

Research Article

Antibiotic resistance profile of *Escherichia coli* from Marikina River in the Philippines: Environmental and public health implications

Aaron Jan Palmares*Department of Medical Technology, Institute of Health Sciences and Nursing,
Far Eastern University, Nicanor Reyes St., Manila, 1015, Philippines**Kim Joshua Tacsagon**Department of Medical Technology, Institute of Health Sciences and Nursing,
Far Eastern University, Nicanor Reyes St., Manila, 1015, Philippines**Francesca Clare Taopo**Department of Medical Technology, Institute of Health Sciences and Nursing,
Far Eastern University, Nicanor Reyes St., Manila, 1015, Philippines**Xhaira Taroma**Department of Medical Technology, Institute of Health Sciences and Nursing,
Far Eastern University, Nicanor Reyes St., Manila, 1015, Philippines**Sheena Toledo**Department of Medical Technology, Institute of Health Sciences and Nursing,
Far Eastern University, Nicanor Reyes St., Manila, 1015, Philippines**Ivy Sophia Torres**Department of Medical Technology, Institute of Health Sciences and Nursing,
Far Eastern University, Nicanor Reyes St., Manila, 1015, Philippines**Jamaica Red Ventura**Department of Medical Technology, Institute of Health Sciences and Nursing,
Far Eastern University, Nicanor Reyes St., Manila, 1015, Philippines**Angela Marie Villanueva**Department of Medical Technology, Institute of Health Sciences and Nursing,
Far Eastern University, Nicanor Reyes St., Manila, 1015, Philippines**Leslie Gayle Yamat**Department of Medical Technology, Institute of Health Sciences and Nursing,
Far Eastern University, Nicanor Reyes St., Manila, 1015, Philippines**Francisco Gellecanao**Department of Medical Technology, Institute of Health Sciences and Nursing,
Far Eastern University, Nicanor Reyes St., Manila, 1015, Philippines

*Corresponding author. E-mail: apalmares@feu.edu.ph

Article Info<https://doi.org/10.31018/jans.v17i2.6552>

Received: January 18, 2025

Revised: May 12, 2025

Accepted: May 17, 2025

How to CitePalmares, A. J. *et al.* (2025). Antibiotic resistance profile of *Escherichia coli* from Marikina River in the Philippines: Environmental and public health implications. *Journal of Applied and Natural Science*, 17(2), 614 - 621. <https://doi.org/10.31018/jans.v17i2.6552>**Abstract**

The Marikina River in the Philippines is faced with problems caused by urbanization through the indiscriminate discharge of agricultural and municipal effluents, thus making the river potentially take part in the transmission of antimicrobial resistance (AR). This study, therefore, focused on determining the concentration and AR profile of *Escherichia coli* isolated from surface waters across various river sites. The results showed that the concentration of *E. coli* from all six sampling sites, including one within a Philippine-protected landscape, has far exceeded the upper microbial limit for all water body classifications designated for beneficial use, including Class D water (400 CFU/100 mL). The estimated average *E. coli* and coliform concentrations from all sampling sites were 1.9×10^5 CFU/100 mL and 12.0×10^5 CFU/100 mL. Even in a protected area, average concentrations of *E. coli* (1.5×10^5 CFU/100 mL) and coliforms (5.6×10^5 CFU/100 mL) were far above the Class A water limit of 100 CFU/100 mL. Moreover, 18.8% and 22.9% of the isolates were classified as multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL) producers. Some isolates from the most upstream sites exhibited resistance to third-generation cephalosporins. These findings underscore the need for further surveillance, particularly in upstream areas, to better understand how human activities are contributing to the progression of AR in the river. Such studies are essential to keep authorities and policy-makers informed and to guide more effective management and remediation efforts.

Keywords: Antimicrobial resistance, Coliform, *Escherichia coli*, Marikina river

INTRODUCTION

When Wawa Dam was constructed on the upper Marikina River, it became Metro Manila's primary water source from 1909 until its function was supplanted by Angat Dam in 1970. Despite being replaced, Wawa Dam still retains the capacity to supply up to 50 million liters of water per day, a volume that could help alleviate the chronic water shortages affecting Metro Manila and nearby provinces (Berkman International Inc., 2015; Santillan *et al.*, 2013). This potential, however, is tempered by the possible contamination of the dam's water supply with antibiotic-resistant (AR) fecal bacteria from surface runoff of nearby human settlements and small-scale livestock farming (Alawi *et al.*, 2022; Ghosh & Panigrahi, 2023; Iloba *et al.*, 2021; Larsson & Flach, 2022). If Wawa Dam were to be reinstated as a source of potable water for Metro Manila, the risk of AR bacteria reaching water treatment facilities could increase, posing a serious public health risk. These bacteria may survive standard treatment processes, potentially leading to infections that prove difficult to treat with conventional antibiotics (Dela Peña *et al.*, 2022; Hasan & Shakir, 2025; Kundan & Slathia, 2018; Yoneda *et al.*, 2022). Given the growing threat of AR bacteria, surveillance programs like the Philippines' Antimicrobial Resistance Surveillance Program (ARSP) have been established to monitor resistance trends in human pathogens (ARSP, 2023, 2024). However, these programs predominantly focus on bacteria isolated from clinical settings, leaving a gap in knowledge about the current AR profiles of bacteria in water bodies (Cho *et al.*, 2010; Palmares *et al.*, 2024). To address this gap, the present study aimed to investigate the concentration of total coliforms and the AR profiles of *Escherichia coli* (*E. coli*)—a key fecal indicator—at various sites along the Marikina River, including the Wawa Dam. This research aimed to provide necessary information to private water concessionaires and concerned government agencies to possibly implement mitigation measures, especially for water bodies designated for multiple beneficial uses.

MATERIALS AND METHODS

Sample collection

The Marikina River, approximately 31 km long, is situated east of the National Capital Region (Metro Manila). Six sampling sites located beneath bridges along the river were selected for this study. These included three upstream sites (U1, U2, and U3) within Rizal Province, outside of Metro Manila, and three downstream sites (D1, D2, and D3) located within Metro Manila, as shown in Fig. 1. The distance between the most upstream and the most downstream collection sites is 30 kilometers. The non-point sources along the river were targeted for

sampling, and site coordinates were approximated using Google Maps (Fig. 1). At each of the six locations, four grab water samples (50 mL each) were collected at depths ranging from 1 to 15 cm, resulting in 24 samples per batch. The first batch of samples was collected during the first week of February 2024, and the second batch in the last week of March 2024, for a total of 48 samples. All water samples were collected in sterile conical tubes, immediately placed on ice, and transported for processing within 4 hours of collection.

Microbial quality testing

Each water sample underwent selective isolation of *E. coli* and other coliforms using 3M Petrifilm *E. coli*/Coliform Count (EC) Plates (3M Microbiology, St. Paul, Minnesota), following the manufacturer's instructions. A 1-mL aliquot of a 20:1000 dilution (prepared with sterile 0.9% saline solution) was dispensed onto the media. The EC plates were incubated at $37\pm0.1^{\circ}\text{C}$ for 48 ± 2 hours, with a maximum stack of four plates. After incubation, colonies were counted using a colony counter. Blue colony-forming units (CFUs) associated with gas bubbles were considered presumptive *E. coli*, while both blue and red CFUs with gas bubbles were counted as total coliforms (Azuma *et al.*, 2022). To calculate CFUs per mL, colony counts were multiplied by the dilution factor of 50. For the final CFU count per 100 mL, the results were further multiplied by 100 (Z. Chen *et al.*, 2017).

Purification of presumptive *Escherichia coli* colonies

Representative blue colonies with gas bubbles that grew in EC plates were selected for further identification and antimicrobial susceptibility testing (AST). By isolation streaking, four to five presumptive *E. coli* colonies were subcultured onto Eosin Methylene Blue (EMB) agar (HiMedia Laboratories, Mumbai, India). The EMB agar was then incubated at $37\pm0.5^{\circ}\text{C}$ for 24 hours. On EMB agar, presumptive *E. coli* colonies appeared purple, with or without a green metallic sheen. By isolation and streaking, four to five well-isolated colonies displaying the characteristic green metallic sheen were then subcultured onto MacConkey (MAC) agar (HiMedia Laboratories, Mumbai, India). The MAC agar was then incubated at $37\pm0.5^{\circ}\text{C}$ for 18 hrs. On MAC agar, presumptive colonies of *E. coli* appeared as pink colonies with or without a pink halo (Palmares and De los Reyes, 2016).

Identification and antimicrobial susceptibility testing

Bacterial suspensions were prepared from presumptive *E. coli* colonies on MAC agar by emulsifying the colonies in 0.5% sodium chloride. Using the VITEK DensiCHEK™ (bioMérieux), the turbidity of the bacterial suspension was adjusted to match the 0.55 McFarland

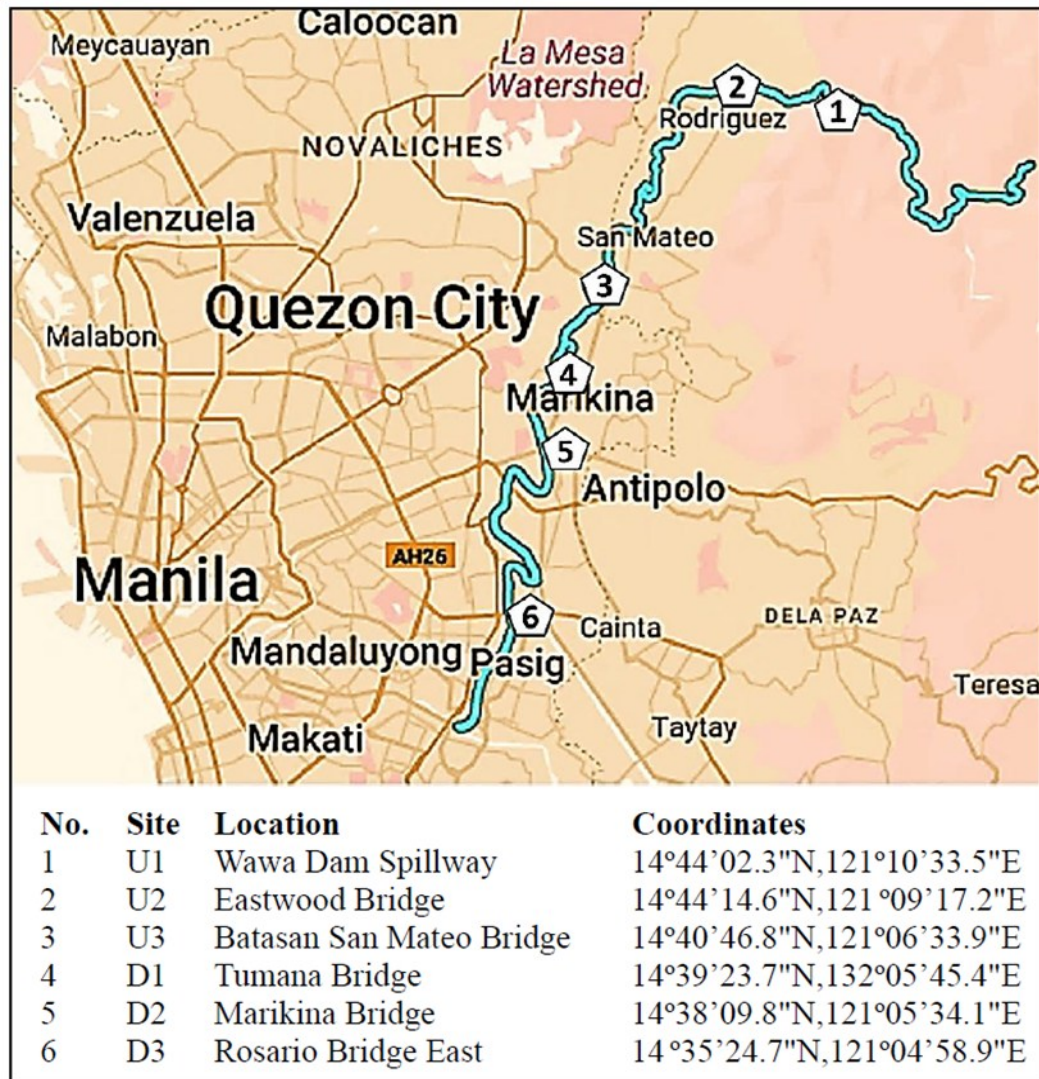


Fig. 1. Schematic Diagram of Sampling Points, Collection Sites, and Coordinates. U, upstream, D, downstream

standard. Afterwards, the bacterial suspension and the VITEK 2 ID-N261 card were placed into the VITEK 2 system (bioMérieux, Durham, NC, USA) for the identification of *E. coli*. Antibiotic susceptibility testing (AST) was then performed by VITEK 2 using the AST-N261 card with software version 9.03.3, according to the manufacturer's instructions. The VITEK 2 ESBL (Extended-Spectrum Beta-Lactamase) test was included on the AST-N261 card for *E. coli*. Isolates were tested with seventeen antibiotics covering seven different antimicrobial classes or subclasses. This included the following: a. β -lactams such as: i.) Penicillin's [amoxicillin/clavulanic acid (AMC), ampicillin (AMP), and piperacillin/tazobactam (TZP)], ii.) Cephalosporins [cefepime (FEP), cefoxitin (FOX), ceftazidime (CAZ), ceftriaxone (CRO), cefuroxime (CXM), and cefuroxime axetil (CXMA)] and, iii.) Carbapenems [(ertapenem (ETP), imipenem (IMP), and meropenem (MEM)]; b. aminoglycosides such as amikacin (AMK) and gentamicin (GEN); c. quinolones such as ciprofloxacin (CIP); d. polymyxin such as colistin (CST), and e. sulfonamides

such as cotrimoxazole (SXT). The isolates were then classified as resistant, intermediate, or sensitive based on their minimum inhibitory concentrations (MIC's) following the CLSI (Clinical and Laboratory Standards Institute) guidelines (CLSI, 2021). In addition, a Sensititre™ plate (Sensititre™, Thermo Fisher, Dardilly, France) was used. AST was further determined for additional five antibiotics covering penicillin [ampicillin/sulbactam (SAM)], cephalosporins [cefotaxime (CTX), ceftazidime/avibactam (CZA)], and carbapenems [imipenem/relebactam (IMIREL), and meropenem/vaborbactam (MEMV)] for a total of twenty-two antibiotics. The results were interpreted according to the EUCAST guidelines (Bonnin *et al.*, 2022; Thermo Fisher Scientific, 2018).

Calculation of multiple antibiotic resistance indices

The multiple antibiotic resistance (MAR) index of *E. coli* was then calculated using the formula: MAR index = a/b , where 'a' refers to the number of antibiotics to which *E. coli* showed resistance, and 'b' refers to the total

Table 1. Concentration of *Escherichia coli* and coliforms according to the site of collection

Site	<i>E. coli</i> CFU/100 mL	Coliform CFU/100 mL
	Mean $\pm$ SD (10^4)	Mean $\pm$ SD (10^5)
U1	0.2 $\pm$ 0.1	0.1 $\pm$ 0.01
U2	15.9 $\pm$ 3.3	6.6 $\pm$ 3.4
U3	32.1 $\pm$ 0.7	15.9 $\pm$ 1.6
D1	18.9 $\pm$ 6.0	12.6 $\pm$ 0.7
D2	17.3 $\pm$ 1.3	16.9 $\pm$ 1.9
D3	28.4 $\pm$ 1.5	19.7 $\pm$ 1.1
<i>p</i> value	<0.001	<0.001
U	16.1 $\pm$ 13.5	7.5 $\pm$ 6.9
D	21.5 $\pm$ 5.4	16.4 $\pm$ 3.3
<i>p</i> value	0.069	<0.001

CFU, colony forming units; D, downstream; NS, not significant; SD, standard deviation; U, upstream

number of antibiotics to which the *E. coli* was tested (Titilawo *et al.*, 2015). In addition, the isolates were further classified according to their AR levels, such as antibiotic-resistant (AR, if resistant to 1 or 2 antimicrobials); multiple antibiotic-resistant (MAR, if resistant to ≥ 3 antimicrobials), and multidrug-resistant (MDR, if resistant to at least one antimicrobial belonging to ≥ 3 different classes/subclasses). They were also further determined if they were an ESBL producer (ARSP, 2023; Ham *et al.*, 2012).

Data analysis

The association between collection sites and the isolation frequencies of ESBL, MAR, and MDR *E. coli* and the differences in *E. coli* and coliform concentrations among the sites were analyzed using SPSS version 28.0 (SPSS Inc., Chicago, IL, USA). Fisher's exact test was employed for categorical data, while T-Test or ANOVA was used to compare group means. If ANOVA indicated significant differences, Tukey's post-hoc test was performed for further comparison. A *p*-value of ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Coliform and *Escherichia coli* concentrations from the water samples

Water samples from the six sites had an estimated average *E. coli* and coliform concentrations of 1.9×10^5 CFU/100 mL and 12.0×10^5 CFU/100 mL, respectively. *E. coli* and coliform concentrations from each sampling site are shown in Table 1. Post-hoc comparisons revealed that *E. coli* concentrations at site U3 (32.1×10^4 CFU/100 mL) were significantly higher than all the other sites ($p \leq 0.001$). In contrast, site U1 (0.2×10^4 CFU/100 mL) has a significantly lower *E. coli* concentration than all the other sampling sites ($p \leq 0.001$). This is attributed to U1's surroundings, which remain largely forested and sparsely inhabited, resulting in less fecal contamination than other sites with dense populations and commercial developments (Berkman International

Inc., 2015). Nonetheless, all of the sampling sites (including U1) have far exceeded the upper microbial limit of 100, 200, and 400 CFU/100 mL of *E. coli* for Class B (primary contact recreation), Class C (fishery, non-contact recreation, industrial), and Class D (navigable) water, respectively (DENR, 2015, 2016; EPA, 2012; "Guidelines on Recreational Water Quality. Volume 1: Coastal and Fresh Waters. Geneva," 2021). Despite the claims for Wawa Dam as a potential additional water source for Metro Manila, its current microbial quality is poor due to fecal pollution from nearby livestock farms and informal settlers, rendering it unsuitable for beneficial use (Clemente, 2020). There was no significant difference in *E. coli* concentrations between upstream and downstream sites (16.1×10^4 CFU/mL vs. 21.5×10^4 CFU/mL, $p = 0.069$). This lack of distinction was mainly due to the very high *E. coli* concentrations observed at the urbanized U2 and U3 sites.

Antimicrobial susceptibility profile of *Escherichia coli*

Antimicrobial susceptibility testing (AST) was performed on 48 *E. coli* isolates. The antibiotics with the highest resistance rates were AMP at 54.2%, SXT at 27.1%, CXMA/CXM at 22.9%, CRO at 22.9%, and CIP at 20.8% (see Fig. 2). However, all isolates were fully susceptible to 10 other antibiotics from five different classes or subclasses, including penicillins (TZP, SAM), cephalosporins (FEP), carbapenems (ETP, IMP, MEM, IMIREL, MEMV), polymyxins (CST), and aminoglycosides (AMK), and thus were not included in Fig. 2. The ranking of resistance rates was similar to the data from the 2023 Antimicrobial Resistance Surveillance Program (ARSP) of the Department of Health (DOH), Philippines (ARSP, 2023, 2024). Based on their data for *E. coli* with commonly used oral agents, the highest resistance rates were also with AMP (78%), SXT (56%), CIP (43%), CXM (42%) and CRO (37%) (ARSP, 2023, 2024). The higher resistance rates in clinical isolates are attributed to the greater selective pressure they face from patients treated with broad-spectrum antibiotics (Collignon & McEwen, 2019; Rousham *et al.*, 2018).

Table 2. Frequencies and percentage of antimicrobial resistance (AMR) profiles

Result	Frequency (Percent, %)				
	FS	AR	MAR	MDR	ESBL
Positive	11 (22.9)	22 (45.8)	15 (31.3)	9 (18.8)	11 (22.9)
Negative	37 (77.1)	26 (54.2)	33 (68.7)	39 (81.2)	37 (77.1)

AR, antibiotic-resistant; ESBL, extended spectrum beta-lactamase producer; FS, fully susceptible; MAR, multiple antibiotic resistant; MDR, multidrug resistant

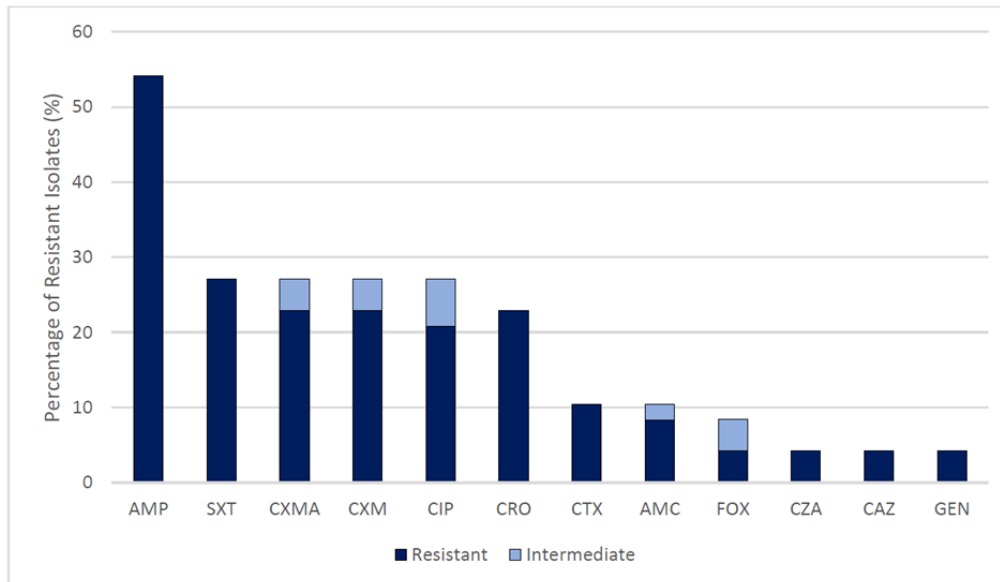


Fig. 2. Antibiotic resistance profile of *E. coli* from water samples in order of resistance level. AMP, ampicillin; SXT, sulfamethoxazole-trimethoprim; CXMA, cefuroxime axetil; CXM, cefuroxime; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; AMC, amoxicillin/clavulanic acid; FOX, ceftazidime; CZA, ceftazidime/avibactam; CAZ, ceftazidime; and GEN, gentamicin

Nonetheless, MAR and MDR *E. coli* were observed in the water samples, with 31.1% and 18.8% of isolates, respectively (Table 2). Given the high coliform and *E. coli* concentrations, it is likely that many AR isolates originated from human and animal waste through sewage discharge from nearby urban communities and medical facilities rather than from naturally occurring antibiotics produced by microorganisms (Ancheta, 2021; Chen *et al.*, 2023; Essert *et al.*, 2023; Larsson and Flach, 2022; Suzuki *et al.*, 2020).

Frequency of antibiotic-resistant (AR), multiple antibiotic-resistant (MAR), multidrug-resistant (MDR), and extended-spectrum beta-lactamase (ESBL) *Escherichia coli*

Among the 48 isolates tested, only 11 (22.9%) were fully susceptible (FS) to all antibiotics, while 22 (45.8%) were AR, 15 (31.3%) were MAR, 9 (18.8%) were MDR, and 11 (22.9%) were ESBL producers (see Table 2). Six isolates had a MAR index of ≥ 0.2 , with 3, 1, and 2 isolates coming from sites U3, D1, and D3, respectively. However, there was no significant association between AR patterns and collection sites ($P \geq 0.461$) (Table 3). This could be attributed to the similar AR profiles of *E. coli* from wastewater affecting both upstream and downstream locations (Azzam *et al.*, 2017).

Comparing our results to a study by Vital *et al.* (2017), which examined *E. coli* isolates from irrigation water in Bulacan (a neighboring province), 58.2% of their isolates were MDR and 1.3% were FS, showing a 39% higher MDR, and 21.6% more FS isolates than in our study (Vital *et al.*, 2017). Moreover, Vital *et al.* (2018) reported a MAR prevalence of only 25.3% in *E. coli* from Metro Manila irrigation water, 6% lower than our findings (Vital *et al.*, 2018). This discrepancy may be explained by the fact that their study tested only 9 antibiotics, compared to the 22 used in ours. Regardless, despite local laws, ordinances, and public awareness campaigns on sanitation, the concentration of *E. coli* in the Marikina River remains extremely high. Wastewater

Table 3. Frequency of multiple antibiotic-resistant (MAR), multidrug-resistant (MDR), and extended-spectrum beta-lactamase (ESBL) *Escherichia coli* by the site of collection

Result	Frequency per site, n = 24		p value
	Upstream	Downstream	
MAR	7	8	0.755
MDR	3	6	0.461
ESBL	7	4	0.494

ESBL, extended-spectrum beta-lactamase producer; MAR, multiple antibiotic resistant; MDR, multidrug resistant; n, sample size per site

discharge continues to degrade the river's quality, likely contributing to the persistence of MDR *E. coli* (Bringula *et al.*, 2015). Notably, two *E. coli* isolates from the U1 site were resistant towards a second (CRO) and third (CXM) generation cephalosporin and were classified as MDR and ESBL producers. This resistance may be linked to agricultural activity, particularly to the small-scale hog and poultry farms in upland areas (Berkman International Inc., 2015). Thus, increased surveillance in upstream sites may be necessary to assess how the human settlements and local farming practices in the area influence the development of AR.

Limitations of the study

Despite the results, the study's limited two-month sampling period (February and March 2024) may overlook seasonal variations and temperature influence on antibiotic resistance (AR) and microbial concentrations. For instance, rainy seasons may increase runoff, altering bacterial loads and resistance profiles. Thus, data from other seasons, such as the rainy season, is needed to capture year-round fluctuations (Jiang *et al.*, 2021; Liang *et al.*, 2020). Furthermore, the study's sampling was limited to six sites along the Marikina River. This restricted geographic scope may introduce bias, as it might not reflect other sites along the river with different pollution levels, potentially overestimating the prevalence of MDR and ESBL isolates. Local factors like the density of informal settlements and hospital wastewater could skew the results; thus, a more extensive special coverage would offer a more complete picture of antibiotic resistance (Quintela-Baluja *et al.*, 2019; Wang *et al.*, 2020; Zheng *et al.*, 2021). Finally, sampling at shallow depths (1 to 15 cm) may not capture the full range of microbial activity, potentially missing AR bacteria at greater depths and leading to an incomplete understanding of their distribution (Fu *et al.*, 2022; Zhang *et al.*, 2022).

Conclusion

The study's findings revealed that even in the relatively isolated and sparsely populated forested regions along the Marikina River, surface waters contain alarmingly high concentrations of MAR and extended-ESBL-producing *E. coli*. This indicated that these remote, seemingly pristine areas around Wawa Dam may still serve as reservoirs for AR bacteria, posing a potential public health risk. Suppose water from this source were to be used directly for Metro Manila's supply; in that case, there is a real risk of introducing AR *E. coli* into water treatment facilities managed by private concessionaires and other providers. This could potentially lead to the spread of AR bacteria within the broader water distribution network. To mitigate these risks, it is crucial to investigate further the spatial and temporal

distribution of AR *E. coli* in the most upstream regions. Such research could offer deeper insights that may prompt policymakers to regulate and limit human activities in these areas while implementing remediation efforts to protect water sources from the upper reaches of the Marikina River.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Medical Technology, Far Eastern University, for allowing the use of the research facility and the technical assistance of staff for this work.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Alawi, M., Torrijos, T. V., & Walsh, F. (2022). Plasmid-mediated antimicrobial resistance in drinking water. *Environmental Advances*, 8, 100191. <https://doi.org/10.1016/j.envadv.2022.100191>
2. Ancheta, A. A. (2021). Sustaining Estuarial Creeks: Water Corridors in Mitigating Flooding in Manila, Philippines. *IOP Conference Series: Earth and Environmental Science*, 799(1). <https://doi.org/10.1088/1755-1315/799/1/012004>
3. ARSP. (2023). Antimicrobial Resistance Surveillance Program Annual Report - 2022. In *Department of Health - Research Institute for Tropical Medicine*.
4. ARSP. (2024). Antimicrobial Resistance Surveillance Program Annual Report - 2023. In *Department of Health - Research Institute for Tropical Medicine*.
5. Azuma, T., Uchiyama, T., Zhang, D., Usui, M., & Hayashi, T. (2022). Distribution and characteristics of carbapenem-resistant and extended-spectrum β -lactamase (ESBL) producing *Escherichia coli* in hospital effluents, sewage treatment plants, and river water in an urban area of Japan. *Science of the Total Environment*, 839, 156232. <https://doi.org/10.1016/j.scitotenv.2022.156232>
6. Azzam, M. I., Ezzat, S. M., Othman, B. A., & El-DougDoug, K. A. (2017). Antibiotics resistance phenomenon and virulence ability in bacteria from water environment. *Water Science*, 31(2), 109–121. <https://doi.org/10.1016/j.wsj.2017.10.001>
7. Berkman International Inc. (2015). Formulation of an Integrated River Basin Management and Development Master Plan for Marikina river Basin. In *River Basin Control Office Department of Environment and Natural Resources - Department of Environment and Natural Resources River Basin Control Office* (Vol. 2).
8. Bonnin, R. A., Bernabeu, S., Emeraud, C., Creton, E., Vanparis, O., Naas, T., Jousset, A. B., & Dortet, L. (2022). Susceptibility of OXA-48-producing Enterobacterales to imipenem/relebactam, meropenem/vaborbactam and ceftazidime/avibactam. *International Journal of Antimicrobial Agents*, 60(4), 106660. <https://doi.org/10.1016/j.ijantimicag.2022.106660>
9. Bringula, R. P., Canlas, R. L., Afable, J. M., Gajo, R., San-

- tos, M. C., & Ancheta, A. A. (2015). "How do people view the estuary and the technology management practices to rehabilitate it?": The case of Estero de Paco in Manila. *Proceedings of 2014 2nd International Conference on Technology, Informatics, Management, Engineering and Environment, TIME-E 2014*, 128–134. <https://doi.org/10.1109/TIME-E.2014.7011605>
10. Chen, J., Hsu, B., Ko, W., & Wang, J. (2023). Ecotoxicology and Environmental Safety Comparison of antibiotic-resistant *Escherichia coli* and extra-intestinal pathogenic *E. coli* from main river basins under different levels of the sewer system development. *Ecotoxicology and Environmental Safety*, 263(138), 115372. <https://doi.org/10.1016/j.ecoenv.2023.115372>
11. 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. *Frontiers in Microbiology*, 8, 1–11. <https://doi.org/10.3389/fmicb.2017.01133>
12. Cho, K. H., Cha, S. M., Kang, J. H., Lee, S. W., Park, Y., Kim, J. W., & Kim, J. H. (2010). Meteorological effects on the levels of fecal indicator bacteria in an urban stream: A modeling approach. *Water Research*, 44(7), 2189–2202. <https://doi.org/10.1016/j.watres.2009.12.051>
13. Clemente, E. D. (2020). Evaluating the Water Quality Contribution of Estero de Paco to Pasig River and Manila Bay, Philippines. *E3S Web of Conferences*, 148, 1–7. <https://doi.org/10.1051/e3sconf/202014807010>
14. CLSI. (2021). *M100 Performance Standards for Antimicrobial Susceptibility Testing* (31st ed.). Clinical Laboratory Standards Institute.
15. Collignon, P. J., & McEwen, S. A. (2019). One health-its importance in helping to better control antimicrobial resistance. *Tropical Medicine and Infectious Disease*, 4(1). <https://doi.org/10.3390/tropicalmed4010022>
16. Dela Peña, L. B. R. O., Nacario, M. A. G., Bolo, N. R., & Rivera, W. L. (2022). Multiple Antibiotic Resistance in *Escherichia coli* Isolates from Fecal and Water Sources in Laguna Lake, Philippines. *Water*, 14(9). <https://doi.org/10.3390/w14091517>
17. DENR. (2015). *DENR MC 2015-006 Guidelines for Recreational Waters Monitoring Program*. Department of Environment and Natural Resources, Republic of the Philippines.
18. DENR. (2016). *DENR AO 2016-08. Water Quality Guidelines and General Effluent Standards of 2016*. Department of Environment and Natural Resources, Republic of the Philippines.
19. EPA. (2012). Recreational Water Quality Criteria. In U. S. *Environmental Protection Agency*. Health and Ecological Criteria Division, Office of Science and Technology, United States (U.S.).
20. Essert, S. M., Zacharias, N., Precht, T., Pankratz, D., Funken, K., Mutters, N. T., Kistemann, T., & Schreiber, C. (2023). Persistence of MRSA and ESBL-producing *E. coli* and *K. oxytoca* in river water. *Hygiene and Environmental Health Advances*, 7(December 2022), 100072. <https://doi.org/10.1016/j.heha.2023.100072>
21. Fu, C., Xu, B., Chen, H., Zhao, X., Li, G., Zheng, Y., Qiu, W., Zheng, C., Duan, L., & Wang, W. (2022). Occurrence and distribution of antibiotics in groundwater, surface water, and sediment in Xiong'an New Area, China, and their relationship with antibiotic resistance genes. *Science of the Total Environment*, 807, 151011. <https://doi.org/10.1016/j.scitotenv.2021.151011>
22. Ghosh, P., & Panigrahi, A. K. (2023). Evaluation of water quality of Mundeswari River in eastern India: a water quality index (WQI) based approach. *Journal of Applied and Natural Science*, 15(1), 379–390. <https://doi.org/10.31018/jans.v15i1.4340>
23. Guidelines on recreational water quality. Volume 1: coastal and fresh waters. Geneva: (2021). In Geneva: *World Health Organization*. <https://www.who.int/publications/i/item/9789240031302>
24. Ham, Y. S., Kobori, H., Kang, J. H., Matsuzaki, T., Iino, M., & Nomura, H. (2012). Distribution of antibiotic resistance in urban watershed in Japan. *Environmental Pollution*, 162, 98–103. <https://doi.org/10.1016/j.envpol.2011.11.002>
25. Hasan, S. A. R., & Shakir, B. K. (2025). Isolation and identification of pathogenic bacteria from drinking tap water and Tigris River water sources in Baghdad. *Journal of Applied and Natural Science*, 17(1), 96–104. <https://doi.org/10.31018/jans.v17i1.5963>
26. Iloba, K. I., Akawo, N. O., & Godwin, P. I. (2021). Assessment of Anwai river water quality using the weighted arithmetic water quality index (WQI) in delta state, Nigeria. *Journal of Applied and Natural Science*, 13(3), 913–922. <https://doi.org/10.31018/jans.v13i3.2758>
27. Jiang, X., Liu, L., Chen, J., Fan, X., Xie, S., Huang, J., & Yu, G. (2021). Antibiotic resistance genes and mobile genetic elements in a rural river in Southeast China: Occurrence, seasonal variation and association with the antibiotics. *Science of the Total Environment*, 778, 146131. <https://doi.org/10.1016/j.scitotenv.2021.146131>
28. Kundan, P., & Slathia, D. (2018). Investigation of water quality changes in drinking water supplied from Sitlee water treatment plant on River Tawi to Old Jammu City, Jammu, J&K, India. *Journal of Applied and Natural Science*, 10(2), 601–607. <https://doi.org/10.31018/jans.v10i2.1742>
29. Larsson, D. G. J., & Flach, C. F. (2022). Antibiotic resistance in the environment. *Nature Reviews Microbiology*, 20(5), 257–269. <https://doi.org/10.1038/s41579-021-00649-x>
30. Liang, X., Guan, F., Chen, B., Luo, P., Guo, C., Wu, G., Ye, Y., Zhou, Q., & Fang, H. (2020). Spatial and seasonal variations of antibiotic resistance genes and antibiotics in the surface waters of Poyang Lake in China. *Ecotoxicology and Environmental Safety*, 196, 110543. <https://doi.org/10.1016/j.ecoenv.2020.110543>
31. Palmares, A. J., & De los Reyes, J. (2016). *Diagnostic Microbiology Workbook* (1st ed.). C&E Publishing, Inc.
32. Palmares, A. J., Rongalirios, J. J., Santos, M., Siggaoat, M. J., Sucgang, P. L., Tipa, N. N., Torres, J. C. A., Vasquez, C. A., Viernes, A. M., & Gellecanao, F. (2024). The Antibiotic Resistance Profile of *Escherichia coli* Isolated from Estuaries in the City of Manila, Philippines. *Asian Journal of Water, Environment and Pollution*, 21(6), 15–22. <https://doi.org/DOI.10.3233/AJW240068>
33. Quintela-Baluja, M., Abouelnaga, M., Romalde, J., Su, J. Q., Yu, Y., Gomez-Lopez, M., Smets, B., Zhu, Y. G., & Graham, D. W. (2019). Spatial ecology of a wastewater network defines the antibiotic resistance genes in down-

- stream receiving waters. *Water Research*, 162, 347–357. <https://doi.org/10.1016/j.watres.2019.06.075>
34. Rousham, E. K., Unicomb, L., & Islam, M. A. (2018). Human, animal and environmental contributors to antibiotic resistance in low-resource settings: Integrating behavioural, epidemiological and one health approaches. *Proceedings of the Royal Society B: Biological Sciences*, 285 (1876). <https://doi.org/10.1098/rspb.2018.0332>
35. Santillan, J., Ramos, R., David, G., & Recamadas, S. (2013). *Development, calibration and validation of a flood model for Marikina River Basin, Philippines and its applications for flood forecasting, reconstruction, and hazard mapping*. <https://doi.org/10.13140/RG.2.1.3059.2161>
36. Suzuki, Y., Nazareno, J., Nakano, R., Mondoy, M., Nakano, A., & Bugayong, P. (2020). Environmental Presence and Genetic Characteristics of Carbapenemase-Producing Enterobacteriaceae from Hospital Sewage and River Water in the Philippines. *Applied and Environmental Microbiology*, 86(2), 1–10.
37. Thermo Fisher Scientific. (2018). *Thermo Scientific Sensititre Plate Guide for Antimicrobial Susceptibility Testing*.
38. Titilawo, Y., Sibanda, T., Obi, L., & Okoh, A. (2015). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of faecal contamination of water. *Environmental Science and Pollution Research*, 22(14), 10969–10980. <https://doi.org/10.1007/s11356-014-3887-3>
39. Vital, P. G., Caballes, M. B. D., & Rivera, W. L. (2017). Antimicrobial resistance in *Escherichia coli* and *Salmonella* spp. Isolates from fresh produce and the impact to food safety. *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes*, 52(9), 683–689. <https://doi.org/10.1080/03601234.2017.1331676>
40. Vital, P. G., Zara, E. S., Paraoan, C. E. M., Dimasupil, M. A. Z., Abello, J. J. M., Santos, I. T. G., & Rivera, W. L. (2018). Antibiotic resistance and extended-spectrum beta-lactamase production of *Escherichia coli* isolated from irrigation waters in selected urban farms in Metro Manila, Philippines. *Water*, 10(5), 1–11. <https://doi.org/10.3390/w10050548>
41. Wang, Z., Han, M., Li, E., Liu, X., Wei, H., Yang, C., Lu, S., & Ning, K. (2020). Distribution of antibiotic resistance genes in an agriculturally disturbed lake in China: Their links with microbial communities, antibiotics, and water quality. *Journal of Hazardous Materials*, 393, 122426. <https://doi.org/10.1016/j.jhazmat.2020.122426>
42. Yoneda, I., Rozanah, U. N., Nishiyama, M., Mith, H., & Watanabe, T. (2022). Detection and genetic analysis of *Escherichia coli* from Tonle Sap Lake and its tributaries in Cambodia: Spatial distribution, seasonal variation, pathogenicity, and antimicrobial resistance. *Environmental Pollution*, 315(September), 120406. <https://doi.org/10.1016/j.envpol.2022.120406>
43. Zhang, H., Wang, Y., Liu, P., Sun, Y., Dong, X., & Hu, X. (2022). Unveiling the occurrence, hosts and mobility potential of antibiotic resistance genes in the deep ocean. *Science of the Total Environment*, 816, 151539. <https://doi.org/10.1016/j.scitotenv.2021.151539>
44. Zheng, D., Yin, G., Liu, M., Chen, C., Jiang, Y., Hou, L., & Zheng, Y. (2021). A systematic review of antibiotics and antibiotic resistance genes in estuarine and coastal environments. *Science of the Total Environment*, 777, 146009. <https://doi.org/10.1016/j.scitotenv.2021.146009>