

Abundance and Spatial Distribution of Third Generation Cephalosporin Resistant *Escherichia coli* in Wastewater and impact on the receiving Korle Lagoon, Accra, Ghana

Lady Asantewah Boamah Adomako¹, Appiah-Korang Labi², Noah Obeng-Nkrumah³, Japheth Awuletey Opintan²

Affiliations:

¹Environmental Biology, Biotechnology & Health Division, Council for Scientific and Industrial Research - Water Research Institute (CSIR-WRI), Achimota, Accra, Ghana

²Department of Medical Microbiology, University of Ghana Medical School, College of Health Sciences, University of Ghana, Accra, Ghana

³Department of Medical Laboratory Sciences, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, Accra, Ghana

Corresponding author: Japheth Awuletey Opintan

Email: jaopintan@ug.edu.gh

Tel: +233 26 525 9997

Abstract

Antimicrobial resistance in the environment is a major public health threat, particularly in low- and middle-income countries where there is inadequate wastewater treatment infrastructure. Third generation cephalosporin resistant *Escherichia coli* (3GCR *E. coli*) are of particular concern as they have clinical significance and may spread associated resistance genes in environment. This study quantified 3GCR *E. coli* in wastewater discharged into the Korle Lagoon in Accra, Ghana, and assessed the influence of these discharges on the lagoon. In a cross-sectional study conducted from November 2023 to October 2024, wastewater and lagoon water samples were collected monthly from four discharge points (the Korle Bu Teaching Hospital (KBTH) mortuary drain, the Korle Bu wastewater pumping station, the Mudor Wastewater Treatment Plant and the Lavender Hill Faecal Treatment Plant) and from paired upstream and downstream lagoon sites at each point. Isolates were enumerated by membrane filtration on a selective chromogenic medium, confirmed by MALDI-TOF mass spectrometry, and tested for antimicrobial susceptibility in accordance with CLSI (2024) guidelines. Counts were highest in overflow wastewater from the Korle Bu pumping station (median 3.75×10^6 CFU/100 mL) and in Mudor effluent (1.24×10^6 CFU/100 mL) and differed significantly across sampling sites (Kruskal-Wallis $H = 19.6$, $p = 0.003$). Paired comparisons consistently showed higher counts downstream than upstream of the discharge points, most markedly at the KBTH mortuary drain (4.03×10^5 versus 2.36×10^5 CFU/100 mL; $p = 0.018$) and the Korle Bu pumping station overflow ($p = 0.012$). The elevated downstream burden indicates active dissemination of resistant 3GCR *E. coli* along the lagoon

continuum. These findings underscore the urgent need to upgrade wastewater treatment infrastructure and to integrate AMR surveillance within wastewater systems in Ghana.

Keywords: Antimicrobial resistance; Third generation cephalosporin resistant *Escherichia coli*; wastewater; Korle Lagoon; Ghana

Introduction

Antimicrobial resistance (AMR) is a major global public health threat that has the potential to reverse the progress made in the treatment of infectious disease. As a result, the effectiveness of antibiotics is reduced which results in infections that are difficult to treat with associated high morbidity and mortality (1).

Third-generation cephalosporins such as cefotaxime and ceftazidime are of particular risk due to their widespread use in clinical care which makes resistance to this drug class a matter of particular concern. In *Escherichia coli* and other Enterobacterales resistance to these antibiotics is mostly mediated by Extended Spectrum Beta lactamases (ESBLs) (2–5). Extended spectrum β -lactamases hydrolyse the oxyimino side chain shared by third generation cephalosporins thereby inactivating the entire drug class rather than a single agent (6).

ESBL *E. coli* are increasingly recognized as markers of environmental AMR dissemination and is recommended for surveillance (7,8)

Environmental compartments, including urban wastewater systems, have been acknowledged as key reservoirs and conduits for the dissemination of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARG) (La Rosa et al., 2025; Rizzo et al., 2013). Hospital and municipal wastewater are substantial contributors to the spread of ARBs (Adomako et al., 2021; Hocquet et al., 2016; Hotor et al., 2025; La Rosa et al., 2025; Tettey et al., 2024). These wastewater sources contain high concentrations of antibiotics, resistant bacteria, and resistance genes (Berendonk et al., 2015; Larsson & Flach, 2022a, 2022b; Manaia et al., 2018; Rizzo et al., 2013)

In many low- and middle-income countries, including Ghana, wastewater originating from hospitals, mortuaries, households, and industrial facilities are frequently discharged into drainage systems and nearby surface waters, often with limited or no prior treatment (20–22). This creates a conducive environment for the selection, and spread of resistant bacteria, including third-generation cephalosporin resistant bacteria within the environment. Contaminated water can then serve as a transmission route to humans and animals through direct exposure or through the food chain (23,24)

A recent study at Korle Bu Teaching Hospital (KBTH) in Accra demonstrated that effluents from clinical units contain multidrug resistant organisms including ESBL producing

organisms, thereby highlighting wastewater from KBTH as an important source of ARBs (25). A portion of wastewater from the KBTH reaches the Korle Lagoon untreated or partially treated (African Water Facility, 2024; Onuoha, 2016). The discharge of untreated or partially treated hospital wastewater via these pathways could facilitate the dissemination of AMR in the receiving Lagoon.

The Korle Lagoon, located in southwestern Accra drains into the Gulf of Guinea adjacent to coastal communities in Jamestown. These communities engage in fishing, recreation, and food handling along the shoreline. The continuous discharge of partially treated wastewater into the Lagoon creates potential exposure pathways for humans who are exposed to lagoon or sea water to acquire clinically important resistant pathogens (28). Despite this risk, 3GCR *E. coli* in wastewater entering the Korle Lagoon and how these influences antibiotic resistance in the lagoon remains unknown. This study assessed 3GCR *E. coli* in wastewater discharged into the Korle Lagoon and evaluated the influence of these wastewater effluents on the prevalence of 3GCR *E. coli* within the lagoon ecosystem.

Methods

Study design and Setting

This study was a cross-sectional study conducted on wastewater discharged from the KBTH mortuary, Korle Bu pumping station overflow, Mudor WWTP and Lavender Hill FTP and the receiving Korle Lagoon in Accra, Ghana. Ghana has a population of about 30 million, with about 5.4 million living in Accra (29). Accra is a rapidly urbanizing city with a high population density. High volumes of wastewater is generated in Accra. However, wastewater treatment infrastructure is inadequate or lacking, which results in the discharge of untreated or partially treated effluents into the environment.

The Korle Lagoon is situated in the southwestern part of Accra. The lagoon covers a total surface area of about 0.6 km², drains a total catchment area of about 400 km² and is fed principally by the Odaw River and several associated drains, which that passes through Accra, before discharging into the Gulf of Guinea (Karikari et al., 2009; Owusu Boadi & Kuitunen, 2002). Due to rapid urbanisation, inadequate sanitation infrastructure, and widespread discharge of domestic and industrial wastes into surface drains and waterways, the Korle Lagoon has become highly polluted (Karikari et al., 2009; Owusu Boadi & Kuitunen, 2002)

The KBTH is the largest teaching and referral hospital in Ghana and serves as critical point for antibiotic use. Wastewaters generate spanning effluent from wards, clinical units, laboratories, and mortuary and sanitary operations generated here contains high concentrations of antibiotic residue and resistant bacteria. The KBTH mortuary generates wastewater from body preparation, cleaning, and sanitary activities, which is discharged into a drain that connects to the Korle Lagoon. A portion of the wastewater generated at the Korle Bu Teaching Hospital is carried through the municipal sewer network to the Korle Bu pumping station and treated at the Mudor WWTP. However, not all hospital wastewater is captured by this system, and some is discharged untreated or partially treated into nearby drains that ultimately flow into the Korle Lagoon (Onuoha, 2016). Furthermore, during periods of high flow or system malfunction, overflow from the pumping station is directly released into the Korle Lagoon resulting to intermittent discharge of untreated or partially treated hospital effluent into the Korle Lagoon. This practice exacerbates pollution levels and potentially establishing the lagoon as a potential hotspot for antimicrobial resistance.

In addition to hospital derived wastewater, the Korle Lagoon also receives effluent from the Mudor WWTP and Lavender Hill FTP. These facilities, which serve substantial portions of the Accra metropolitan area discharge treated municipal wastewater into the lagoon (34). Although these facilities are intended to remove microbial and chemical contaminants before wastewater

is discharged, their effectiveness can vary due to differences in treatment technologies, operational conditions, and maintenance practices(35–37). As a result, treated effluents may still contain antibiotic resistant bacteria and antibiotic resistance genes, which can be released into receiving water bodies and contribute to the environmental spread of antimicrobial resistance (Adomako et al., 2021; Delgado-Blas et al., 2022; Marutescu et al., 2023; Udoh et al., 2025).

Sample collection

Wastewater and surface samples were collected on a monthly basis over a twelve-month period (November 2023– October 2024). Sample collection was carried out according to procedures outlined in (42) Wastewater were collected from KBTH mortuary drain, Korle Bu pumping station, Mudor Wastewater WWTP effluent and Lavender Hill FTP effluent. Lagoon water samples were collected from multiple sites upstream and downstream of the discharge outfall of the KBTH mortuary drain, the Korle Bu pumping station overflow, as well as downstream of the outfall of Mudor WWTP and Lavender Hill FTP (Figure 1). Due to the close spatial proximity of the Mudor WWTP and Lavender Hill FTP discharge points to upstream sampling locations along the lagoon continuum, distinct upstream reference sites could not be established for these two facilities. Only downstream sites were sampled for these locations. A total of 126 grab 500ml samples were systematically collected in the morning between 8 a.m and 10 a.m, with each sample representing a composite of three sub samples collected at 15-minute intervals from a single site to account for short-term variability. Wastewater and lagoon water samples were aseptically collected, approximately 30 cm below the water surface into sterile 500ml WhirlPak bags. Physicochemical parameters were measured at each sampling point during every sampling round from November 2023 to October 2024. Temperature, pH, total dissolved solids and electrical conductivity were determined in situ using a Multi-Parameter PCSTestr™ 35 Eutech Instruments OakTon) calibrated before each sampling round according

167 to the manufacturer's instructions. Values reported are means of the measurements taken across
168 the sampling period. All samples were promptly transported on ice to the Council for Scientific
169 and Industrial Research-Water Research Institute CSIR-Water Research Institute (CSIR-WRI)
170 microbiology laboratory and analyzed immediately upon arrival.

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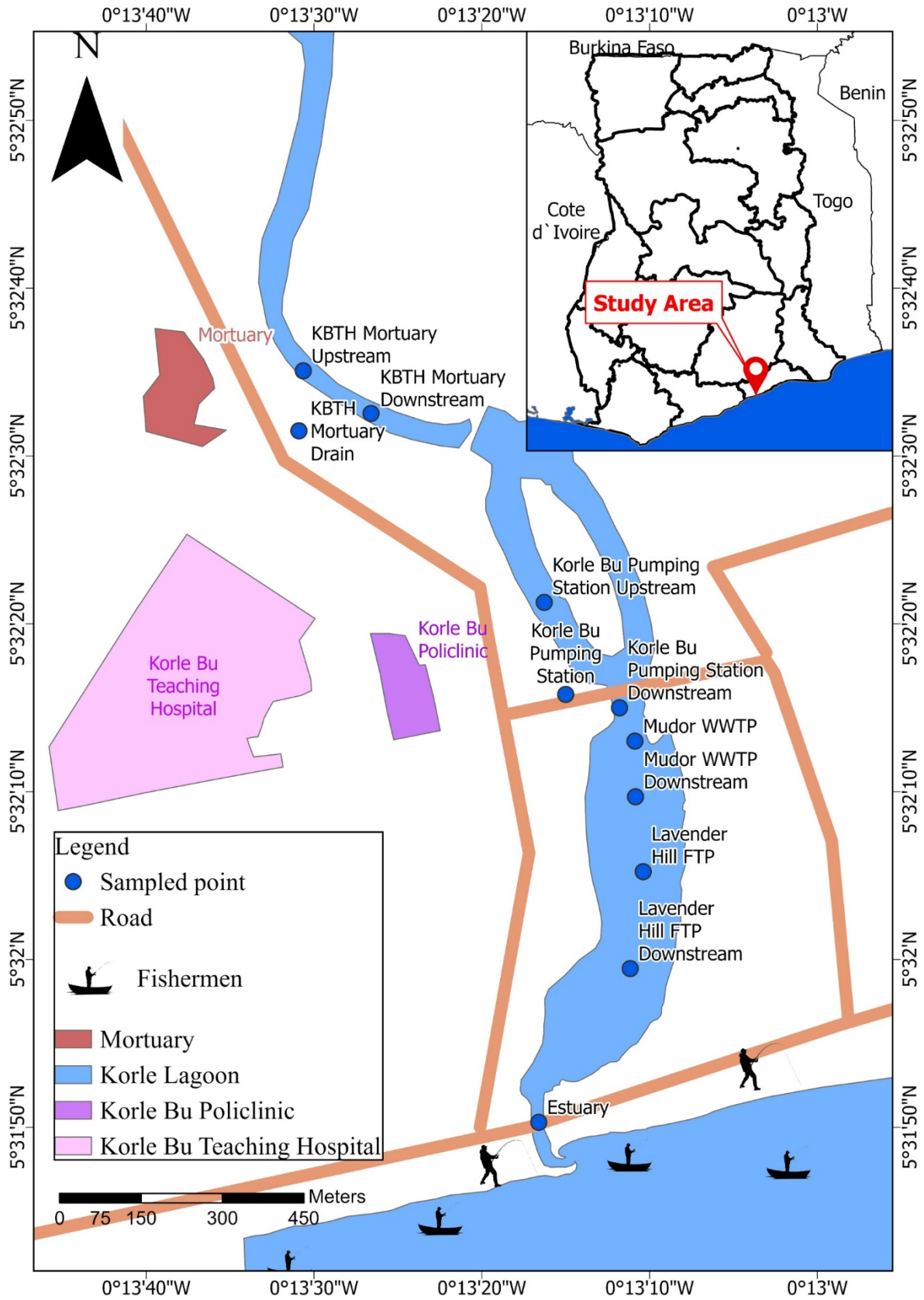


Fig 1 Schematic Map showing water sampling sites in the Korle Lagoon.

178 *Laboratory analysis*

179 All samples were serially diluted by ten-fold serial dilutions in phosphate buffered saline
180 solution and analyzed using membrane filtration. Each sample was plated on Tryptone Bile X-
181 Glucuronide (TBX) agar base (Oxoid Ltd., Basingstoke, UK) supplemented with 4 µg/mL
182 Cefotaxime for the isolation of presumptive 3GCR *E. coli*. Inoculated plates were incubated
183 for 24 h at 37 °C., Colonies appearing as blue-green colonies on TBX agar were considered
184 presumptive 3GCR *E. coli*, enumerated and expressed as colony-forming units (CFU)/100 mL.
185 For each positive sample, we selected three to five dominant representative colonies per
186 morphotype for further work. Presumptive 3GCR *E. coli* isolates were identified using a
187 Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) Biotyper (Bruker
188 Daltonics GmbH, Bremen, Germany) and stocked in skim milk, tryptone soya broth glycerol,
189 and glucose (STGG) and stored at -20°C.

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191 *Antimicrobial susceptibility testing*

192 A total of 404 3GCR-Ec isolates were selected to determine their susceptibility to 6 antibiotics
193 using disk diffusion method in accordance with CLSI M02 standards (43) and results were
194 interpreted using CLSI M100 breakpoints(43). The following antibiotics were used
195 amoxicillin–clavulanate (20/10 µg), meropenem (10 µg), gentamicin (10 µg), ciprofloxacin (5
196 µg), ceftazidime (30 µg), and trimethoprim–sulphamethoxazole (1.25/23.75 µg) (Oxoid Ltd.,
197 Basingstoke, UK).

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199 *ESBL phenotype detection*

200 Extended-spectrum beta-lactamase production in *E. coli* was screened using the double-disk
201 synergy test (DDST) according to CLSI M100. Disks containing cefotaxime (30 µg) and
202 ceftazidime (30 µg), alone and in combination with clavulanic acid (cefotaxime-clavulanic

acid, 30/10 µg; ceftazidime-clavulanic acid, 30/10 µg), were placed on Mueller-Hinton agar inoculated with the test isolate. ESBL production was confirmed by an increase in inhibition zone diameter of ≥ 5 mm for either cephalosporin tested in combination with clavulanic acid compared with the cephalosporin alone.

Quality Control

All culture media were prepared according to the manufacturer's instructions. Each batch was tested for sterility and capacity to support growth prior to use. Reference strains, *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were employed as quality control organisms to serve as negative and positive controls, respectively.

Statistical Analysis

The study variables included sample/isolate identifiers and dates, sewage sample sources, surface water samples, bacterial species isolated, bacterial counts, and antibiotic resistance profiles. Data were recorded in a laboratory notebook at the microbiology laboratory of CSIR-WRI and subsequently double entered into Excel database on a password-protected laptop. All entries in the Excel file were cross checked against the raw data in the laboratory notebooks to ensure accuracy and consistency and subsequently imported into Jamovi version 2.3.28 for analysis. The abundance and spatial distribution of third-generation cephalosporin resistant (3GCR) *E. coli* in wastewater sources discharging into the Korle Lagoon and at sampling locations along the lagoon were assessed. Antibiotic susceptibility profiles and ESBL production among 3GCR *E. coli* isolates were also evaluated. Bacterial concentrations were summarized using the median and interquartile range (IQR; 25th–75th percentiles). Antibiotic resistance patterns and ESBL prevalence were expressed as frequencies and percentages. The median and interquartile range (IQR) were used to summarise the 3GCR *E. coli* loads in

wastewater and lagoon water samples over the study period due to the non-normal distribution of microbiological data. The antibiotic resistance profiles of 3GCR *E. coli* isolated from all wastewater and lagoon water samples were summarised using frequencies and percentages. Differences in 3GCR *E. coli* counts among all sampling sites were assessed using the Kruskal–Wallis test. Upstream downstream paired sites were compared using Wilcoxon signed-rank tests. Statistical significance was determined at a threshold of $p < 0.05$. Paired comparisons were not possible for the Mudor WWTP and Lavender Hill FTP discharge points, as no corresponding upstream sampling sites were included for these locations.

Ethical considerations

The study received ethical approval from the Ghana Health Service Ethics Review Committee with IRB number-GHS-ERC-006/08/22.

Results

A total of 126 samples comprising wastewater ($n = 42$) and lagoon surface water ($n = 84$) were collected and analysed over the 12-month study period. Results describe the bacterial counts and spatial distribution of 3GCR *E. coli* in wastewater sources discharged into the Korle Lagoon and along the lagoon from upstream of the KBTH mortuary drain to the estuary, as well as the antibiotic susceptibility profiles and ESBL production of 3GCR *E. coli* isolated from wastewater and lagoon water samples.

Physicochemical Characteristics of Wastewater and Receiving Water Bodies

Physicochemical parameters across the wastewater and Korle Lagoon showed variations. These parameters are presented as average values for the sampling period from November 2023

to October 2024. The pH levels ranged from 6.63 to 7.08. Temperature ranged from 28.94°C to 31.36°C. Total dissolved solids of wastewater were between 952 ppm and 1970 ppm. Higher total dissolved solids levels were observed in the Korle Lagoon. For example, Total dissolved solids increased from 2454ppm to 4328 ppm upstream of the Korle Bu overflow to downstream respectively. The estuary had the highest total dissolved solids (6109 ppm). Conductivity also varied across the sampling sites. Wastewater had lower conductivity values compared to lagoon water (Table 1). Higher conductivity was observed in downstream locations and the estuary. The highest conductivity was recorded at the estuary (20,745 µS/cm), which indicates strong saline influence.

Table 1 Physicochemical Characteristics of Wastewater, Receiving Waters, and Estuarine Sampling Sites

Sampling Site	pH	Temperature (°C)	Total dissolved solids (ppm)	Conductivity (µS/cm)
Korle Bu overflow wastewater	6.82	30	1140	1227
Mortuary drain wastewater	7.08	30	952	1047
Mudor WWTP effluent	7.06	31	1644	2450
Lavender Hill WWTP Effluent	7.06	29	1970	3000
Mortuary Upstream	6.78	31	1945	2116
Mortuary Downstream	6.74	30	1718	6862
Korle Bu overflow Upstream	6.72	30	2454	4627
Korle Bu overflow Downstream	6.66	30	4328	4023
Mudor downstream	6.96	31	3320	5455
Lavender Hill downstream	6.78	31	3866	6265
Estuary	6.63	31	6109	20745

3GCR *E. coli* counts in wastewater

Median concentrations of 3GCR *E. coli* across wastewater discharged into the Korle Lagoon measured over the 12-month period revealed marked variation among the different wastewater samples (Figure 2). The highest concentration of 3GCR *E. coli* was observed at the Korle Bu pumping station overflow (3.75×10^6 CFU/100 mL; IQR: 2.57×10^6 - 2.04×10^6) followed by Mudor WWTP effluent (1.24×10^6 CFU/100 mL; IQR: 7.02×10^5 – 1.45×10^6). The KBTH

mortuary drain wastewater contained 2.11×10^5 CFU/100 mL; IQR: $8.3 \times 10^6 - 1.86 \times 10^6$). In contrast, the Lavender Hill FTP effluent recorded the lowest 3GCR *E. coli* (5.5×10^3 CFU/100 mL; IQR: $1.0 \times 10^2 - 5.55 \times 10^5$).

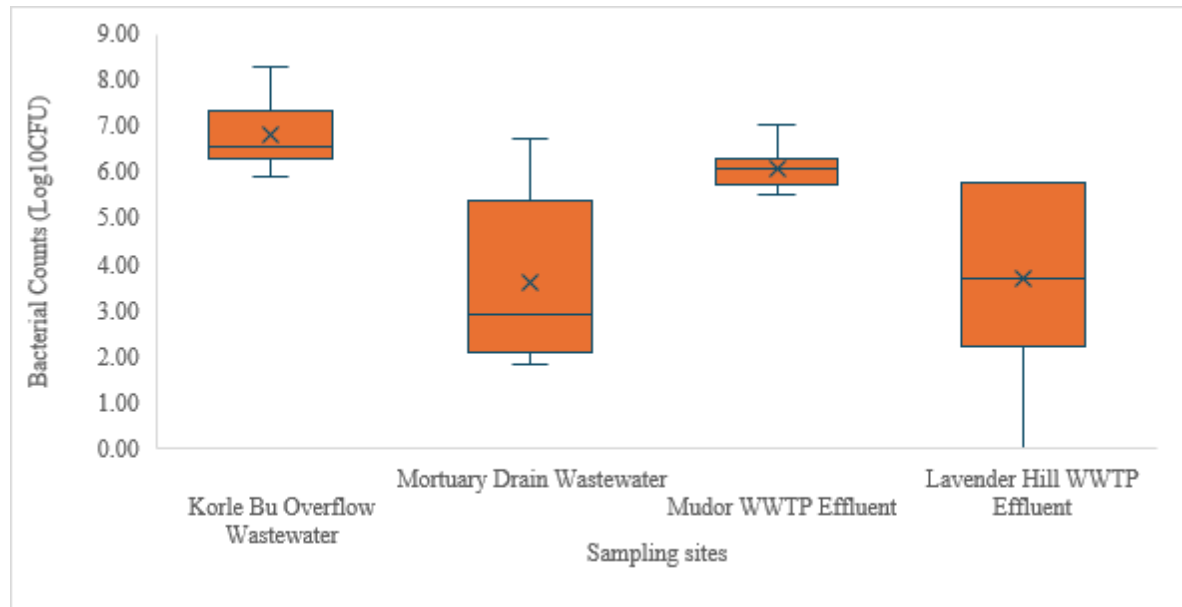


Fig 2 Box plots showing 3GCR *E. coli* counts (Log10 CFU/mL) in wastewater and effluent samples from key sources discharging into the Korle Lagoon between November 2023 and October 2024

3GCR *E. coli* counts in the Korle Lagoon

Median counts of 3GCR *E. coli* showed distinct spatial variation across the Korle Lagoon continuum (Figure 3). 3GCR *E. coli* counts were typically greatest in proximity to wastewater discharge points and diminishing toward the estuary. The Mudor WWTP downstream had the highest median counts of resistant 3GCR *E. coli* (6.61×10^5 CFU/100 mL; IQR: $3.70 \times 10^5 - 3.33 \times 10^6$), followed by KBTH mortuary downstream (4.03×10^5 CFU/100 mL; IQR: $3.38 \times 10^5 - 5.49 \times 10^5$) and Korle Bu pumping station overflow downstream (3.88×10^5 CFU/100 mL; IQR: $2.33 \times 10^5 - 5.53 \times 10^5$). Furthermore, the Lavender Hill FTP effluent downstream also displayed comparatively elevated counts (3.53×10^5 CFU/100 mL; IQR: $2.50 \times 10^5 - 4.99 \times 10^5$). In contrast, the KBTH mortuary upstream and the estuary had lower median counts of (2.36×10^5 CFU/100 mL (IQR: $1.63 \times 10^5 - 3.17 \times 10^5$) and 8.88×10^4 CFU/100 mL

respectively. 3GCR *E. coli* counts varied significantly among sampling sites (Kruskal-Wallis $H = 19.6$, $p = 0.003$). Paired upstream downstream comparisons using the Wilcoxon signed-rank test revealed significantly higher 3GCR *E. coli* counts at downstream sites particularly, at the KBTH mortuary ($p = 0.018$) and Korle Bu pumping station overflow ($p = 0.012$).

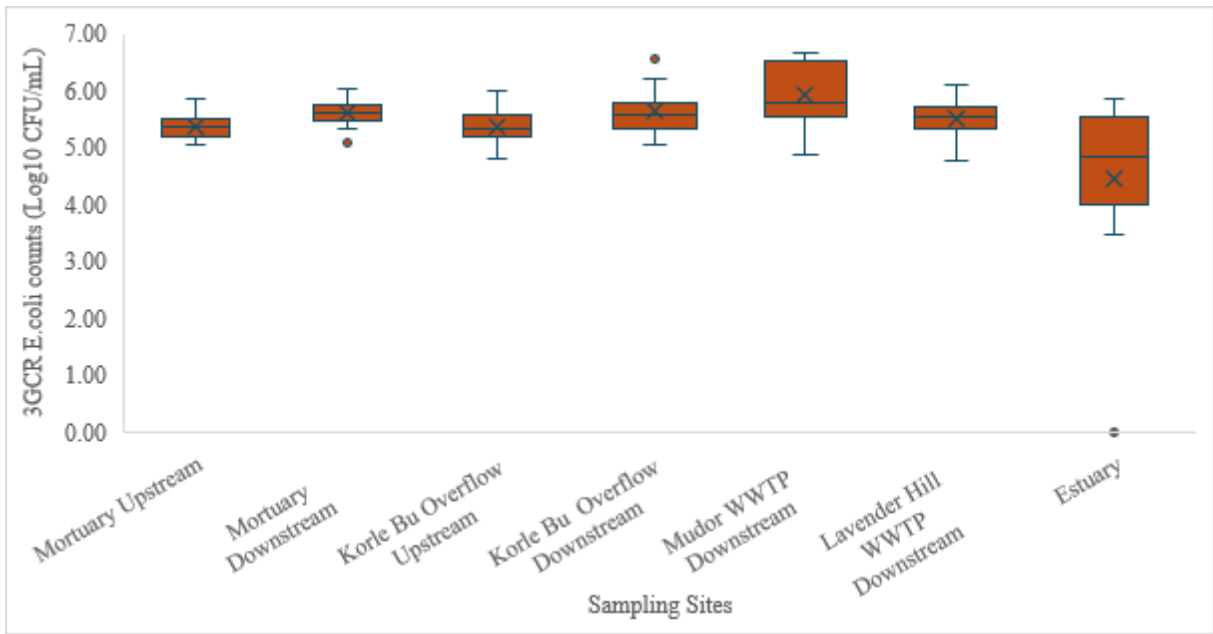


Fig 3 Box plots showing 3GCR *E. coli* counts (Log10 CFU/mL) at upstream and downstream points of major wastewater discharge sites along in the Korle Lagoon between November 2023 and October 2024

Antimicrobial Resistance Patterns of 3GCR *E. coli* in Wastewater and Korle Lagoon Samples

A total of 404 *E. coli* isolates were recovered from across the sampling sites. Of these 140 were from wastewater: Korle Bu overflow wastewater (30), KBTH mortuary drain wastewater (42), Mudor WWTP effluent (45), Lavender Hill FTP (23). The remaining 264 were from the Korle Lagoon: KBTH mortuary upstream (36), KBTH mortuary downstream (47), Korle Bu overflow upstream (34) Korle Bu overflow downstream (42), Mudor downstream (29), Lavender Hill FTP downstream (31), Estuary (45) (Table 2 and 3).

Table 2: Antibiotic resistance profiles of 3GCR *E. coli* isolates recovered from wastewater sources discharging into the Korle Lagoon, Accra Ghana from November 2023 to October 2024

Sampling sites	Total number of Isolates	MEM	SXT	GN	AMC	CAZ	CIP	MDR	MAR Index	ESBL Producing
		n (%)								
Korle Bu overflow wastewater	30	2(7)	24(80)	23(77)	23(77)	28(93)	28(93)	30(100)	0.75	28(93)
KBTH Mortuary Drain wastewater	42	3(7)	32(76)	36(86)	36(86)	39(93)	42(100)	42(100)	0.77	39(93)
Mudor WWTP effluent	45	2(4)	37(81)	37(81)	37(81)	45(98)	44(96)	45(100)	0.79	39(87)
Lavender Hill WWTP Effluent	23	1(4)	16(70)	23(100)	18(78)	20(87)	22(96)	22(95)	0.71	20(87)

n: number of 3GCR *E. coli* tested; %: percentage of 3GCR *E. coli*; Meropenem; SXT = Trimethoprim-Sulfamethoxazole; GN = Gentamicin; AMC = Amoxicillin-Clavulanic Acid; CAZ = Ceftazidime; CIP = Ciprofloxacin; MDR=Multi drug resistant (Resistant to more than 3 classes of antibiotics); MAR= Multiple Antibiotic Resistance Index; ESBL Producing = percentage of 3GCR *E. coli* isolates confirmed as extended-spectrum β -lactamase

Across all sampling sites, ciprofloxacin resistance was the most prevalent (> 90%), followed by sulfamethoxazole-trimethoprim (75-97%) and amoxycillin clavulanate (67-89%). In contrast, meropenem resistance was consistently low, remaining below < 8%.

Resistance rates in wastewater sources varied across the four primary wastewater sample sites (Table 2). Korle Bu Overflow Wastewater showed high resistance to ciprofloxacin (93%), followed by sulfamethoxazole-trimethoprim and gentamicin (80% and 77%) respectively.

E. coli isolates from KBTH mortuary drain wastewater were completely resistant to ciprofloxacin (100%) and near-complete resistance to amoxicillin-clavulanate (86%). Mudor WWTP Effluent recorded particularly high resistance to ciprofloxacin (96%), whereas meropenem resistance remained relatively low (4%). Meropenem resistance in Lavender Hill FTP was also low at 4%, but high resistance to gentamicin (100%) and ciprofloxacin (96%) was seen in isolates.

330

331 *Comparative analysis of Upstream and Downstream Sites in the Korle Lagoon*

332 Table 3: Antibiotic resistance profiles of *E. coli* isolates recovered from upstream and downstream points of major
333 wastewater discharge sites along the Korle Lagoon, Accra Ghana from November 2023 to October 2024
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Sampling sites	Number of Isolates	MEM	SXT	GN	AMC	CAZ	CIP	MDR	MAR Index	ESBL Producing (%)
n (%)										
KBTH Mortuary upstream	36	1(3)	35(97)	26(72)	24(67)	34(94)	34(94)	35(97)	0.73	33(92)
KBTH Mortuary downstream	47	5(11)	39(83)	36(77)	39(83)	45(96)	44(94)	47(100)	0.78	45(96)
Korle Bu overflow upstream	34	4(12)	30(88)	27(79)	27(79)	34(100)	32(94)	34(100)	0.79	34(100)
Korle Bu Overflow downstream	42	4(10)	40(95)	31(74)	33(79)	38(90)	41(98)	42(100)	0.78	39(93)
Mudor downstream	29	2(7)	28(97)	25(86)	25(86)	28(97)	27(93)	29(100)	0.81	28(97)
Lavender Hill downstream	31	1(3)	27(87)	23(74)	24(77)	30(97)	31(100)	31(100)	0.77	30(97)
Estuary	45	3(7)	36(80)	36(80)	40(89)	41(91)	42(93)	43(95)	0.77	41(91)

335 n: number of 3GCR *E. coli* tested; %: percentage of 3GCR *E. coli*; Meropenem; SXT = Trimethoprim-
336 Sulfamethoxazole; GN = Gentamicin; AMC = Amoxicillin-Clavulanic Acid; CAZ = Ceftazidime; CIP =
337 Ciprofloxacin; MDR=Multi drug resistant (Resistant to more than 3 classes of antibiotics); MAR= Multiple
338 Antibiotic Resistance Index; ESBL Producing = percentage of 3GCR *E. coli* isolates confirmed as extended-
339 spectrum β -lactamase

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342 A comparative analysis of paired upstream and downstream sites showed higher resistance
343 frequencies downstream for some antibiotics. Isolates from the KBTH mortuary upstream
344 showed low meropenem resistance (3%) but high sulfamethoxazole-trimethoprim (97%) and
345 ceftazidime (94%) resistance. Downstream of the KBTH mortuary drain outfall (n = 47),
346 meropenem resistance rose to 11%, and resistance to ciprofloxacin and ceftazidime remained
347 high (94-96%). Korle Bu Overflow Upstream had a ciprofloxacin resistance of 94%, while
348 downstream samples showed increased ciprofloxacin resistance (98%) and meropenem
349 resistance (10%). Mudor WWTP Downstream and Lavender Hill FTP Downstream isolates
350 had high resistance to ciprofloxacin (93-100%), mirroring the patterns seen in wastewater
351 themselves. Spatially, downstream sampling points had slightly higher resistance frequencies

than their corresponding upstream sites; however, these differences were not statistically significant for any of the antibiotics tested (Chi-square test, $p > 0.05$)

Discussion

This study demonstrates that wastewater releases substantial amounts of 3GCR *E. coli* which enter the Korle Lagoon ecosystem. The continuous high counts of 3GCR *E. coli* in wastewater effluents and downstream lagoon water samples demonstrate that wastewater, especially hospital wastewater, may play a vital role in maintaining and increasing antibiotic resistance in the Korle Lagoon.

The marked differences in 3GCR *E. coli* levels found between wastewater sources and lagoon sampling points indicate variable contributions of contamination inputs and subsequent environmental dilution and attenuation processes within the lagoon system. The 3GCR *E. coli* concentrations in Lavender Hill FTP effluent samples were lower than the Mudor WWTP sources but still present significant risks for both environmental health and public safety. The highest 3GCR *E. coli* levels were found in Korle Bu pumping station overflow and Mudor WWTP effluent samples because these discharges contain wastewater from the KBTH which is likely to contain very resistant bacteria due to the complexity of care delivered at the hospital. The Mudor plant relies on primary treatment followed by an upflow anaerobic sludge blanket (UASB) process without a dedicated disinfection or tertiary polishing stage (Ahmed et al., 2018). The anaerobic configurations used are optimised for organic load reduction and biogas recovery rather than the elimination of microbial contaminants. Available evidence indicates that UASB based systems achieve small and inconsistent reductions of antibiotic-resistant bacteria and their resistance genes and under certain conditions, anaerobic environments may even permit the survival or selective enrichment of

resistant organisms (45). The persistence of elevated 3GCR *E. coli* in the Mudor effluent observed here is therefore consistent with the recognized limitations of conventional anaerobic treatment. Inadequate treatment of wastewater means high concentrations of clinically important resistant bacteria are released into the receiving Korle Lagoon. These findings make a compelling case for integrating advanced or alternative treatment technologies which are capable of specifically targeting resistant bacteria and their genetic determinants.

The presence of elevated 3GCR *E. coli* concentrations at all wastewater discharge points demonstrates that both treated and untreated effluents increase microbial contamination and antibiotic resistance spread throughout the lagoon system.

The high levels of co-occurrence of ciprofloxacin resistance are expected with ESBL *E. coli* is expected. ESBL are frequently associated with plasmid mediated resistance determinants that co-carry resistance to fluoroquinolones (Partridge et al., 2018). As a result, ESBL *E. coli* are usually multidrug-resistant and frequently co-resistant to fluoroquinolones such as ciprofloxacin (47,48)). This pattern reduces therapeutic options, since both first-line β -lactams and a key alternative class are compromised in the same isolate.

The prevalence of ESBL *E. coli* across sampling sites mirror global reports that highlight the spread of ESBL Enterobacteriaceae in wastewater and surface water (49,50). This reflects the environmental persistence and spread of MDR Enterobacteriaceae.

Meropenem resistance rate remained below 8% which suggests that carbapenem resistant strains have not yet become widely established in the environment. This finding may reflect the comparatively restricted use of carbapenems in Ghana, where these Reserve antibiotics remain less frequently prescribed than other antibiotic classes. Nonetheless, the detection of carbapenem resistance even at low levels, is concerning as carbapenems are last resort

antibiotics and their resistance genes (e.g., blaNDM, blaKPC) can easily spread via horizontal gene transfer in aquatic systems (51,52).

The observed increase in resistance frequencies at some downstream sites compared to upstream points provides evidence of the dissemination of AMR along the Korle Lagoon from wastewater discharge. Although these increases were not statistically significant, the trend suggests a potential influence of wastewater inputs on resistance patterns, which may not have reached statistical significance due to limited sample size and the already high baseline resistance among the selected third-generation cephalosporin-resistant *E. coli* isolates. This pattern aligns with prior studies showing progressive accumulation of resistant bacteria and resistance genes downstream of effluent discharges (Adomako et al., 2021; Banu et al., 2021; Elmarghani et al., 2025). Wastewater plumes act as hotspots for the spread of resistance, where high concentrations of nutrients, antibiotic residues, and resistant microorganisms create selective environments that favour the survival and horizontal gene transfer of resistance determinants (56). Over time, these conditions can lead to the creation of persistent AMR reservoirs within sediments and the water column.

The estuary where freshwater from the lagoon mixes with sea water, showed substantial resistance to multi antibiotics. It also showed high 3GCR *E. coli* suggesting that 3GCR *E. coli* may reach the marine interface. High resistance in lagoon and estuary water poses long-term ecological and public health risks to surrounding communities that depend on the lagoon and sea for livelihood and recreation. Residents may become colonized with these resistant bacteria through contact during fishing, washing, and recreation. Such carriage can persist in the gut and later act as a reservoir for hard-to-treat community acquired infections (57)

However, the overall bacterial load at the estuary was lower compared to other sites, possibly due to the dilutional effect of tidal seawater exchange; this can reduce bacterial counts without necessarily eliminating antimicrobial resistance.

The findings of this study underscore the urgent need for the improvement of wastewater management and AMR surveillance in Ghana. Continuous routine monitoring of AMR in wastewater systems should be integrated into national surveillance programmes. This could serve as an early warning system for emerging resistance trends, which could complement clinical surveillance efforts within a One Health framework.

The high prevalence of *3GCR E. coli* in WWTP effluents indicates that the current treatment infrastructure at the Mudor WWTP and Lavender Hill FTP, is inadequate for controlling the dissemination of AMR into the Korle Lagoon. The Integration of advanced treatment technologies has the potential to enhance the removal efficiency of bacteria, antibiotic resistant bacteria and genes. Studies have shown that integrating advanced tertiary treatment technologies including ozonation, ultraviolet (UV) disinfection, membrane filtration, and antimicrobial blue light (aBL) irradiation can significantly improve removal efficiencies of ARB and ARGs (58–63). This is achieved through the synergistic effects of oxidative, physical, and photonic inactivation processes. Ozonation and UV-based advanced oxidation processes have shown multi-log reductions in ARGs and ARB under optimized conditions (64). Furthermore, membrane technologies such as ultrafiltration and nanofiltration, offer effective physical barriers, although concentrated retentates may necessitate additional treatment (62) Antimicrobial blue light disinfection has also shown promise in inactivating ARB and degrading resistance genes (58).

This study has some limitations. Although each sample was a composite of three grab samples collected at 15-minute intervals to reduce short-term variability, this approach only

partially mitigates the temporal limitations inherent to grab based sampling. A 45-minute composite window cannot capture diurnal fluctuations in bacterial abundance and resistance gene prevalence that may occur over the course of a full day given the heterogeneity of wastewater discharge patterns. Grab sampling captures only a single point in time, which may not represent temporal fluctuations in bacterial abundance and resistance genes in heterogeneous environmental samples (65,66). Time of day, temperature, flow, and total dissolved solids are factors that can influence microbial recovery and CFU counts. Another limitation of this study is the lack of periodic data on water inflow volumes into the lagoon. This restricts the ability to assess how variations in hydrological input influence bacterial concentrations and distribution within the lagoon system over time.

Conclusion

This study is the first comprehensive assessment of 3GCR *E. coli* in wastewater discharged into the Korle Lagoon and its potential effect on the Lagoon. The findings highlight high concentrations of 3GCRE in wastewater effluents entering the Korle lagoon, with evidence on significant influence in AMR concentrations in the lagoon. Wastewater discharges into the lagoon may have significant ecological and public health implications. To address this issue there is the need for continued improvement of wastewater treatment including exploring advanced treatment modalities aimed at reducing antibiotic resistant bacteria and genes.

Acknowledgement

We acknowledge Mr George Duker Hayfon for his support with the laboratory work as well as staff of the Department of Medical Microbiology of the University of Ghana Medical School and the CSIR-Water Research Institute Microbiology Laboratory and Biomedical Unit. Further

thanks go to the management of the Mudor Wastewater Treatment Plant, Sewerage Systems
Ghana limited and Accra Sewerage Improvement Project.

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Author Contributions

LABA: Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. A-KL: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. NO-K: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. JO: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Disclaimer

The funders had no role in the study design, data collection, analysis, interpretation, or manuscript writing.

Financial Support

This research was funded by the International Center for Antimicrobial Resistance Solutions (ICARS) under the JPIAMR funding program.

730 **Potential Conflict of Interest**

731 All authors declare no conflict of interest

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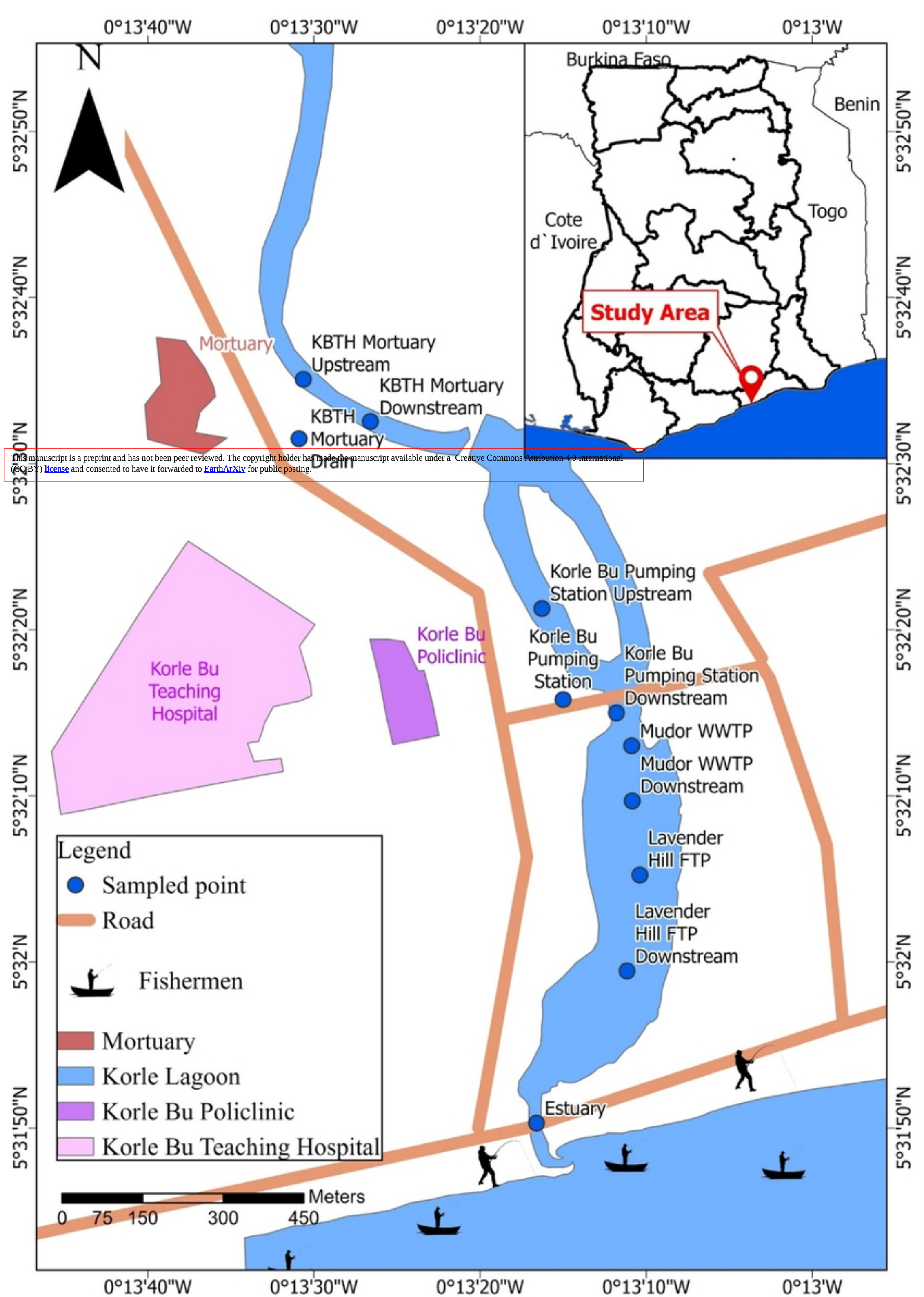


Figure1

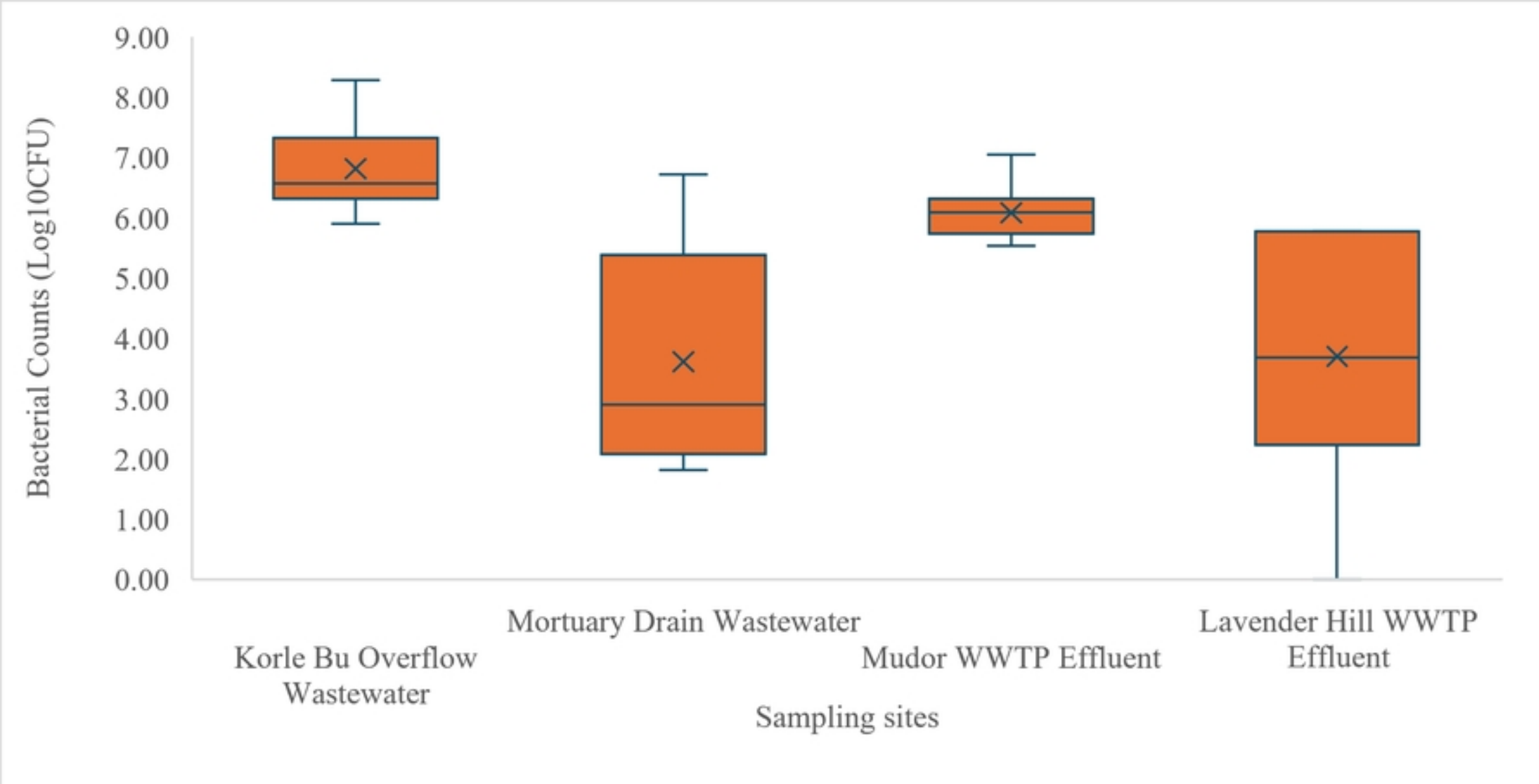


Figure2

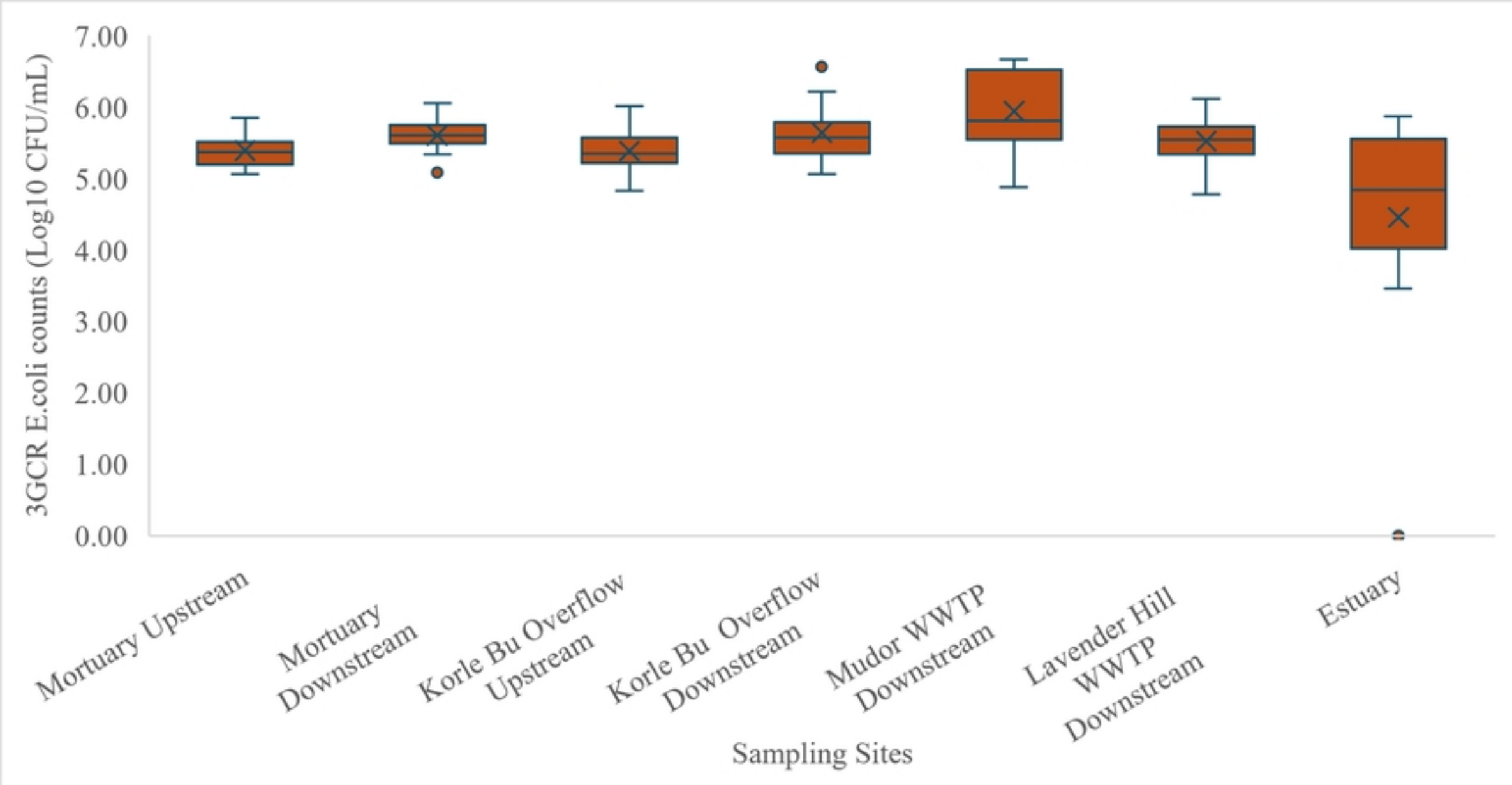


Figure3