a molecular cornerstone of antimicrobial resistance in the One Health era. Nat Rev Microbiol 22:18-32. https://doi.org/10.1038/s41579-023-00926-x

Chavarria K, Batista J, Saltonstall K (2024) Widespread occurrence of fecal indicator bacteria in oligotrophic tropical streams. Are common culture-based coliform tests appropriate? PeerJ 12:e18007. https://doi.org/10.7717/peerj.18007

Chen Z, Yu D, He S, Ye H, Zhang L, Wen Y, Zhang W, Shu L, Chen S (2017) Prevalence of antibiotic-resistant *Escherichia coli* in drinking water sources in hangzhou city. Front

Microbiol 8:1133. https://doi.org/10.3389/fmicb.2017.01133

Cui Q, Huang Y, Wang H, Fang T (2019) Diversity and abundance of bacterial pathogens in urban rivers impacted by domestic sewage. Environ Pollut 249:24-35. https://doi.org/10.1016/j.envpol.2019.02.094

Devane ML, Moriarty E, Weaver L, Cookson A, Gilpin B (2020) Fecal indicator bacteria from environmental sources; strategies for identification to improve water quality monitoring. Water Res 185. https://doi.org/10.1016/j.watres.2020.116204

JANIS (Japan nosocomial infections surveillance) (2024) JANIS Open Report. https://janis.mhlw.go.jp/english/report/index.html#

Jang J, Hur HG, Sadowsky MJ, Byappanahalli MN, Yan T, Ishii S (2017) Environmental *Escherichia coli*: ecology and public health implicationsa review. J Appl Microbiol 123:570-581. https://doi.org/10.1111/jam.13468

Kemper MA, Veenman C, Blaak H, Schets FM (2023) A membrane filtration method for the enumeration of *Escherichia coli* in bathing water and other waters with high levels of background bacteria. J Water Health 21:995-1003. https://doi.org/10.2166/wh.2023.004

Korajkic A, Wanjugi P, Brooks L, Cao Y, Harwood Valerie J (2019) Persistence and decay of fecal microbiota in aquatic habitats. Microbiol Mol Biol Rev 83:10.1128/mmbr.00005-00019. https://doi.org/10.1128/mmbr.00005-19

Lange B, Strathmann M, Oßmer R (2013) Performance validation of chromogenic coliform agar for the enumeration of *Escherichia coli* and coliform bacteria. Lett Appl Microbiol 57:547-553. https://doi.org/10.1111/lam.12147

Leclerc H, Mossel DAA, Edberg SC, Struijk CB (2001) Advances in the bacteriology of the coliform group: Their suitability as markers of microbial water safety. Annu Rev Microbiol 55:201-234. https://doi.org/10.1146/annurev.micro.55.1.201

Lee J, Beck K, Burgmann H (2022) Wastewater bypass is a major temporary point-source of antibiotic resistance genes and multi-resistance risk factors in a Swiss river. Water Res 208:117827. https://doi.org/10.1016/j.watres.2021.117827

Lenaker PL, Corsi SR, De Cicco LA, Olds HT, Dila DK, Danz ME, McLellan SL, Rutter TD

(2023) Modeled predictions of human-associated and fecal-indicator bacteria concentrations and loadings in the Menomonee River, Wisconsin using in-situ optical sensors. PLoS One 18:e0286851. https://doi.org/10.1371/journal.pone.0286851

Ma CY, Sugie Y, Yu Z, Okuno Y, Tanaka H, Ihara M (2022) Occurrence of *E. coli* and antibiotic-resistant *E. coli* in the southern watershed of Lake Biwa, including in wastewater treatment plant effluent and inflow rivers. Chemosphere 301:134372. https://doi.org/10.1016/j.chemosphere.2022.134372

Matsui K, Miki T (2023) Microbial community composition and function in an urban waterway with combined sewer overflows before and after implementation of a stormwater storage pipe. PeerJ 11:e14684. https://doi.org/10.7717/peerj.14684

McConn BR, Kraft AL, Durso LM, Ibekwe AM, Frye JG, Wells JE, Tobey EM, Ritchie S, Williams CF, Cook KL, Sharma M (2024) An analysis of culture-based methods used for the detection and isolation of *Salmonella* spp., *Escherichia coli*, and *Enterococcus* spp. from surface water: A systematic review. Sci Total Environ 927:172190. https://doi.org/10.1016/j.scitotenv.2024.172190

McGinnis S, Spencer S, Firnstahl A, Stokdyk J, Borchardt M, McCarthy DT, Murphy HM (2018) Human Bacteroides and total coliforms as indicators of recent combined sewer overflows and rain events in urban creeks. Sci Total Environ 630:967-976. https://doi.org/10.1016/j.scitotenv.2018.02.108

Nnadozie CF, Odume ON (2019) Freshwater environments as reservoirs of antibiotic resistant bacteria and their role in the dissemination of antibiotic resistance genes. Environ Pollut 254:113067. https://doi.org/10.1016/j.envpol.2019.113067

Osaka Prefectural Government (2022). 2022 Neyagawa basin bottom improvement measures study group (in Japanese). Osaka Prefectural Government official website. ; https://www.pref.osaka.lg.jp/o130110/kasenkankyo/teishitsushingikai-1/r4teishitubukai.html Accessed 8 October 2025

Passerat J, Ouattara NK, Mouchel JM, Rocher V, Servais P (2011) Impact of an intense combined sewer overflow event on the microbiological water quality of the Seine River. Water Res 45:893-903. https://doi.org/10.1016/j.watres.2010.09.024

Rochelle-Newall E, Nguyen TMH, Le TPQ, Sengteheuanghoung O, Ribolzi O (2015) A short review of fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions. Front Microbiol 6. https://doi.org/10.3389/fmicb.2015.DD3D8

Rumball NA, Alm EW, McLellan SL (2023) Genetic determinants of *Escherichia coli* survival in beach sand. Appl Environ Microbiol 89. https://doi.org/10.1128/aem.01423-22

Sidrach-Cardona R, Hijosa-Valsero M, Marti E, Balcazar JL, Becares E (2014) Prevalence of antibiotic-resistant fecal bacteria in a river impacted by both an antibiotic production plant and urban treated discharges. Sci Total Environ 488-489: 220-227. https://doi.org/10.1016/j.scitotenv.2014.04.100

The AMR One Health Surveillance Committee (2024) Nippon AMR One Health Report (NAOR) 2024. Tokyo: Division of Infectious Diseases Prevention and Control, Department of Infectious Disease Prevention and Control, Public Health Bureau, Ministry of Health, Labour and Welfare. https://www.mhlw.go.jp/content/10900000/001477833.pdf

Uranishi K, Uranishi Y, Tsujimoto M, Shiroyama J (2022) Comparison of quantification methods and study on *Escherichia coli* and coliforms in Yamato River. J Jpn Soc Water Environ 45:29-39 (in Japanese). https://doi.org/10.2965/jswe.45.29

WHO (World Health Organization) (2021a) Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report: 2021. WHO: https://www.who.int/publications-detail-redirect/9789240027336

WHO (World Health Organization) (2021b) Guidelines on recreational water quality: Volume 1 Coastal and fresh waters. WHO: https://www.who.int/publications/i/item/9789240031302

Figure legends

Fig. 1. Coliforms (mauve) and *E. coli* (blue) colonies on a membrane filter, as visualized using CHROMagar ECC^{TM}. Nontarget organisms formed colorless colonies, were easily distinguishable, and were excluded from analysis in this study.

Fig. 2. Temporal variation in daily rain and (a) water quality; (b) pH, (b) water temperature, (c) conductivity, and (d) dissolved oxygen at the Hirano River sampling site.

Fig. 3. Temporal variation in colony formation units (CFUs) of coliforms (mauve) and *E. coli* (blue) at the Hirano River sampling site.

Fig. 4. Spearman correlation matrix showing relationships between environmental parameters and bacterial indicators. Positive correlations are shown in red, negative ones are shown in blue, with color intensity proportional to correlation strength. Only the lower triangle of the matrix is displayed for clarity.

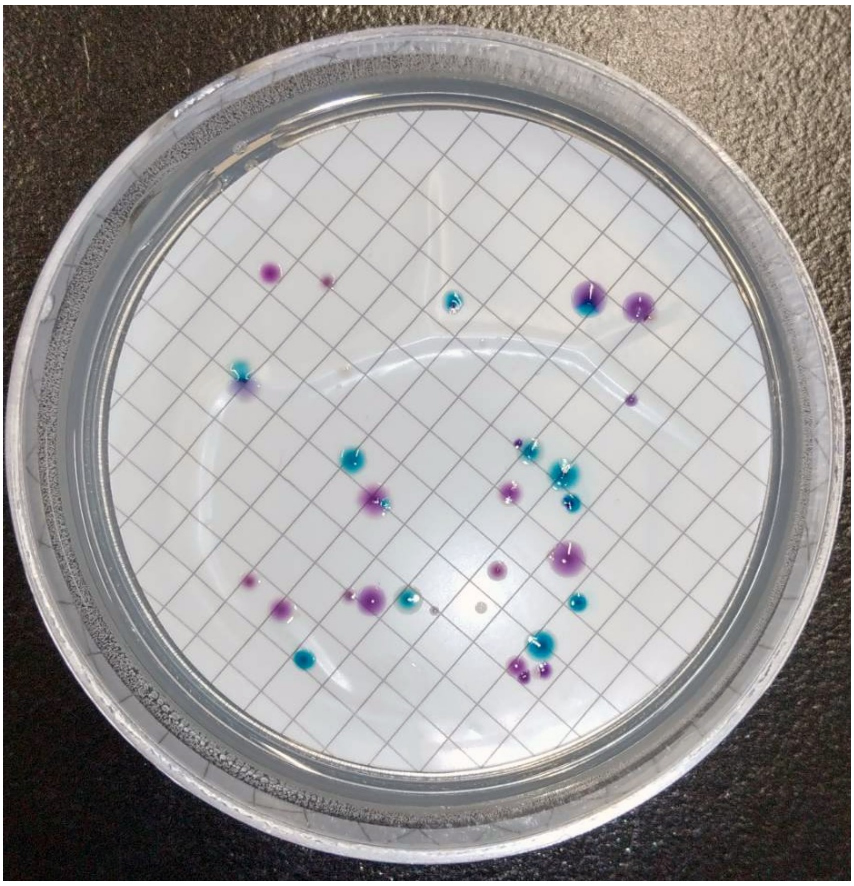
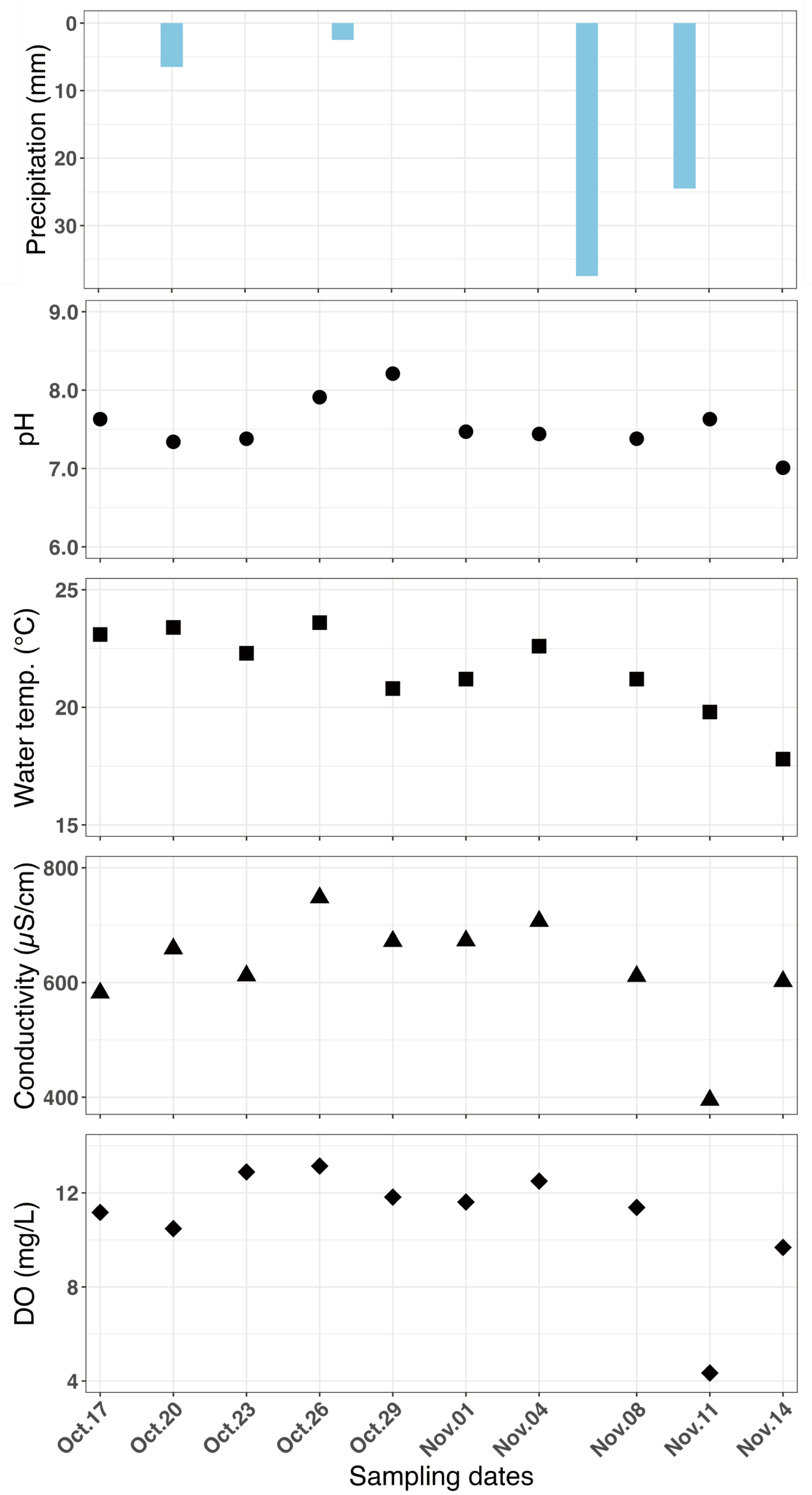


Fig. 1



Precipitation (mm)
0
10
20
30
pH
9.0
8.0
7.0
6.0
Water temp. (°C)
25
20
15
Conductivity (µS/cm)
800
600
400
DO (mg/L)
12
8
4
Oct.17
Oct.20
Oct.23
Oct.26
Oct.29
Nov.01
Nov.04
Nov.08
Nov.11
Nov.14
Sampling dates

Fig. 2

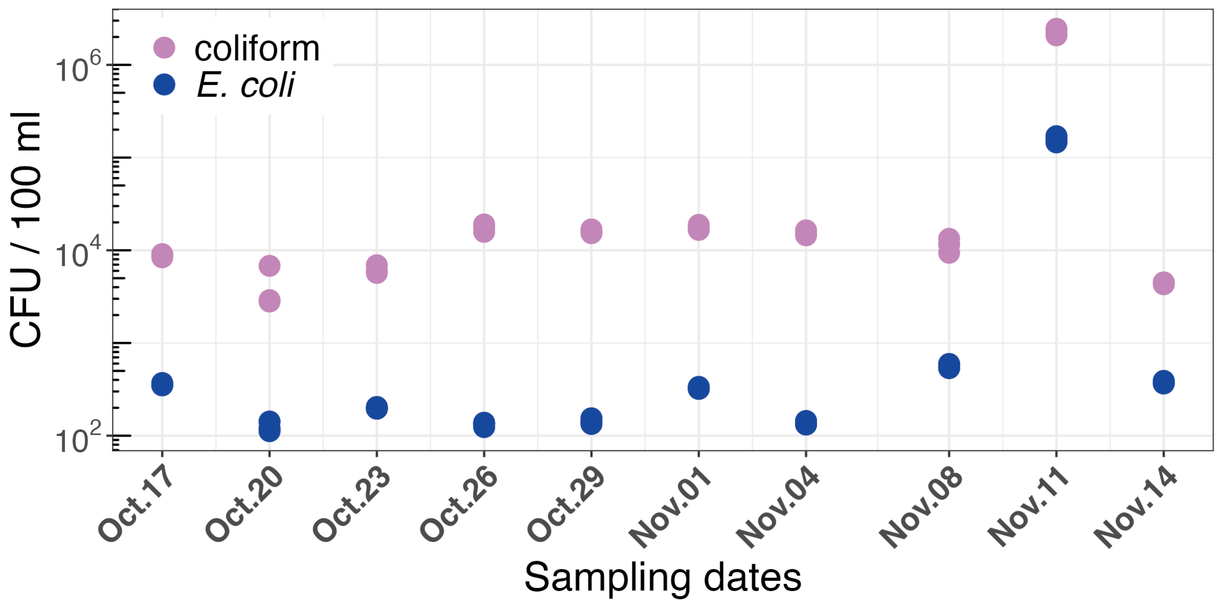

coliform
E. coli
CFU / 100 ml
10^6
10^4
10^2
Oct.17
Oct.20
Oct.23
Oct.26
Oct.29
Nov.01
Nov.04
Nov.08
Nov.11
Nov.14
Sampling dates

Fig. 3

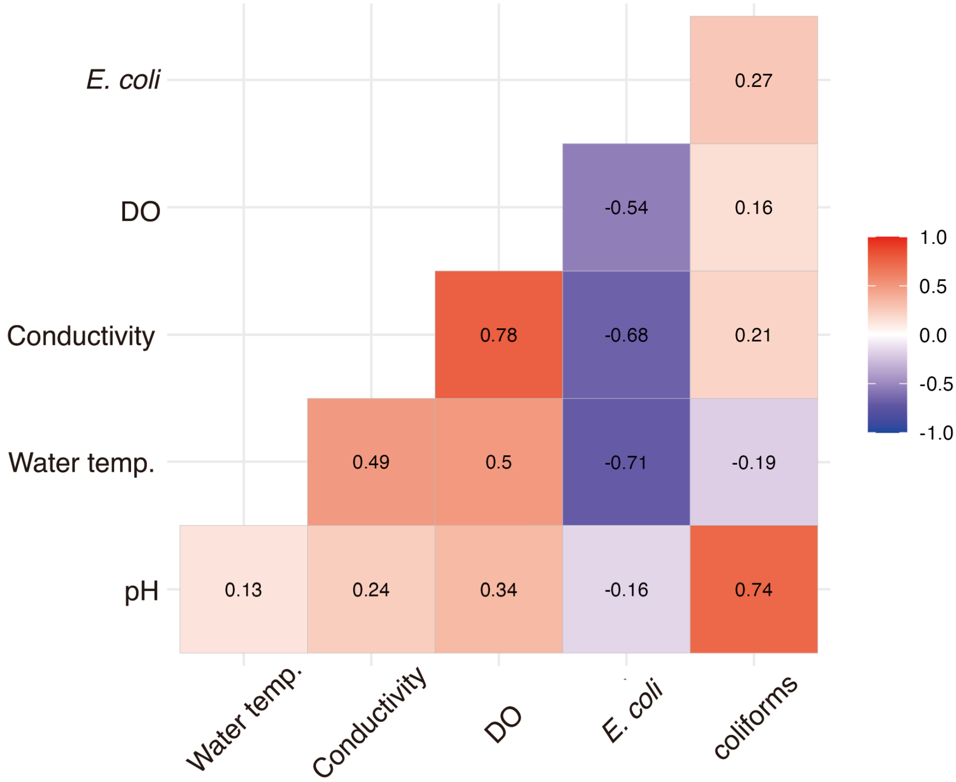

E. coli
DO
Conductivity
Water temp.
pH
Water temp.
Conductivity
DO
E. coli
coliforms
0.27
-0.54
0.16
0.78
-0.68
0.21
0.49
0.5
-0.71
-0.19
0.13
0.24
0.34
-0.16
0.74
1.0
0.5
0.0
-0.5
-1.0

Fig. 4

Table 1. Antibiotic resistance rates of coliforms and *E. coli*

Sampling date	Ampicillin		Tetracycline	
	coliforms (%)	*E. coli* (%)	coliforms (%)	*E. coli* (%)
2023/10/17	3.1	18.8	0.9	9.1
2023/10/20	6.6	18.7	1.2	12.7
2023/10/23	4.2	27.4	0.6	13.9
2023/10/26	1.2	16.2	0.3	10.8
2023/10/29	0.7	14.4	0.2	14.0
2023/11/1	1.5	32.4	0.3	10.7
2023/11/4	1.8	28.0	0.2	12.3
2023/11/8	3.0	22.4	1.7	13.0
2023/11/11	7.6	28.2	0.5	9.1
2023/11/14	7.2	24.5	1.8	17.9

Table 1