ARTICLE

Fecal coliform assessment and detection of *bla*$_{TEM}$, *bla*$_{SHV}$, and *tetA* in *Escherichia coli* isolated from selected river waters of Tacloban City, Leyte, Philippines

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ABSTRACT

Fecal coliforms (FCs) are generally used as indicators of fecal pollution in water systems. High levels of FC pollution in water bodies may also be associated with antibiotic resistance. *Escherichia coli*, a member of the FC group, is generally commensal and harmless. However, some strains can cause various diseases in humans. The prevalence of antibiotic-resistant *E. coli* has been reported in several river systems worldwide. Herein, we report the most probable number (MPN) of FC and *E. coli* and the first detection of *bla*$_{TEM}$, *bla*$_{SHV}$, and *tetA* antibiotic resistance genes (ARGs) as well as ARG combinations (i.e. *bla*$_{TEM}$ + *tetA* and *bla*$_{SHV}$ + *tetA*) in *E. coli* isolates from river waters with high levels of FC in Tacloban City. MPN was determined using a multiple-tube fermentation technique, and ARG was detected using polymerase chain reaction (PCR). Results show that the Bagacay River had the highest MPN of FC and *E. coli* (both >160,000 MPN/100 mL), followed by the Burayan Creek (>160,000 MPN/100 mL; 160,000 MPN/100 mL), the Mangonbangon River (92,000 MPN/100 mL; 54,000 MPN/100 mL), and the Tigbao River (35,000 MPN/100 mL; 24,000 MPN/100 mL). Among the 113 *uidA* gene-confirmed *E. coli* isolates, *bla*$_{TEM}$, *bla*$_{SHV}$, and *tetA* were detected in 39 (34.5%), 7 (6.2%), and 83 (73.5%) *E. coli* isolates, respectively. Moreover, 34 (30%) of the isolates possess both *bla*$_{TEM}$ and *tetA*, and five (4.4%) of the isolates have both *bla*$_{SHV}$ and *tetA*. These results imply that the rivers are unsafe for anthropogenic use and the presence of ARGs in *E. coli* isolates pose a threat for locals. Hence, the public is urged to ensure safety and awareness in relation to these water environments. Further monitoring and source tracking are recommended to enhance environmental and public health safety among locals.

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INTRODUCTION

Fecal coliforms (FCs) are a subset of the total coliform (TC) bacteria that primarily originate in feces (US EPA 2012). FCs, also known as thermotolerant coliform bacteria (Cisneros 2011),

KEYWORDS

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generally indicate the magnitude of fecal pollution and sewage contamination in aquatic environments (Gokul et al. 2019). FCs are generally not harmful, but their presence in a particular environment suggests the coexistence of other pathogens that can also be found in human and animal feces (US EPA 2012; Mishra et al. 2018).

Escherichia coli is a species within the FC group and has been recommended as the best indicator of health risk in waters (Cookson et al. 2022). Similar to FCs, *E. coli* thrives in the guts of humans and animals (Martinson and Walk 2020). *E. coli* is generally commensal and harmless, but also has pathogenic forms (pathotypes), such as the extraintestinal pathogenic *E. coli* (ExPEC) (Manges et al. 2019), adherent invasive *E. coli* (AIEC) (Mirsepasi-Lauridsen et al. 2019), and Shiga-toxin producing *E. coli* (STEC) (Breyer et al. 2022; Cookson et al. 2022), among others. Pathogenic *E. coli* may cause a wide array of diseases. Although they mainly thrive in the vertebrate gut, they can also be extraintestinal opportunistic pathogens (Denamur et al. 2021; Wells and Whiteford 2022). Bacteremia and urinary tract infection (Bonten et al. 2021; Haghighatpanah and Mojtahedi 2019) are some of the extraintestinal diseases caused by *E. coli*. Antibiotics and antimicrobials are used to treat infected patients. However, recent studies show that *E. coli* exhibits resistance against the antibiotics and/or antimicrobials making it difficult or impossible to treat them (CDC 2022). Antibiotic-resistant *E. coli* has been observed to be prevalent in humans, animals, food, and environment (Pormohammad et al. 2022).

Antibiotic resistance can be associated with FCs in aquatic environments (Reynolds et al. 2020). Freshwater bodies, especially rivers, play an important role in disseminating antibiotic-resistant *E. coli* (Al Salah et al. 2020; Amarasiri et al. 2020; Reddy et al. 2022). River waters accelerate the occurrence of antibiotic resistance genes (ARGs) and mobile genetic elements (Reddy et al. 2022), which can be transmitted to other pathogens mainly through horizontal gene transfer (HGT) (Khan et al. 2013; Jian et al. 2021; Nava et al. 2022). *bla* is an ARG that encodes for β-lactamase (Perez-Llarena et al. 1997), which inactivates β-lactam antibiotics (Majiduddin et al. 2002), such as penicillins, cephalosporins, carbapenems, and monobactams. On the other hand, *tetA* is an ARG that codes for resistance against tetracycline antibiotics (Jahantigh et al. 2020; Perewari et al. 2022). Furthermore, river waters can be discharged to marine environments and contaminate seafoods (Ghosh et al. 2019; You et al. 2023), which implies serious problems in foodborne diseases (Beyari et al. 2021; Kusunur et al 2022). These occurrences are not well monitored by authorities responsible for controlling water quality (Grenni 2022). Hence, river waters are considered as emerging hotspots of antibiotic resistance especially in the urban setting (Al Salah et al. 2020; Mishra et al. 2018; Nava et al. 2022). The prevalence of antibiotic-resistant FCs in the rivers varies according to season and geographic location (Mishra et al. 2018).

Tacloban City is the capital city of Leyte, located in the Eastern Visayas region (Region VIII) of the Philippines. It has a low elevation and is boarded by mountains on its north and west, San Juanico Strait on its east, and San Pedro Bay on its south (Lagmay et al. 2015). The city is situated in a geographic location where rainfall is evenly distributed throughout the year, and with no dry season (Type IV weather classification) (JICA 2015; Giles et al. 2019; NEDA 2021). Tacloban City is a home of more than 250,000 people. The city's strategic location makes it a center of multiculturalism of people from different parts of Eastern Visayas and even the Philippines. There are six major hospitals in the city, making it the healthcare hub of the region. Years after being devastated by Super Typhoon Haiyan (Yolanda), the city showed significant improvements in terms of infrastructures that cater primarily to the business and economic sector, thus attracting more people in the city for either residential or recreational purposes (City Government of Tacloban 2019).

Anthropogenic activities, such as tourism (Soumastre et al. 2022) and open defecation (Niyoyitungiye et al. 2020), further aggravate the magnitude of FC contamination and the prevalence of virulence factors (Soumastre et al. 2022; Xie et al. 2023; You et al. 2023). Domestic wastewater and livestock sewerage can be primary sources of FC pollution (Paule-Mercado et al. 2022; Xie et al. 2023), in addition to regional development and rapid urbanization (Zhang et al. 2020; Zhang et al. 2021) and population increase (Li et al. 2022). Hence, despite the advancements exhibited by Tacloban City, it may also imply the high risk of FC contamination and dissemination of antimicrobial resistance in the rivers within the city. To the best of our knowledge, no known scientifically published literature is available on the prevalence of FC and antibiotic resistance in the city's aquatic environments before this study. In this study, we report the prevalence of FCs and ARGs (i.e., bla_{TEM}, bla_{SHV}, and *tetA*), as well as ARG combinations (i.e. bla_{TEM} + *tetA*, and bla_{SHV} + *tetA*) harbored by *E. coli* isolates from four selected river waters of Tacloban City.

MATERIALS AND METHODS

Sampling sites

Four river waters in the city, namely, Bagacay Creek, Tigbao River, Mangonbangon River, and Burayan Creek, were selected in this study. These were selected in relation to hospitals and household areas that are possible sources of FCs and antibiotic resistance. Bagacay Creek, Mangonbangon River, and Tigbao River drain toward San Juanico Strait (Toda et al. 2015; Deocaris et al. 2022), while Burayan Creek drain toward Cancabato Bay. Both marine bodies of water are economically important for fisheries and aquaculture purposes (Toralde et al. 2021; Yu et al. 2024). Accessible and relatively equidistant sampling points in relation to the city's coastline were strategically selected to ensure well representation of the geographical coverage of Tacloban City (Figure 1).

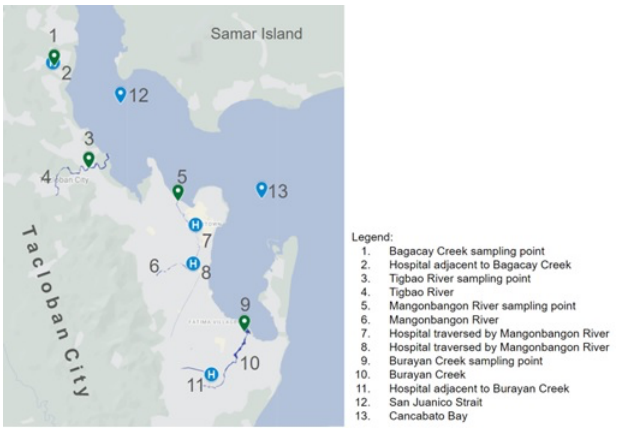

Figure 1: Site map showing the sampling points per river. Coordinates (lat, lon): Bagacay Creek (11.2870626996844, 124.95868383174), Tigbao River (11.2575733409783, 124.968835302344), Mangonbangon River (11.2481601882399, 124.995128898999), Burayan River (11.2106261983878, 125.014625334664).

Water sample collection

Sterile water samplers were used to collect 680 mL of water samples. The samples were then contained in sterile glass jars, which were then stored in an ice chest maintaining a cool temperature and were immediately transported to the Regional Standards and Testing Laboratory of the Department of Science and Technology Regional Office VIII in Palo, Leyte for FC and

E. coli quantification and isolation.

FC and *E. coli* quantification

The most probable number (MPN) of FCs and *E. coli* per 100 mL of water sample was determined through multiple fermentation tubes method following the protocols stated in the Standard Methods for the Examination of Water and Wastewater, 23rd edition (APHA, AWWA, WEF 2017). Briefly, 10 mL of water sample was serially diluted up to 10^{-5} in sterile peptone water. Moreover, 10 mL of diluted samples were pipetted to tubes containing 10 mL double-strength lauryl sulfate broth (LSB) (HiMedia, India). Vigorous shaking was done to the water bottles prior to dispensing, to ensure well representation of the samples. The LSB tubes containing the inoculum were incubated at 35°C for 24 to 48 h. Tubes indicating the presence of coliform growth (i.e., presence of bubbles for gas production and the broth's color change to yellow for acidic reaction) were identified. Two loopfuls of inoculum from the LSB tubes positive for coliform growth were inoculated into *E. coli* (EC) broth medium (HiMedia, India) and tryptone water (Merck, USA) and were incubated at 44.5°C for 24 h. EC broth medium tubes indicating the presence of fecal coliform growth (i.e., presence of bubbles) were counted. To detect the presence of *E. coli*, an indole-production test was performed on the incubated tryptone water by applying three to five drops of Kovac's reagent. The MPN/100 mL quantities were then determined based on the scale indicated in Standard Methods for the Examination of Water and Wastewater, 23rd edition (APHA, AWWA, WEF 2017).

***E. coli* isolation**

Following the procedures of Kumar et al. (2005), Lima (2017), Phyo (2019), and Verawaty et al. (2020), a loopful of inoculum from the EC broth medium tubes with positive indication of *E. coli* was streaked on eosin methylene blue agar (EMBA; Merck, USA) plates and was incubated at 37°C for 18 to 24 h. Colonies with green-metallic sheen growing on the EMBA plates were further inoculated on nutrient agar plates for purification and were incubated for another 18 to 24 h. Purified isolates were further inoculated to tryptic soy broth (TSB; Merck, USA) medium contained in sterile 1.5 mL microcentrifuge tubes and were incubated at 37°C for another 18 to 24 h. Twenty percent of triple-sterilized glycerol was loaded to the TSB tubes, vortexed, and stored in the freezer until transport to the Pathogen–Host–Environment Interactions Research Laboratory (PHEIRL) of the Institute of Biology, University of the Philippines Diliman for further analyses. Supplementary Figure 1 describes the procedural interconnection of FC and *E. coli* MPN determination and *E. coli* isolation. Packaging and transport of the isolates were carried out applying the protocols of the International Air Transport Association.

DNA extraction

A loopful of inoculum was taken from the cultures preserved in glycerol stocks to fresh TSB and was incubated at 37°C for 18 to 24 h. The DNA of the revived isolates was extracted through boil-lysis method (Salvador-Membreve and Rivera 2021). Briefly, revived isolates were centrifuged for 10 min at 10,000 x g. In each sample, the supernatant was decanted and the pellet was washed with 1 mL sterile distilled water. Bacterial cell lysis was performed in a heat block at 100°C for 15 min. Then, 50 µL of supernatant was transferred in sterile tubes accordingly, and refrigerated at 2°C for storage and further analyses.

Amplification of *uidA* gene and targeted ARGs

Detection of *uidA* gene was also performed in various studies, such as that of Martins et al. (1993), Godambe et al. (2017), and Salvador-Membreve and Rivera (2021), to genetically confirm the identity of the isolates as *E. coli*. *uidA* gene was amplified through polymerase chain reaction (PCR) with the conditions specified in Table 1. *E. coli* ATCC 25922 and no template mix were used as positive and negative controls, respectively.

Table 1: The following are the specific PCR conditions applied to amplify *uidA* (ECN) gene and the ARGs.

Step	ECN[1]		*bla*[2]		*tetA*[3]	
	Temp (°C)	Time (min:s)	Temp (°C)	Time (min:s)	Temp (°C)	Time (min:s)
Initial denaturation	98	02:00	95	03:00	94	05:00
Denaturation[4]	95	00:30	95	00:30	94	01:00
Annealing	63	01:00	60	00:30	57	01:00
Extension	72	01:00	72	01:00	72	01:00
Final extension	72	05:00	72	10:00	72	07:00
Hold	12	∞	12	∞	12	∞

[1]Labrador et al. 2020
[2]Multiplex PCR for *bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{SHV}$ (Monstein et al. 2007).
[3]Ng et al. 2001
[4]Number of cycles: 35 for *uidA* and *tetA* and 29 for *bla*

tetA, bla$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{SHV}$ were the targeted ARGs in this study due to the high prevalence rates of *E. coli* with tetracycline resistance, and extended-spectrum β-lactamase (ESBL) production. *bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{SHV}$ ARG amplification was done through multiplex PCR, while *tetA* was amplified through singleplex PCR (Salvador-Membreve and Rivera 2021) with the conditions specified in Table 1. *Salmonella* sp. was used as positive control for *bla*$_{TEM}$ and *bla*$_{CTX-M}$, while *Klebsiella pneumoniae* (ATCC 700603) was used as positive control for *bla*$_{SHV}$. A *tetA*-positive *E. coli* isolate provided by PHEIRL was used as positive control for *tetA*. No template mix was used as negative control for the ARGs. Table 2 lists the sequence of primers used to amplify the abovementioned genes.

Table 2: Primer sequences of the targeted genes

Gene	Primers	Reference
ECN	Forward: 5′—GCAAGGTGCACGGGAATATT—3′ Reverse: 5′—CAGGTGATCGGACGCGT—3′	Labrador et al. 2020
bla$_{TEM}$	Forward: 5′—TCGCCGCATACACTATTCTCAGAATGA—3′ Reverse: 5′—ACGCTCACCGGCTCCAGATTTAT—3′	Monstein et al. 2007
bla$_{CTX-M}$	Forward: 5′—ATGTGCAGYACCAGTAARGTKATGGC—3′ Reverse: 5′—TGGGTRAARTARGTSACCAGAAYCAGC—3′	Boyd et al. 2004
bla$_{SHV}$	Forward: 5′—ATGCGTTATATTCGCCTGTG—3′ Reverse: 5′—TGCTTTGTTATTCGGGCCAA—3′	Paterson et al. 2003
tetA	Forward: 5′—GCTACATCCTGCTTGCCTTC—3′ Reverse 5′—CATAGATCGCCGTGAAGAGG—3′	Ng et al. 2001

Agarose gel electrophoresis

Three microliters of generated amplicons and controls and 5 µL of 100 bp DNA ladders (Hyperladder™ Bioline, USA) were loaded into 2% agarose gel stained with SYBR safe DNA gel stain (Invitrogen, USA). Visualization of the amplicons was done through gel electrophoresis for 30 min at 280 V in Tris-Acetate-EDTA (TAE) buffer and through UV transillumination.

RESULTS AND DISCUSSION

Prior to this study, levels of dissolved oxygen and heavy metal contamination were investigated in Bagacay Creek (Garcia 1972) and Mangonbangon River (Decena et al. 2018; Deocaris et al. 2022), respectively. Moreover, species A rotaviruses were detected in Mangonbangon River and Burayan Creek (Imagawa et al. 2020). In this study, we report the FC and *E. coli* MPN counts and the presence of bla_{TEM}, bla_{SHV}, and *tetA* ARGs from the four selected water bodies in Tacloban City.

FC and *E. coli* MPN count

As mentioned earlier, FCs are indicators of fecal pollution in aquatic environments. Although generally regarded as harmless, their presence indicates manifestation of other pathogens. *E. coli* is a member of the fecal coliform group that more accurately indicates fecal origin of contamination.

Multiple fermentation tube tests revealed that Bagacay Creek had the highest MPN counts for both FCs and *E. coli* (both >160,000 MPN/100 mL), followed by Burayan Creek (>160,000 MPN/100 mL; 160,000 MPN/100 mL), Mangonbangon River (92,000 MPN/100 mL; 54,000 MPN/100 mL), and Tigbao River (35,000 MPN/100 mL; 24,000 MPN/100 mL). These values exceeded the acceptable levels for anthropogenic use based on the Department of Environment and Natural Resources (DENR) Administrative Order No. 2016-08, which ranged from <1.1 to 400 MPN/100 mL (Figure 2).

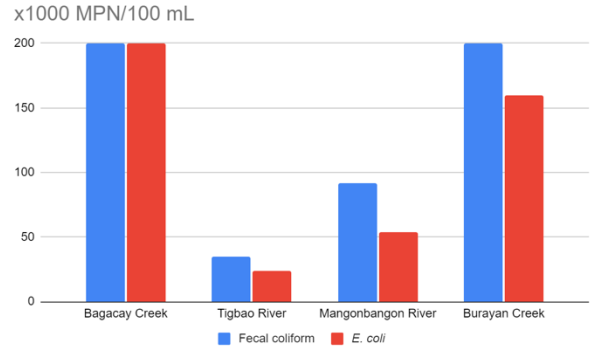

Figure 2: MPN of FCs and *E. coli*. Bagacay Creek (FC and E. coli) and Burayan Creek (*E. coli* only) had counts of >160,000 MPN/100 mL. The acceptable range for human use is <1.1 to 400 MPN/100 mL based on DENR Administrative Order No. 2016-08.

MPN values of FCs that exceed the accepted standards were also observed in other freshwater environments. The FC counts in Pasig River reached up to 256,000,000 MPN/100 mL in 2018. Since then, the number was observed to be increasing until 2019 (Castro and Obusan 2023). Such a trend was attributed to the widespread discharge of human and animal wastes and urban runoff especially during the rainy season. Levels of FC counts were also observed to be exceeding the acceptable threshold from 2009 to 2012 and 2019 to 2020 (Castro and Obusan 2023). High levels of FC counts were also reported in Laguna Lake from 2009 to 2012. Laguna Lake is the largest inland body of water in the Philippines with significant anthropological and economic functions. However, observable decreases became a trend from 2013 to 2020, especially during the years after the Laguna Lake Development Authority followed the acceptable values set by the DENR (Castro and Obusan 2023). Furthermore, it was predicted that fecal pollution and *E. coli* in Laguna Lake originate from sewage contamination, humans, and agricultural sources (de la Peña et al. 2021). FC levels were also reported to scale up to 160,000 MPN/100 mL in San Roque River in Northern Samar (Jarito and Malabarbas 2021) and Padada Watershed in Davao del Sur (Branzuela et al. 2022). FC levels in both water systems are associated with domestic, recreational, industrial, and agricultural practices. Moreover, an interesting trend was observed in Orani river systems in Bataan, where FC counts were highest at the midstream, followed by downstream and upstream areas. Principal component analysis revealed that high nitrogen-containing compounds were observed in high microbial loads. This trend may be attributed to the process of decomposition (Rabadon and Corpuz 2021).

Relatively lower risk levels with respect to TC counts were reported in Pagbanganan River, Salog River, and Palhi River in Baybay City, Leyte. These rivers were recommended to be used with caution and limitation due to elevated levels of TCs (Bitacura 2019; Lumagbas and Bitacura 2022). A similar trend was also observed in Bongoy River in Romblon, as the river was categorized as "moderately impacted/polluted" based on the surveyed coliforms and macroinvertebrates (Maulion 2020).

Detection of bla_{TEM}, bla_{SHV}, and *tetA*

This study reveals the presence of ARGs, specifically bla_{TEM}, bla_{SHV}, and *tetA*, as well as bla_{TEM} + *tetA* and bla_{SHV} + *tetA* ARG combinations in *E. coli* isolates from the river waters of Tacloban City. Thirty (26.5%) *uidA* gene-confirmed *E. coli* were isolated from Mangonbangon River and Bagacay Creek, while there were 29 (25.7%) and 24 (21.2%) from Tigbao River and Burayan Creek, respectively. Of the 113 *uidA* gene-confirmed *E. coli* isolates across the sampling sites, 39 (34.5%) and 7 (6.2%) of them were positive for bla_{TEM} and bla_{SHV}, respectively. Interestingly, bla_{CTX-M} was not detected in any of the isolates. *tetA* was detected in 83 (73.5%) of the isolates. Five (4.4%) of the isolates possessed bla_{TEM} only, and two (1.7%) had bla_{SHV} only. Forty-four (38.9%) of the isolates had *tetA* only. Moreover, 34 (30%) of the isolates possess both bla_{TEM} and *tetA*, and five (4.4%) of the isolates have both bla_{SHV} and *tetA* (Figure 3). *tetA* was mostly observed in Tigbao River (90%), followed by Mangonbangon River (87%), Burayan Creek (63%), and Bagacay Creek (53%). bla_{TEM} was mostly detected in Burayan Creek (23%), followed by Mangonbangon River (37%), Tigbao River (31%), and Bagacay River (23%). bla_{SHV} was the least prevalent among the tested ARGs across all sites, but the highest prevalence was observed in Bagacay Creek (13%), followed by Mangonbangon River (7%) and Burayan Creek. Moreover, bla_{SHV} was not detected in Tigbao River (Figure 4).

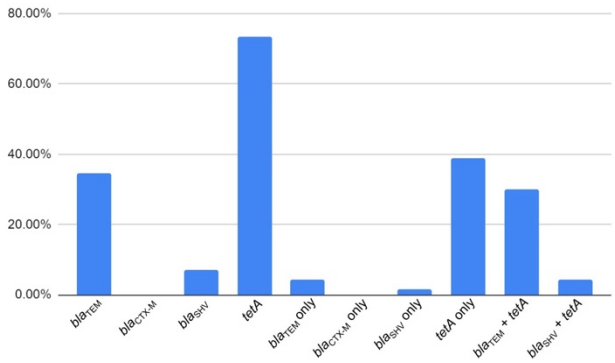

Figure 3: Overall prevalence of the ARGs showing bla_{TEM}, bla_{CTX-M}, bla_{SHV}, and *tetA*, across all *E. coli* isolates. Among the ARGs, *tetA* was the most prevalent either exclusively detected or in combination with another ARG per isolate.

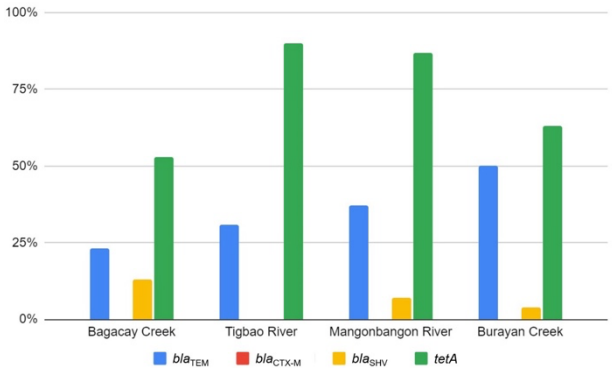

Figure 4: Prevalence of *E. coli*-harbored ARGs per site. The highest prevalence of *bla*$_{TEM}$ and *bla*$_{SHV}$ was observed from *E. coli* isolates from Burayan Creek and Bagacay Creek, respectively. *bla*$_{CTX-M}$ was not detected across all sites. *bla*$_{SHV}$ was not detected in Tigbao River, but interestingly, this site had the highest *tetA* prevalence compared to others.

The pattern of the prevalence rates of the targeted ARGs displays similarities to that of *E. coli* isolated from Laguna Lake (Salvador-Membreve and Rivera 2021) and from the Seven Crater Lakes of San Pablo, Laguna (Mamawal et al. 2023). *tetA* had the highest prevalence, followed by *strA* (an ARG for aminoglycoside resistance), *bla*$_{TEM}$, and *bla*$_{SHV}$. *bla*$_{CTX-M}$ was also not detected in Laguna Lake (Salvador-Membreve and Rivera 2021). In the case of the Seven Crater Lakes of San Pablo, *bla*$_{AmpC}$ had the highest percentage occurrence. *bla*$_{TEM}$ counts were still higher than that of *bla*$_{SHV}$. *bla*$_{CTX-M}$ was detected in minimal amounts (Mamawal et al. 2023). By contrast, *bla*$_{CTX-M-15}$ and *bla*$_{CTX-M-3}$ were detected in carbapenemase-producing *Enterobacteriaceae* isolated from various hospital sewage and river sites in Metro Manila (Suzuki et al. 2020). In addition, *bla*$_{TEM}$, *bla*$_{SHV}$, and *tetA*, as well as *bla*$_{CTX-M}$, *tetB*, *tetU*, *tetW*, *qnrB*, and *qnrS*, were observed to be prevalent in broiler farms (Gundran et al. 2019; Imperial et al. 2022).

Implications

High FC levels and *E. coli* counts with the presence of ARGs in rivers can have substantial implications for the environment and public health. This may contaminate water supplies (Fernando et al. 2016; Mishra et al. 2018; Odonkor et al. 2020), which may lead to waterborne diseases (Some et al. 2021). Elevated contamination levels also pose risk to recreational and anthropogenic activities. Exposure to contaminated waters increases the chances of infections and other health issues (Fakhr et al. 2016; Nnadozie and Odume 2019).

E. coli harboring *bla*$_{TEM}$, *bla*$_{SHV}$, *tetA*, and ARG combinations (i.e. *bla*$_{TEM}$ + *tetA* and *bla*$_{SHV}$ + *tetA*) in rivers may act as reservoir to facilitate these ARGs to other bacteria (Bong et al. 2022) mainly through HGT (Grenni 2022; Kulik et al. 2023). This contributes to the spread of the ARGs in the environment, which increases the risk for the individuals exposed to the contaminated water (Serwecinska 2020). Individuals infected with *E. coli* or any other pathogen harboring these ARGs have limited treatment options (Poirel et al. 2018) since β-lactam and tetracycline antibiotics are most likely ineffective against these pathogens (Khalifa et al. 2021; Perewari et al. 2022).

Increased number of these bacteria can adversely affect aquatic ecosystems (Oporto-Bensig et al. 2014; Paruch et al. 2019). FCs and other associated pathogenic organisms, especially those with ARGs, can be detrimental to the quality of aquaculture and fishing resources (Schar et al. 2021; Cid et al. 2022) implying the risks of economic loss and food-borne diseases (Adinortey et al. 2020; Islam et al. 2021; dos Santos et al. 2022; Leung et al. 2022). Since hospitals and household areas can be sources of FCs and ARGs (Opisa et al. 2012; Skariyachan et al. 2015; Lepesova et al. 2020; Montealegre et al. 2020), these detrimental effects may happen to San Juanico Strait and Cancabato Bay since the studied rivers drain toward these two economically important marine water bodies. This situation is relatively similar to the cases of Manila Bay (Raña et al. 2017), Bandon Bay in Thailand (Chinfak et al. 2023), coastal area of the Red River in Vietnam (Le et al. 2023), and the Mediterranean Sea (Pepi and Forcardi 2021).

CONCLUSION

The results of this study imply that the sampled river waters are unsafe for human use. Caution should be exercised by the locals when dealing with these rivers and their associated environments. The high levels of FC and *E. coli* counts, coupled with the presence of ARGs and ARG combinations in *E. coli* isolates, pose a threat to the public health and environment in the locality. Considering the classification of the weather condition of Tacloban City, the FC levels are relatively similar throughout the year, unless changes in anthropogenic activities occur. Hence, further monitoring and source tracking are recommended to enhance environmental and public health safety among locals. Lastly, to the best of our knowledge, this study is the first to report on the detection of ARGs in *E. coli* isolates from river waters with high levels of FC counts in Tacloban City and perhaps also outside the Luzon area. This may contribute to the current existing knowledge of the prevalence of ARGs within the Philippines.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Adinortey CA, Aheto DW, Boateng AA, Agbeko R. Multiple antibiotic resistance-coliform bacteria in some selected fish farms of the central region of Ghana. Scientifica 2020; 6641461. https://doi.org/10.1155/2020/6641461

Al Salah DMM, Ngweme GN, Laffite A, Otamonga J-P, Mulaji C, Pote J. Hospital wastewaters: A reservoir and source of clinically relevant bacteria and antibiotic resistant genes dissemination in urban river under tropical conditions. Ecotoxicol Environ Saf 2020; 200:110767. https://doi.org/10.1016/j.ecoenv.2020.110767

Amarasiri M, Sano D, Suzuki S. Understanding human health risk caused by antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water environments: Current knowledge and questions to be answered. Crit Rev Environ Sci Technol 2020; 50(19):2016–2059. https://doi.org/10.1080/10643389.2019.1692611

American Public Health Association, American Water Works Association, Water Environment Federation [APHA, AWWA, WEF]. In: Baird RB, Eaton AD, Rice EW, eds. Standard Methods for the Examination of Water and Wastewater. 23rd Ed. Washington DC, 2017; 9221 B—G.

Beyari EA, Aly MM, Jastaniah SD. Incidence of foodborne bacteria that cause serious health hazards in fish: A review. Ann Med Health Sci Res 2021; 11:60–66.

Bitacura JG. Influence of environmental disturbances on the bacteriological quality of Pagbanganan River in Baybay City, Leyte, Philippines. Ann Trop Res 2019; 41(1):81–91.

Bong CW, Low KY, Chai LC, Lee CW. Prevalence and diversity of antibiotic resistant *Escherichia coli* from anthropogenic-impacted Larut River. Front Public Health 2022; 10:794513. https://doi.org/10.3389/fpubh.2022.79513

Bonten M, Johnson JR, van den Biggelaar AHJ, Georgalis L, Geurtsen J, de Palacios PI, Gravenstein S, Verstraeten T, Hermans P, Poolman JT. Epidemiology of *Escherichia coli* bacteremia: A systematic literature review. Clin Infect Dis 2021; 72(7):1211–1219. https://doi.org/10.1093/cid/ciaa210

Boyd DA, Tyler S, Christianson S, McGeer A, Muller MP, Willey BM, Bryce E, Gardam M, Nordmann P, Mulvey MR, Canadian Nosocomial Infection Surveillance Program, Health Canada. Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrob Agents Chemother 2004; 48(10):3758–3764. https://doi.org/10.1128/AAC.48.10.3758–3764.2004

Branzuela NE, Germo RLR, Gorgonio CT, Branzuela WT. Water quality assessment of Padada Watershed, Davao del Sur, Philippines. J Environ Sci Eng 2022; 11:31–36. https://doi.org/10.17265/2162-5263/2022.02.001

Breyer GM, de Carli S, Inácio NV, Ribeiro VMF, da Silva FRC, Daudt C, Siqueira FM. Wild capybaras as reservoir of shiga toxin-producing *Escherichia coli* in urban Amazonian Region. Lett Appl Microbiol 2022; 75(1):10–16. https://doi.org/10.1111/lam.13694

Castro AE, Obusan MCM. Microbial quality and emerging pollutants in freshwater systems of Mega Manila, Philippines: A scoping review. Urban Water J 2023; 20(6):639–651. https://doi.org/10.1080/1573062X.2023.2209559

Centers for Disease Control and Prevention [CDC]. 2022 https://www.cdc.gov/drugresistance/about.html#:~:text=Antimicrobial%20resistance%20happens%20when%20germs,and%20sometimes%20impossible%2C%20to%20treat

Chinfak N, Charoenpong C, Sompongchaiyakul P, Wu Y, Supcharoen R, Zhang J. Environmental factors influencing the distribution of fecal coliform bacteria in Bandon Bay, Thailand. Reg Stud Mar Sci 2023; 68:103277. https://doi.org/10.1016/j.rsma.2023.103277

Cid AMT, Sarapuddin SB, Santos RM, Fernandez JS, Divinagracia AML, Garcia CJS, Untalan ACL, Albelda PB, Cano EAA. A systematic review on the bacterial infestation of aquaculture farms in selected regions of the Philippines and its impact on the fisheries industry and public health. Asian J Bio Life Sci 2022; 11(2):256–266. https://doi.org/10.5530/ajbls.2022.11.35

Cisneros BJ, Safe sanitation in low economic development areas. In Wilderer P, ed. Treatise on Water Science: Elsevier, 2011; 147–200. https://doi.org/10.1016/B978-0-444-53199-5.00082-8

City Government of Tacloban. 2019; https://tacloban.gov.ph/

Cookson AL, Marshall JC, Biggs PJ, Rogers LE, Collis RM, Devane M, Stott R, Wilkinson DA, Kamke J, Brightwell G. Whole-genome sequencing and virulome analysis of *Escherichia coli* isolated from New Zealand environments of contrasting observed land use. Appl Environ Microbiol 2022; 88:9. https://doi.org/10.1128/aem.00277-22

Decena SCP, Arguilles MS, Robel LL. Assessing heavy metal contamination in surface sediments in an urban river in the Philippines. Pol J Environ Stud 2018; 27(5):1983–1995. https://doi.org/10.15244/pjoes/75204

de la Peña LBRO, Labrador KL, Nacario MAG, Bolo NR, Rivera WL. Microbial source tracking of fecal contamination in Laguna Lake, Philippines using the library-dependent method, rep-PCR. J Water Health 2021; 19(5):762–774. https://doi.org/10.2166/wh.2021.119

Denamur E, Clermont O, Bonacorsi S, Gordon D. The population genetics of pathogenic *Escherichia coli*. Nat Rev Microbiol 2021; 19:37–54. https://doi.org/10.1038/s41579-020-0416-x

Deocaris CC, Diwa RR, Tucio PB. Assessment of heavy metal levels in an urban river in the Philippines using an unconstrained ordination- and GIS-based approach: evidence of the return of past pollution after the 2013 Typhoon Haiyan (Yolanda). H_2Open J 2022; 5(3):412–423. https://doi.org/10.2166/h2oj.2022.012

Department of Environmental and Natural Resources. DENR Administrative Order No. 2016-08: Water quality guidelines and general effluent standards of 2016; 2016.

dos Santos FS, Krepsky N, Teixeira VL, Martins VB, da Silva PM, Neves RAF. Fecal pollution increases susceptibility to diseases in brown mussel *Perna perna* from cultured and wild populations. Aquaculture 2022; 551:737922. https://doi.org/10.1016/j.aquaculture.2022.737922

Fakhr AE, Gohar MK, Atta AH. Impact of some ecological factors on fecal contamination of drinking water by diarrheagenic antibiotic-resistant *Escherichia coli* in Zagazig City, Egypt. Int J Microbiol 2016; 2016:6240703. https://doi.org/10.1155/2016/6240703

Fernando DM, Tun HM, Poole J, Patidar R, Li R, Mi R, Amarawansha GEA, Fernando WGD, Khafipour E, Farenhorst A, Kumar A. Detection of antibiotic resistance genes in source drinking water samples from a first nations community in Canada. Appl Environ Microbiol 2016; 82(15). https://doi.org/10.1128/AEM.00798-16

Garcia RG. Tolerance of *Oncomelania hupensis quadrasi* to varying concentrations of dissolved oxygen and organic pollution. Bull World Health Organ 1972; 47(1): 59–70.

Ghosh SK, Lekshmi M, Das O, Kumar S, Nayak BB. Occurrence of human enteric adenoviruses in fresh tropical seafood from retail markets and landing centers. J Food Sci 2019; 84(8):2256–2260. https://doi.org/10.1111/1750-3841.14735

Giles J, Macandong PB, Sova C, Seriño MNV, Ruales JH, Enerlan WC, Palao LK, Balanza JG, Hildebrand J, Grosjean G. Climate-resilient agriculture in the Philippines: climate risk profile, Visayas. Department of Agriculture—Adaptation and Mitigation Initiative in Agriculture, Government of the Philippines; The Food and Agriculture Organization of the United Nations (FAO), Manila, Philippines. 2019.

Godambe LP, Bandekar J, Shashidhar R. Species specific PCR based detection of *Escherichia coli* from Indian foods. 3 Biotech 2017; 7:130. https://doi.org/10.1007/s13205-017-0784-8

Gokul MS, Dahms H-U, Muthukumar K, Kaviarasan T, Henciya S, Vignesh S, Kumar TD, James RA. Multimarker pollution studies along the east coast of Southern India. In: Ramkumar M, James A, Menier D, Kumaraswamy K, eds. Coastal Zone Management. Elsevier, 2019; 331–346. https://doi.org/10.1016/B978-0-12-814350-6.00013-6

Grenni P. Antimicrobial resistance in rivers: A review of the genes and new challenges. Toxicol Environ Chem 2022; 41(3):687–714. https://doi.org/10.1002/etc.5289

Gundran RS, Cardenio PA, Villanueva MA, Sison FB, Benigno CC, Kreausukon K, Pichpol D, Punyapornwithaya V. Prevalence and distribution of bla_{CTX-M}, bla_{SHV}, bla_{TEM} genes in extended-spectrum β-lactamase-producing *E. coli* isolates from broiler farms in the Philippines. BMC Vet Res 2019; 15:227. https://doi.org/10.1186/s12917-019-1975-9

Haghighatpanah M, Mojtahedi. Characterization of antibiotic resistance and virulence factors of *Escherichia coli* strains from Iranian inpatients with urinary tract infections. Infect Drug Resist 2019; 12:2747–2754.

Imagawa T, Saito M, Yamamoto D, Saito-Obata M, Masago Y, Ablola AC, Tandoc AO III, Segubre-Mercado E, Lupisan SP, Oshitani H. Genetic diversity of species A rotaviruses detected in clinical and environmental samples, including porcine-like rotaviruses from hospitalized children in the Philippines. Infect Genet Evol 2020; 85:104465. https://doi.org/10.1016/j.meegid.2020.104465

Imperial IC, Pabustan PM, Valencia KA, Nicdao MA, Ibana J. Emergence of resistance genes in fecal samples of antibiotic-treated Philippine broilers emphasizes the need to review local farming practices. Trop Biomed 2022; 39(1):150–159. https://doi.org/10.47665/tb.39.1.020

Islam SR, Ahsan ME, Haque MM, Razzak MA. Microbial water quality in pangasius and tilapia aquaculture systems in five regions of Bangladesh. Malays J Microbiol 2021; 17(4):435 – 451. https://doi.org/10.21161/mjm.211132

Jahantigh M, Samadi K, Dizaji RE, Salari S. Antimicrobial resistance and prevalence of tetracycline resistance genes in *Escherichia coli* isolated from lesions colibacillosis in broiler chickens in Sistan, Iran. BMC Vet Res 2020; 16:267. https://doi.org/10.1186/s12917-020-02488-z

Japan International Cooperation Agency. The urgent development study on the project on rehabilitation and recovery from Typhoon Yolanda in the Philippines. 2015.

Jarito EL, Malabarbas GT. Biophysical characteristics and the anthropogenic activities in San Roque River, Northern Samar. Int J Trend Sci Res Dev 2021; 5(3):199–208.

Jian Z, Zeng L, Xu T, Sun S, Yan S, Yang L, Huang Y, Jia J, Dou T. Antibiotic resistance genes in bacteria: Occurrence, spread, and control. J Basic Microbiol 2021; 61(12):1049–1070. https://doi.org/10.1002/jobm.202100201

Khalifa SM, Abd El-Aziz AM, Hassan R, Abdelmegeed ES. β-lactam resistance associated with β-lactamase production and porin alteration in clinical isolates of *E. coli* and *K. pneumoniae*. PLoS ONE 2021; 16(5): e0251594. https://doi.org/10.1371/journal.pone.0251594

Khan GA, Berglund B, Khan KM, Lindgren P-E, Fick J. Occurrence and abundance of antibiotics and resistance genes in rivers, canal and near drug formulation facilities – A study in Pakistan. PLoS ONE 2013; 8(6):e62712. https://doi.org/10.1371/journal.pone.0062712

Kulik K, Lenart-Boron A, Wyzykowska K. Impact of antibiotic pollution on the bacterial population within surface water with special focus on mountain rivers. Water 2023; 15(5):975. https://doi.org/10.3390/w15050975

Kumar HS, Parvathi A, Karunasagar I, Karunasagar I. Prevalence and antibiotic resistance of *Escherichia coli* in tropical seafood. World J Microbiol Biotechnol 2005; 21:619–623. https://doi.org/10.1007/s11274-004-3555-8

Kusunur AB, Kuraganti GK, Mogilipuri SS, Vaiyapuri M, Narayanan SV, Badireddy MR. Multidrug resistance of *Escherichia coli* in fish supply chain: A preliminary investigation. J Food Saf 2022; 42(3):e12972. https://doi.org/10.1111/jfs.12972

Labrador KL, Nacario MAG, Malajacan GT, Abello JJM, Galarion LH, Rivera WL. Selecting rep-PCR markers to source track fecal contamination in Laguna Lake, Philippines. J Water Health 2020; 18(1): 19–29. https://doi.org/10.2166/wh.2019.042

Lagmay AMF, Agaton RP, Bahala MAC, Briones JBLT, Cabacaba KMC, Caro CVC, Dasallas LL, Gonzalo LAL, Ladiero CN, Lapidez JP, Mungcal MTF, Puno JVR, Ramos MMAC, Santiago J, Suarez JK, Tablazon JP. Devastating storm surges of Typhoon Haiyan. Int J Disaster Risk Reduct 2015; 11:1–12. https://doi.org/10.1016/j.ijdrr.2014.10.006

Le ND, Hoang TTH, Nguyen TMH, Rochelle-Newall E, Pham TMH, Phung TXB, Duong TT, Nguyen TAH, Dinh LM, Duong TN, Nguyen TD, Le TPQ. Microbial contamination in the coastal aquaculture zone of the Ba Lat River mouth, Vietnam. Mar Pollut Bull 2023; 192:115078. https://doi.org/10.1016/j.marpolbul.2023.115078

Lepesova K, Olejnikova P, Mackul'ak T, Cverenkarova K, Krahulcova M, Birosova L. Hospital wastewater—important source of multidrug resistant coliform bacteria with ESBL-production. Int J Environ Res Public Health 2020; 17(21),7827. https://doi.org/10.3390/ijerph17217827

Leung HM, Sung KC, Peng XL, Cheung KC, Au CK, Yung KKL, Li WC. Evaluating seasonal variations of fecal coliform colonization in *Magallana hongkongensis* and *Crassostrea rhizophorae*: a preliminary study of oyster quality grown in Hong Kong aquaculture farms. Mar Pollut Bull 2022; 178:113583.https://doi.org/10.1016/j.marpolbul.2022.113583

Li Y, Ma L, Li Y, Uulu SA, Abuduwaili J. Exploration of the driving factors and distribution of fecal coliform in rivers under a traditional agro-pastoral economy in Kyrgyzstan,

Central Asia. Chemosphere 2022; 286:131700. https://doi.org/10.1016/j.chemosphere.2021.131700

Lima CM, Souza IEGL, Alves TdS, Leite CC, Evangelista-Barreto NS, Almeida RCdC. Antimicrobial resistance in diarrheagenic *Escherichia coli* from ready-to-eat foods. J Food Sci Technol 2017; 54(11):3612–3619. https://doi.org/10.1007/s13197-017-2820-4

Lumagbas RMC, Bitacura JG. Bacteriological examination of the water and sediments in Palhi and Salog rivers in Western Leyte, Philippines. Ann Trop Res 2022; 44(2):49–66. https://doi.org/10.32945/atr4424.2022

Majiduddin FK, Materon IC, Palzkill TG. Molecular analysis of beta-lactamase structure and function. Int J Med Microbiol 2002; 292(2):127–137. https://doi.org/10.1078/1438-4221-00198

Mamawal DRD, Calayo JDV, Gandola KP, Nacario MAG, Vejano MRA, dela Peña LBRO, Rivera WL. Genotypic detection of β-lactamase-producing *Escherichia coli* isolates obtained from the Seven Crater Lakes of San Pablo, Laguna, Philippines. J Water Health 2023; 21(10):1518–1529. https://doi.org/10.2166/wh.2023.157

Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. Global extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. Clin Microbiol Rev 2019; 32(3). https://doi.org/10.1128/cmr.00135-18

Martins MT, Rivera IG, Clark DL, Stewart MH, Wolfe RL, Olson BH. Distribution of *uidA* gene sequences in *Escherichia coli* isolates in water sources and comparison with the expression of beta-glucuronidase activity in 4-methylumbelliferyl-beta-D-glucuronide media. Appl Environ Microbiol 1993; 59(7): 2271–2276. https://doi.org/10.1128/aem.59.7.2271-2276.1993

Martinson JNV, Walk ST. *Escherichia coli* residency in the gut of healthy human adults. EcoSal Plus 2020; 9(1). https://doi.org/10.1128/ecosalplus.esp-0003-2020

Maulion AF. Rapid water quality assessment of Bongoy River in Odiongan, Romblon using macro invertebrates and fecal coliform presence as bioindicators. Int J Sci: Basic Appl Res 2020; 54(5):69–79.

Mirsepasi-Lauridsen HC, Vallance BA, Krogfelt KA, Petersen AM. *Escherichia coli* pathobionts associated with inflammatory bowel disease. Clin Microbiol Rev 2019; 32(2). https://doi.org/10.1128/cmr.00060-18

Mishra M, Arukha AP, Patel AK, Behera N, Mohanta TK, Yadav D. Multi-drug resistant coliform: Water sanitary standards and health hazards. Front Pharmacol 2018; 9:311. https://doi.org/10.3389/fphar.2018.00311

Monstein HJ, Ostholm-Balkhed A, Nilsson MV, Nilsson M, Dornbusch K, Nilsson E. Multiplex PCR amplification assay for the detection of bla_{SHV}, bla_{TEM}, and bla_{CTX-M} genes in *Enterobacteriaceae*. APMIS 2007; 115(12):1400–1408. https://doi.org/10.1111/j.1600-0463.2007.00722.x

Montealegre MC, Rodriguez AT, Roy S, Hossain MI, Islam MA, Lanza VF, Julian TR. High genomic diversity and heterogenous origins of pathogenic and antibiotic-resistant *Escherichia coli* in household settings represent a challenge to reducing transmission in low-income settings. mSphere 2020; 5(1): 1128/msphere.00704-19. https://doi.org/10.1128/msphere.00704-19

Nava AR, Daneshian L, Sarma H. Antibiotic resistant genes in the environment–exploring surveillance methods and sustainable remediation strategies of antibiotics and ARGs. Environ Res 2022; 215:114212. https://doi.org/10.1016/j.envres.2022.114212

National Economic and Development Authority. Eastern Visayas water supply and sanitation regional databook and roadmap. 2021.

Ng LK, Martin I, Alfa M, Mulvey M. Multiplex PCR for the detection of tetracycline resistant genes. Mol Cell Probes 2001; 15:209 – 215. https://doi.org/10.1006/mcpr.2001.0363

Niyoyitungiye L, Giri A, Ndayisenga M. Assessment of coliforms bacteria contamination in Lake Tanganyika as bioindicators of recreational and drinking water quality. South Asian J Res Microbiol 2020; 6(3):9–16.

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

Odonkor ST, Mahami T. *Escherichia coli* as a tool for disease risk assessment of drinking water sources. Int J Microbiol 2020; 2534130. https://doi.org/10.1155/2020/2534130

Opisa S, Odiere MR, Jura WGZO, Karanja DMS, Mwinzi PNM. Fecal contamination of public water sources in informal settlements of Kisumu City, western Kenya. Water Sci Technol 2012; 66(12):2674–2681. https://doi.org/10.2166/wst.2012.503

Oporto-Bensig E, Flores MJL, Maglangit FF. Fecal and coliform levels as indicative factors in deterioration of the water quality of Lahug River, Cebu City, Philippines. IAMURE Int J Ecol Conserv 2014; 10: 71–83.

Paruch L, Paruch AM, Eiken HG, Sorheim R. Faecal pollution affects abundance and diversity of aquatic microbial community in anthropo-zoogenically influenced lotic ecosystems. Sci Rep 2019; 9:19469. https://doi.org/10.1038/s41598-019-56058-x

Paterson DL, Hujer KM, Hujer AM, Yeiser B, Bonomo MD, Rice LB, Bonomo RA, the International *Klebsiella* Study Group. Extended-spectrum β-lactamases in *Klebsiella pneumoniae* blood stream isolates from seven countries: Dominance and widespread prevalence of SHV- and CTX-M type β-lactamases. Antimicrob Agents Chemother 2003; 47(11). https://doi.org/10.1128/aac.47.11.3554-3560.2003

Paule-Mercado MC, Salim I, Sajjad RU, Memon SA, Sukhbaatar C, Lee B-Y, Lee C-H. Quantifying the effects of land use change and aggregate stormwater management practices on fecal coliform dynamics in a temperate catchment. Sci Total Environ 2022; 838:155608. https://doi.org/10.1016/j.scitotenv.2022.155608

Pepi M, Focardi S. Antibiotic-resistant bacteria in aquaculture and climate change: A challenge for health in the Mediterranean Area. Int J Environ Res Public Health 2021; 18(11):5723. https://doi.org/10.3390/ijerph18115723

Perewari DO, Otokunefor K, Agbagwa OE. Tetracycline-resistant genes in *Escherichia coli* from clinical and non-clinical sources in Rivers State, Nigeria. Int J Microbiol 2022; 9192424. https://doi.org/10.1155/2022/9192424

Perez-Llarena F, Martin JF, Galleni M, Coque JJ, Fuente JL, Frere JM, Liras P. The *bla* gene of the cephamycin cluster of *Streptomyces clavuligerus* encodes a class A beta-lactamase of low enzymatic activity. J Bacteriol 1997; 179(19):6035–6040. https://doi.org/10.1128/jb.179.19.6035-6040.1997

Phyo SSM, Yu SS, Saing KM. Bacteriological examination of bottled drinking water by MPN method. Haya Saudi J Life Sci 2019; 4(7):227–232.

Poirel L, Madec J-Y, Lupo A, Schink A-K, Kieffer N, Nordmann P, Schwarz S. Antimicrobial resistance in *Escherichia coli*. Microbiol Spectr 2018; 6(4). https://doi.org/10.1128/microbiolspec.arba-0026-2017

Pormohammad A, Nasiri MJ, Azimi T. Prevalence of antibiotic resistance in *Escherichia coli* strains simultaneously isolated from humans, animals, food, and environment: A systematic review and meta-analysis. Infect Drug Resist 2022; 12:1181–1197. https://doi.org/10.2147/IDR.S201324

Rabadon MLL, Corpuz MNC. Multivariate analyses of selected hydro-bacterial variables along the longitudinal gradient of Orani River, Philippines. IOP Conf Ser: Earth Environ Sci 2021; 798:012004. https://doi.org/10.1088/1755-1315/798/1/012004

Raña JA, Domingo JE, Opinion AGR, Cambia FD. Contamination of coliform bacteria in water and fishery resources in Manila Bay aquaculture farms. Philipp J Fish 2017; 24(2): 98–126.

Reddy S, Kaur K, Barathe P, Shriram V, Govarthanan M, Kumar V. Antimicrobial resistance in urban river ecosystems. Microbiol Res 2022; 263:127135. https://doi.org/10.1016/j.micres.2022.127135

Reynolds LJ, Sala-Comorera L, Martin NA, Nolan TM, Stephens JH, Gitto A, O'Hare GMP, O'Sullivan JJ, Meijer WG. Correlation between antimicrobial resistance and fecal coliform contamination in small urban streams and bathing waters. Sci Total Environ 2020; 739:140242. https://doi.org/10.1016/j.scitotenv.2020.140242

Salvador-Membreve DM, Rivera WL. Predominance of bla_{TEM} and *tetA* genes in antibiotic-resistant *Escherichia coli* isolates from Laguna Lake, Philippines. J Water Sanit Hyg Dev 2021; 11(5): 814–823. https://doi.org/10.2166/washdev.2021.067

Schar D, Cheng Z, Wang Y, Larsson DGJ, Gilbert M, Van Boeckel TP. Twenty-year trends in antimicrobial resistance from aquaculture and fisheries in Asia. Nat Commun 2021; 12:5384. https://doi.org/10.1038/s41467-021-25655-8

Serwecinska L. Antimicrobials and antibiotic-resistant bacteria: A risk to the environment and to public health. Water 2020; 12(12): 3313. https://doi.org/10.3390/w12123313

Some S, Mondal R, Mitra D, Jain D, Verma D, Das S. Microbial pollution of water with special reference to coliform bacteria and their nexus environment. Energy Nexus 2021; 1:100008. https://doi.org/10.1016/j.nexus.2021.100008

Soumastre M, Piccini J, Rodriguez-Gallego L, Gonzalez L, Rodriguez-Graña L, Calliari D, Piccini C. Spatial and temporal dynamics and potential pathogenicity of fecal coliforms in coastal shallow groundwater wells. Environ Monit Assess 2022; 194:89. https://doi.org/10.1007/s10661-021-09672-0

Skariyachan S, Mahajanakatti AB, Grandhi NJ, Prasanna A, Sen B, Sharma N, Vasist KS, Narayanappa R. Environmental monitoring of bacterial contamination and antibiotic resistance patterns of the fecal coliforms isolated from Cauvery River, a major drinking water source in Karnataka, India. Environ Monit Assess 2015; 187:279. https://doi.org/10.1007/s10661-015-4488-4

Suzuki Y, Nazareno PJ, Nakano R, Mondoy M, Nakano A, Bugayong MP, Bilar J. Environmental presence and genetic characteristics of carbapenemase-producing *Enterobacteriaceae* from hospital sewage and river water in the Philippines. Appl Environ Microbiol 2020; 86(2). https://doi.org/10.1128/AEM.01906-19

Toda L, Orduña JR, Lasco R, Santos CT. Assessing and mapping barangay level social vulnerability of Tacloban City and Ormoc City to climate-related hazards. Climate Disast Dev J 2015; 1(1): 26–41.

Toralde CB, Silaras MLS, Garcia MMM, Yap-Dejeto LG. Biology and ecology of wild and cultured green mussel, *Perna viridis* in Eastern Visayas, Philippines. Philipp J Nat Sci 2021; 26: 20–31.

United States Environmental Protection Agency [US EPA]. 2012; https://archive.epa.gov/water/archive/web/html/vms511.htm

Verawaty M, Apriani N, Tarigan LR, Aprian ET, Laurenta WC, Muhanri. Antibiotics resistant *Escherichia coli* isolated from aquatic ecosystems in Palembang, South Sumatra, Indonesia. Biodiversitas J Biol Div 2020; 21(1):86–97. https://doi.org/10.13057/biodiv/d210113

Wells EC, Whiteford LM. The medical anthropology of water and sanitation. In Singer M, Erickson PI, Abadia-Barrero CE, eds. A companion to medical anthropology. Wiley Online Library, 2022. https://doi.org/10.1002/9781119718963.ch9

Xie Z, Chen S, Huang J, Li D, Li X. Patterns and drivers of fecal coliform exports in a typhoon-affected watershed: Insights from 10-year observations and SWAT model. J Clean Prod 2023; 406:137044. https://doi.org/10.1016/j.jclepro.2023.137044

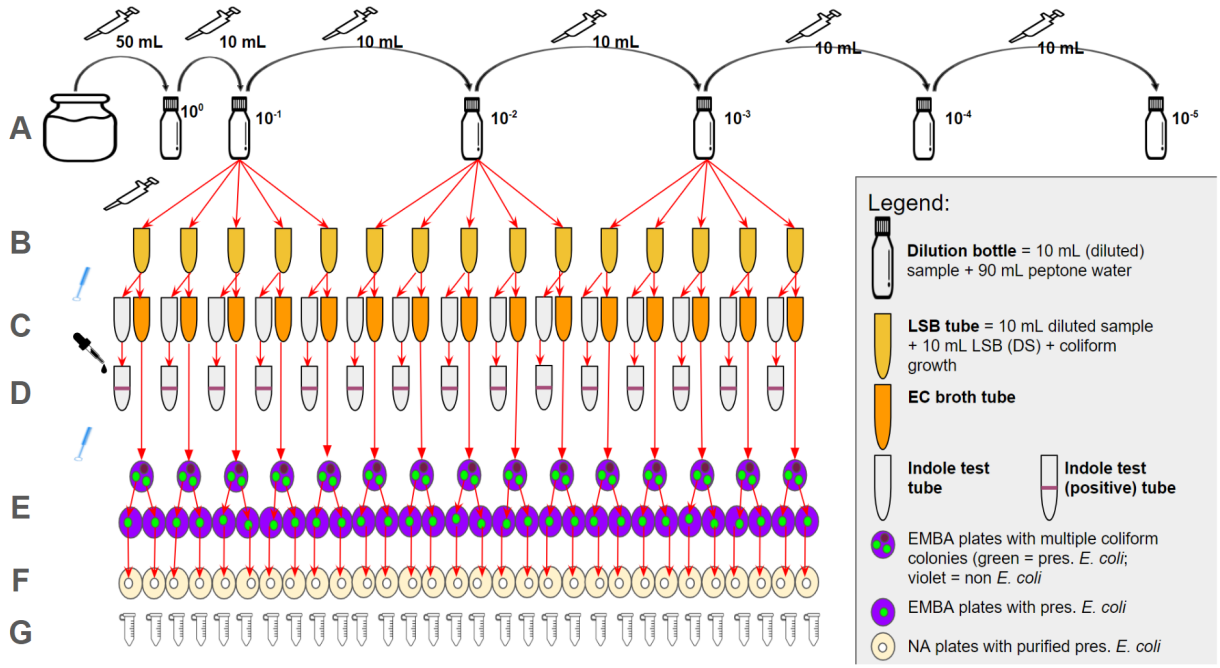
You S, Huang X, Xing L, Lesperance M, LeBlanc C, Moccia LP, Mercier V, Shao X, Pan Y, Zhang X. Dynamics of fecal coliform bacteria along Canada's coast. Mar Pollut Bull 2023; 189:114712. https://doi.org/10.1016/j.marpolbul.2023.114712

Yu AJG, Yap-Dejeto LG, Parilla RB, Elizaga NB. Microplastics in *Perna viridis* and *Venerupis* species: Assessment and impacts of plastic pollution. Int J Environ Sci Technol 2024; 21: 675–686. https://doi.org/10.1007/s13762-023-04982-x

Zhang X, Chen L, Shen Z. Impacts of rapid urbanization on characteristics, sources and variation of fecal coliform at watershed scale. J Environ Manage 2021; 286:112195. https://doi.org/10.1016/j.jenvman.2021.112195

Zhang X, Zhi X, Chen L, Shen Z. Spatiotemporal variability and key influencing factors of river fecal coliform with a typical complex watershed. Water Res 2020; 178:115835. https://doi.org/10.1016/j.watres.2020.115835

SUPPLEMENTARY INFORMATION



Supplementary Figure 1: Workflow of FC and *E. coli* MPN determination and *E. coli* isolation

A. A 680 mL of water sample was collected from the sampling sites, 50 mL was aliquoted from the sample, and 10 mL was serially diluted in sterile peptone water up to 10^{-5}.

B. In each dilution level, 10 mL of diluted sample was pipetted to 10 mL double-strength LSB and was incubated at 35°C for 24 h. LSB tubes were observed for presumptive-positive reaction. Presumptive-positive reactions were indicated by the presence of bubbles trapped in the Durham tube and the color change of the medium into shades of yellow. The bubbles and the yellow-colored broth medium indicate the presence of gas production and acidic reaction, respectively. LSB tubes exhibiting the presumptive absence of FC were further incubated for another 24 h. This also served as an enrichment and recovery phase for the thermotolerant coliforms.

C. Two loopfuls of presumptive-positive LSB were inoculated in EC broth medium, and another two were inoculated in 5 mL tryptone water. Both inoculated media were incubated at 44.5°C for 24 h. After incubation, gas production, which indicates the presence of FCs, were observed. EC broth medium tubes indicating the presence or absence of gas production were quantified and were used to determine the MPN of FCs.

D. Three to five drops of Kovac's reagent were added to the tryptone water after incubation. The presence of *E. coli* was indicated by the appearance of a deep red color ring (which indicates indole production) in the upper layer of the tryptone water. The results of the indole-production test, using tryptone water, were used to determine the MPN of *E. coli*.

E. At the same time, a loopful of positive EC broth medium paralleled with a positive tryptone water was streaked to EMBA plates (EMBA 1) and was incubated at 37°C for 18 to 24 h. From each EMBA plate, two well-isolated colonies with green-metallic sheen (GMS) were further streaked into two new and separate EMBA plates (EMBA 2) and were incubated at 37°C for another 18 to 24 h.

F. A single well-isolated GMS colony was further streaked to nutrient agar and was incubated at 37°C for 18 to 24 h.

G. The purified presumptive *E. coli* isolates were inoculated in 1 mL TSB contained in 1.5 microcentrifuge tubes. These were incubated at 37°C for 18 to 24 h. After incubation, 20% triple-sterilized glycerol was added to TSB tubes with growth. The TSB tubes were vortexed and were stored in the freezer until transport to PHEIRL.