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# Prevalence of Antibiotic Resistance Among Coliform Bacteria Isolated from Surface Water Samples in Kisumu County, Kenya

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## Abstract

# Background

Environmental waters and animal-associated matrices can serve as reservoirs and transmission pathways for antimicrobial-resistant bacteria. Screening for antimicrobial resistance (AMR) across interconnected environmental and animal sources is important for One Health surveillance, particularly in settings where humans, livestock and surface-water systems interact closely.

# Methods

We conducted a cross-sectional environmental study across 13 sampling sites representing urban streams and rivers, rural rivers and Lake Victoria shoreline sites. Water samples were assessed for total coliform contamination, while coliform isolates recovered from water, animal feed and animal waste were identified and subjected to antimicrobial susceptibility testing. Resistance was assessed against seven antibiotics representing major antimicrobial classes. Multiple antibiotic resistance (MAR) indices were calculated. Differences in multi drug resistance (MDR) by sample source were evaluated using logistic regression, with water isolates as the reference category. Site-level coliform burden was correlated with mean MAR index using Spearman's rank correlation.

# Results

A total of 79 coliform isolates were recovered, comprising 46 (58.2%) from water, 11 (13.9%) from animal feed and 22 (27.8%) from animal waste. *Klebsiella pneumoniae* (30/79, 38.0%) and *Escherichia coli* (27/79, 34.2%) were the predominant species. Overall, 20/79 (25.3%) isolates were MDR. Resistance was highest to amoxicillin (68.4%), followed by amoxicillin-clavulanic acid (40.5%) and gentamicin (29.1%), while no meropenem resistance was detected. Prevalence rates of MDR differed substantially by source: 54.5% in animal feed, 36.4% in animal waste, and 13.0% in water. Compared with water isolates, isolates from animal feed had eight-fold greater odds of MDR (OR 8.00, 95% CI 1.89–37.04; $p$ = 0.005), while isolates from animal waste had approximately four-fold greater odds (OR 3.81, 95% CI 1.14–13.51; $p$ = 0.032). Site-level coliform burden was not associated with mean MAR index (Spearman $r$ = 0.106, $p$ = 0.731; n = 13 sites).

# Conclusion

Substantial antimicrobial resistance and MDR were detected across environmental and animal-associated coliform isolates, with particularly elevated MDR among isolates from animal feed and waste. The absence of an association between coliform abundance and MAR index suggests that bacterial contamination burden alone may not reliably indicate AMR risk. These findings support

integrated One Health surveillance incorporating environmental waters, livestock-associated sources and antimicrobial resistance phenotyping.

# Introduction

Antimicrobial resistance (AMR) is a major global public health challenge that extends beyond healthcare settings into interconnected human, animal, and environmental systems [1, 2]. Continuous use of antibiotics in humans and food-producing animals remains an important driver of resistance selection [3, 5]. Environmental compartments can therefore receive antimicrobial residues, resistant bacteria, and antimicrobial resistance genes (ARGs) [6, 7] through wastewater discharge, faecal contamination, agricultural runoff, animal waste, and other anthropogenic inputs [8–12]. Aquatic environments are particularly important because they can provide interfaces for the persistence, mixing, and dissemination of resistant organisms and resistance determinants originating from multiple sources [8, 9, 12].

These environmental pathways may be especially important where rivers, streams, lakes, and ponds support overlapping domestic, agricultural, livestock, recreational, and commercial activities. Enteric bacteria such as *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp. can occur across human, animal, and environmental compartments, including contaminated surface and drinking waters. Research in Kenya has demonstrated clinically relevant AMR across wildlife, livestock, humans, and their shared environments [3], while more recent One Health investigations have identified resistant Enterobacterales across human, animal, water, and soil compartments [7]. Genomic studies from Nairobi have further demonstrated substantial diversity of antimicrobial-resistant *E. coli* among sympatric humans, livestock, and peri-domestic wildlife [13]. Together, this evidence reinforces the importance of considering environmental and animal-associated sources as components of AMR ecology while recognizing that the detection of similar resistance phenotypes across compartments does not, by itself, demonstrate transmission [14].

Multidrug-resistant (MDR) Enterobacterales are of particular concern because resistance across multiple antimicrobial categories can restrict therapeutic options if resistant organisms subsequently cause infections in humans or animals. MDR is conventionally defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories [1]. In addition to categorical MDR classification, the multiple antibiotic resistance (MAR) index provides a quantitative measure of the proportion of tested antimicrobial agents to which an isolate is resistant and has long been used to characterize bacterial populations according to their resistance burden [2]. Combining antimicrobial-specific resistance frequencies, MDR prevalence, and MAR indices can therefore provide complementary measures for characterizing resistance across environmental and animal-associated bacterial populations.

The Lake Victoria basin provides an important setting in which to investigate environmental AMR. Lake Victoria is Africa's largest lake and a major transboundary freshwater ecosystem shared principally by

Kenya, Uganda, and Tanzania [10]. The lake and its tributaries support domestic water use, fisheries, agriculture, livestock production, transport, and other livelihoods, while also receiving inputs associated with expanding urban settlements, wastewater discharge, agricultural activities, and animal waste [10]. These multiple uses create interfaces through which microbial contaminants, antimicrobial residues, resistant organisms, and resistance determinants may enter and persist within the aquatic environment. Studies elsewhere in Kenya have already demonstrated antibiotics in wastewater, receiving surface waters, and sediments at concentrations associated with potential resistance-selection pressure [10], while pharmaceutical contamination has also been documented within the Nairobi River Basin [11].

Emerging evidence indicates that AMR is already an important component of microbial pollution within Lake Victoria [15]. Metagenomic investigation of the Winam Gulf in Kenya demonstrated extensive anthropogenic and microbial pollution [15], followed by detection of a diverse environmental resistome containing ARGs associated with multiple antimicrobial classes [4]. More recently, analysis of the Lake Victoria–Kisumu water and wastewater network identified clinically relevant bacterial taxa, including *Klebsiella pneumoniae* and *E. coli*, together with diverse resistance determinants across lake, river, wastewater, and water-supply compartments [5]. Evidence from drinking-water sources in neighbouring northern Tanzania has similarly demonstrated multidrug-resistant *E. coli* carrying plasmids and resistance genes [16]. These findings indicate that resistant Enterobacterales and their resistance determinants occur within aquatic environments in the wider East African region and reinforce the value of freshwater ecosystems as targets for One Health AMR surveillance.

Conventional environmental microbiological surveillance, however, often focuses on indicators of bacterial contamination rather than the antimicrobial resistance characteristics of the organisms recovered. Measures such as coliform concentrations provide useful information on microbiological water quality but do not directly quantify phenotypic resistance or the underlying environmental resistome. This distinction may be important because environmental AMR is influenced by multiple interacting processes, including antimicrobial residues, wastewater and agricultural inputs, microbial community structure, horizontal gene transfer, and other ecological selection pressures [8–12]. Consequently, a site with substantial bacterial contamination may not necessarily harbour the greatest resistance burden, while comparatively less contaminated environments may still contain MDR organisms. Integrating bacterial abundance with phenotypic resistance, MDR, and MAR indices could therefore provide information that is not captured by conventional microbiological indicators alone.

Despite growing evidence of environmental AMR in Kenya, important gaps remain in the phenotypic characterization of resistant bacteria across interconnected environmental water and animal-associated sources within the Lake Victoria basin. Much of the recent Lake Victoria evidence has relied on metagenomic characterization of aquatic environments [4, 15] or investigation of specific water and wastewater systems [5], with comparatively limited information integrating environmental bacterial burden with culture-based antimicrobial susceptibility across water, animal feed, and animal waste. Furthermore, whether locations with greater bacterial contamination also harbour a greater phenotypic AMR burden remains poorly understood. Addressing these gaps is important because AMR within a

shared freshwater ecosystem may have implications extending beyond individual sampling locations to human and animal health, food production, and environmental health.

This study therefore aimed to characterize environmental coliform contamination and antimicrobial resistance across water and animal-associated sources within a One Health framework in the Lake Victoria basin, Kenya. Specifically, we sought to: (i) characterize coliform contamination across environmentally diverse sampling sites; (ii) describe the distribution of coliform species recovered from water, animal feed, and animal waste; (iii) determine antimicrobial-specific resistance patterns and the prevalence of MDR across sample sources; (iv) compare the odds of MDR among isolates recovered from animal feed and animal waste relative to water isolates; and (v) determine whether site-level coliform burden was associated with the MAR index of recovered isolates.

## Methods

# Study design and setting

A cross-sectional environmental study incorporating a One Health approach to antimicrobial resistance (AMR) surveillance was conducted in Kisumu County, western Kenya. Environmental water samples were collected from 13 different geographical locations in Kisumu County composed of streams rivers and dams (Fig. 4).

Sampling was conducted in January – July 2026, encompassing both dry and rainy seasons. Sites were selected purposively to capture environmental settings with differing potential sources and intensities of human, animal, agricultural, and industrial influence on microbial contamination and AMR dissemination.

## Environmental and animal-associated sampling

A total of 26 water samples were collected from the 13 environmental sampling sites, with between one and four samples collected per site. Samples were collected during the dry and rainy seasons to allow assessment of seasonal variation in bacterial contamination.

Water samples were collected in sterile 50ml falcon tubes, transported to the laboratory in an insulated cooler box with ice packs, and processed within 2 hours of collection. Dilution was done in sterile 0.9% NaCl, vacuum filtration system using 0.45µm sterile filters. Filters were then placed on a McConkey agar.

Total coliform concentrations were determined and expressed as colony forming units per 100 mL (CFU/100 mL) using membrane filtration technique.

In addition to water sampling, animal feed and animal waste samples were collected from vendors and farmers respectively around Kisumu County using sterile tweezers and placed in 50ml falcon tubes to characterize AMR across interconnected environmental and animal-associated compartments.

## Isolation and identification of coliform bacteria

Coliform bacteria (pink/red colonies) were isolated from water, animal feed, and animal waste samples using MacConkey agar and incubated at $37^0$C ± 2 in an inverted position for 18–24 hours. The selected coliforms were streaked on the plate and species identification done using using API 20E strips from biomerieux. The codes were interpreted using apiweb standalone V1.3.2 (biomerieux).

Quality control during bacterial identification was performed using *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 700603 strains.

## Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method using Rosco Neo-Sensitabs.

Each isolate was tested against seven antibiotic drugs representing clinically relevant antimicrobial categories: amoxicillin (penicillins), amoxicillin-clavulanic acid (penicillin/β-lactamase inhibitor combination), ceftazidime (third-generation cephalosporins), ciprofloxacin (fluoroquinolones), doxycycline (tetracyclines), gentamicin (aminoglycosides), and meropenem (carbapenems).

For disk diffusion, inhibition-zone diameters were measured in millimetres and interpreted according to EUCAST and CLSI breakpoints as susceptible, intermediate, or resistant. For the primary resistance analysis, intermediate isolates were treated as non-susceptible.

Quality control of antimicrobial susceptibility testing was performed using *E. coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 700603 strains.

## Definition of MDR and MAR index

Multidrug resistance (MDR) was defined according to the conventional international definition as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories. Isolates meeting this criterion were classified as MDR, while those resistant to fewer than three antimicrobial categories were classified as non-MDR.

The multiple antibiotic resistance (MAR) index was calculated for each isolate as the ratio of the number of antimicrobial agents to which the isolate was resistant to the total number of antimicrobial agents tested:

$$\mathrm{MAR\backslash:index} = \frac{\mathrm{Number\backslash:of\backslash:antimicrobials\backslash:to\backslash:which\backslash:the\backslash:isolate\backslash:was\backslash:resistant}}{\mathrm{Total\backslash:number\backslash:of\backslash:antimicrobials\backslash:tested}}$$

Given that seven antimicrobial agents were tested in this study, individual MAR indices ranged from 0 to 1, with higher values indicating resistance to a greater proportion of the tested antimicrobial panel.

# Statistical analysis

Descriptive statistics were used to summarize bacterial contamination and antimicrobial resistance patterns. Antimicrobial resistance frequencies were reported as counts and percentages for each antimicrobial agent, overall and stratified by sample source (water, animal feed, and animal waste). Multidrug resistance (MDR) prevalence was similarly summarized by sample source. MDR was defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories.

The association between sample source and MDR status was assessed using univariable logistic regression, with MDR status (yes/no) as the binary outcome and sample source as the predictor. Water isolates were specified as the reference category. Effect estimates were reported as odds ratios (ORs) with 95% confidence intervals (CIs) and corresponding **p**-values.

Total coliform concentrations (CFU/100 mL) were highly right-skewed and were therefore summarized using medians and evaluated on a logarithmic scale for graphical presentation. Differences in coliform concentrations between the dry and rainy seasons were assessed using the Wilcoxon rank-sum test. At the sampling-site level, median coliform concentration was calculated for each site and its relationship with the site's mean multiple antibiotic resistance (MAR) index was assessed using Spearman's rank correlation coefficient (ρ) because of the small number of sites and the non-normal distribution of coliform concentrations.

All statistical tests were two-sided, and a $p$-value < 0.05 was considered statistically significant. Analyses were conducted using R statistical software (Version 4.5.3; R Foundation for Statistical Computing, Vienna, Austria).

## Results

# Distribution of bacterial isolates

A total of 79 coliform isolates were recovered across the three sample sources, that included environmental water samples (from 13 sites), animal feeds and waste. Water accounted for 46 (58.2%) isolates, animal waste for 22 (27.8%), and animal feed for 11 (13.9%) (Table 1).

*Klebsiella pneumoniae* was the most frequently identified species (30/79; 38.0%), followed by *Escherichia coli* (27/79; 34.2%). *Enterobacter cloacae* and *Klebsiella oxytoca* each accounted for 7/79 (8.9%) isolates. Less frequently detected organisms included *Cronobacter* spp. and *Serratia odorifera* (Table 1).

Species distribution varied across sample sources. Most *K. pneumoniae* isolates originated from water (23/30), whereas *E. coli* predominated among animal-waste isolates, accounting for 17 of the 22 isolates recovered from this source (Table 1).

Table 1
Distribution of bacterial isolates by sample source and multidrug-resistance status

| Species | Total, n | Overall, % | Water, n | Feed, n | Animal waste, n | MDR, % |
|---|---|---|---|---|---|---|
| *K. pneumoniae* | 30 | 38.0 | 23 | 5 | 2 | 20.0 |
| *E. coli* | 27 | 34.2 | 10 | 0 | 17 | 29.6 |
| *E. cloacae* | 7 | 8.9 | 4 | 1 | 2 | 42.9 |
| *K. oxytoca* | 7 | 8.9 | 5 | 1 | 1 | 28.6 |
| Other | 4 | 5.1 | 3 | 1 | 0 | 0.0 |
| *Cronobacter* spp. | 2 | 2.5 | 1 | 1 | 0 | 0.0 |
| *S. odorifera* | 2 | 2.5 | 0 | 2 | 0 | 50.0 |
| Total | 79 | 100.0 | 46 | 11 | 22 | 25.3 |

**Note: MDR, multidrug resistance.**

# Antimicrobial resistance patterns by sample source

Amoxicillin (68.4%) showed the highest overall antibiotic resistance across all sampled sources (Fig. 1), peaking in animal feed at 82%, while Meropenem shows completely 0% resistance across the sampled sources. In general, animal feed serves as the primary source for the highest resistance rates—specifically dominating in Amoxicillin and Gentamicin—whereas animal waste displays a distinct resistance profile, showing the highest vulnerability to Doxycycline (32%) and acting as the exclusive source of resistance for Ciprofloxacin (23%). Water maintains low-to-moderate resistance levels overall, demonstrating moderate resistance to older beta-lactams but remaining entirely unaffected by Ciprofloxacin and Meropenem.

Figure 1. **Antimicrobial resistance profiles of bacterial isolates by sample source.**

Percentage of isolates resistant to each antimicrobial agent among isolates recovered from water (n = 46), animal feed (n = 11), and animal waste (n = 22). AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; CAZ, ceftazidime; CIP, ciprofloxacin; DOX, doxycycline; GEN, gentamicin; MEM, meropenem.

# Multidrug resistance by sample source

Overall, 20/79 (25.3%) isolates met the definition of multidrug resistance. MDR prevalence varied considerably by sample source, occurring in 6/46 (13.0%) water isolates, 6/11 (54.5%) animal-feed isolates, and 8/22 (36.4%) animal-waste isolates (Table 2).

In univariable logistic regression, sample source was significantly associated with MDR status. Compared with isolates recovered from water, animal-feed isolates had eight-fold higher odds of MDR (OR = 8.00; 95% CI: 1.89–37.04; $p = 0.005$), while animal-waste isolates had approximately four-fold higher odds of MDR (OR = 3.81; 95% CI: 1.14–13.51; $p = 0.032$)

Table 2
Association between sample source and multidrug resistance among bacterial isolates

| Sample source | Total, n | MDR, n (%) | OR | 95% CI | *p*-value |
|---|---|---|---|---|---|
| Water | 46 | 6 (13.0) | 1.00 | Reference | — |
| Animal feed | 11 | 6 (54.5) | 8.00 | 1.89–37.04 | **0.005** |
| Animal waste | 22 | 8 (36.4) | 3.81 | 1.14–13.51 | **0.032** |
| **Overall** | **79** | **20 (25.3)** | — | — | — |

*OR, odds ratio; CI, confidence interval; MDR, multidrug resistance. Odds ratios were estimated using univariable logistic regression with water isolates as the reference category.*

Figure 2. **Association between sample source and multidrug resistance.**Forest plot showing odds ratios (ORs) and 95% confidence intervals (CIs) from univariable logistic regression examining the association between sample source and MDR. Water isolates were used as the reference category.

# Environmental coliform contamination across sampling sites

Coliform contamination was detected across all sampled environmental sites, although concentrations varied considerably (Fig. 3). Median site-level concentrations ranged from approximately 2,000 CFU/100 mL at Kibos Steel Mill to 200,000 CFU/100 mL at Golf Club Bridge. The highest median concentrations were observed at Golf Club Bridge (200,000 CFU/100 mL), CarWash Bridge (150,000 CFU/100 mL), and Obunga Stream (101,000 CFU/100 mL), demonstrating substantial spatial heterogeneity in bacterial contamination across the sampled environments.

Figure 3. **Distribution of total coliform concentrations across environmental sampling sites.**Total coliform concentrations (CFU/100 mL) across the 13 environmental sampling sites. The logarithmic scale is used to accommodate the highly skewed distribution of coliform concentrations.

# Seasonal variation in coliform concentrations

Total coliform concentrations tended to be higher during the dry season than during the rainy season (Fig. 4). However, the difference did not reach the conventional threshold for statistical significance (Wilcoxon rank-sum test, $p = 0.059$). Thus, although the observed distributions suggested a possible seasonal pattern, there was insufficient evidence to conclude that coliform concentrations differed significantly between seasons.

Figure 4. **Total coliform concentrations by sampling season.**Distribution of total coliform concentrations (CFU/100 mL) during the dry and rainy seasons. Differences between seasons were evaluated using the Wilcoxon rank-sum test ($p$ = 0.059). Coliform concentrations are presented on a logarithmic scale.

# Relationship between environmental contamination and antimicrobial resistance

There was no evidence of an association between site-level bacterial contamination and antimicrobial resistance burden (Fig. 5). Median coliform concentration was weakly and positively correlated with mean MAR index, but the association was not statistically significant (Spearman's ρ = 0.106, $p$ = 0.731; n = 13 sites).

Sites with high coliform concentrations did not consistently exhibit high MAR indices, indicating that the magnitude of bacterial contamination and the extent of antimicrobial resistance among recovered isolates were not closely related in this dataset.

Figure 5. **Relationship between site-level coliform concentration and multiple antibiotic resistance index.**

Scatter plot showing the relationship between median total coliform concentration (CFU/100 mL) and mean MAR index across 13 sampling sites. The association was assessed using Spearman's rank correlation (ρ = 0.106, $p$ = 0.731).

# Spatial variation in antimicrobial resistance burden

The antimicrobial resistance burden varied across environmental sampling sites (Fig. 6). Differences were observed in both the proportion of multidrug-resistant isolates and the mean multiple antibiotic resistance (MAR) index, indicating spatial heterogeneity in the distribution of resistance within the study area. Sites with relatively high MDR prevalence did not consistently correspond to those with the highest coliform concentrations, further suggesting that the magnitude of bacterial contamination alone may not reflect the underlying AMR burden.

Given the relatively small number of isolates recovered from some individual sites, these site-level patterns were interpreted descriptively rather than as evidence of statistically significant differences between sites.

Figure 6. **Spatial variation in antimicrobial resistance burden across environmental sampling sites.**Distribution of antimicrobial resistance across sampling sites, presented using site-specific MDR prevalence and/or MAR index. MDR was defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories. MAR, multiple antibiotic resistance index.

# Discussion

This study provides phenotypic evidence of antimicrobial resistance across environmental water and animal-associated bacterial isolates within the Lake Victoria basin and demonstrates substantial heterogeneity in resistance across sample sources and sampling locations. Five principal findings emerged. First, *Klebsiella pneumoniae* and *Escherichia coli* predominated among the recovered isolates. Second, resistance was highest to amoxicillin and amoxicillin-clavulanic acid, whereas no resistance to meropenem was detected. Third, MDR was substantially more frequent among isolates recovered from animal feed and animal waste than among water isolates. Fourth, environmental coliform concentrations varied considerably across sampling sites and tended to be higher during the dry season, although the seasonal difference was not statistically significant. Finally, site-level coliform burden was not associated with mean MAR index. These findings extend the emerging environmental AMR evidence from the Lake Victoria region by integrating culture-based resistance phenotypes with bacterial contamination across water and animal-associated matrices.

**Predominance of Klebsiella pneumoniae and Escherichia coli**

*K. pneumoniae* and *E. coli* accounted for most isolates recovered in this study, although their distribution differed across sample sources. The predominance of *K. pneumoniae* among water isolates is particularly noteworthy given the clinical importance of this species and its ability to persist outside clinical environments. Recent investigation of the Kisumu water and wastewater network similarly identified *K. pneumoniae*, *E. coli*, and other clinically relevant bacterial taxa across Lake Victoria, river, wastewater, and water-supply compartments [5]. Metagenomic investigations of the Winam Gulf have also demonstrated diverse microbial communities and ARGs within the Lake Victoria aquatic ecosystem [4, 6].

The occurrence of *E. coli* across the sampled matrices, particularly animal waste, is consistent with Kenyan One Health evidence. Kavulavu et al. identified resistant Enterobacterales across humans, drinking water, animal faeces, and soil in southwestern Kenya [7]. Studies from Nairobi have similarly documented antimicrobial-resistant *E. coli* among humans, livestock, wildlife, and their shared environments [3, 13]. Our findings therefore add phenotypic evidence from the Lake Victoria basin to an expanding body of Kenyan research demonstrating that clinically relevant Enterobacterales occur across multiple ecological compartments.

# Antimicrobial resistance patterns

Resistance was most frequent to amoxicillin, followed by amoxicillin-clavulanic acid and gentamicin, whereas resistance to doxycycline, ceftazidime, and ciprofloxacin was less common. This predominance of resistance to older β-lactam antimicrobials is broadly consistent with findings from Kenya and the wider East African region. Kavulavu et al. reported substantial ampicillin resistance among Enterobacterales recovered across human, animal, and environmental sources in Kisii [7], while studies at the human–livestock interface in Nairobi have documented resistance to several commonly used

antimicrobial classes [3, 13]. Resistant *E. coli* carrying plasmid-mediated resistance determinants have also been recovered from drinking-water sources in northern Tanzania [14].

Differences between individual antimicrobial resistance frequencies across studies should nevertheless be interpreted cautiously because of variation in bacterial populations, sample sources, antimicrobial panels, breakpoints, and susceptibility-testing methods. The resistance patterns observed here are therefore more informative as evidence of the presence and distribution of resistance within the sampled ecosystem than as direct prevalence comparisons with other settings.

None of the isolates in the present study was resistant to meropenem. This finding is encouraging given the importance of carbapenems for treatment of severe infections caused by resistant Gram-negative bacteria. However, the absence of phenotypic meropenem resistance among 79 cultured isolates does not establish the absence of carbapenem-resistance determinants from the wider environment. Metagenomic studies of the Winam Gulf and the Kisumu water and wastewater network have demonstrated a substantially broader environmental resistome than can be captured through susceptibility testing of selected cultured isolates [4–6]. The combination of culture-based phenotyping and molecular characterization would therefore provide a more complete assessment in future surveillance.

# Greater MDR burden in animal-associated sources

The clearest source-specific finding was the substantially greater MDR burden among animal-associated isolates. More than half of animal-feed isolates and over one-third of animal-waste isolates were MDR, compared with approximately one in eight water isolates. Consistent with these proportions, animal-feed isolates had eight-fold higher odds of MDR and animal-waste isolates had nearly four-fold higher odds compared with water isolates. Although these estimates have relatively wide confidence intervals because of the small numbers of animal-associated isolates, the direction and magnitude of the associations identify these matrices as priorities for further investigation.

The finding is consistent with evidence that livestock production systems can harbour antimicrobial-resistant Enterobacterales. Hassell et al. demonstrated clinically relevant resistance across livestock, humans, wildlife, and their shared environment in Nairobi [3], while genomic investigations have demonstrated diverse resistant *E. coli* populations among sympatric humans and animals in the same setting [13]. A systematic review examining the potential contribution of food animals to resistant *E. coli* in humans similarly highlighted the complexity of resistance exchange between animal and human populations [12]. These studies support inclusion of animal-associated matrices in One Health AMR surveillance rather than focusing exclusively on clinical isolates or environmental water.

The particularly high MDR prevalence among animal-feed isolates warrants further investigation. Feed may become contaminated during production, transportation, storage, handling, or contact with animals and their immediate environment. Antimicrobial use in livestock production may also create selection pressures within animal-production systems. However, antimicrobial-use practices, antimicrobial

residues, and feed-production processes were not measured in this study. The observed association should therefore not be interpreted as evidence that antimicrobial exposure in feed caused the higher MDR prevalence.

Similarly, the present findings cannot establish transmission of resistant organisms between animal feed, animal waste, and environmental water. Demonstrating transmission would require comparison of bacterial lineages and resistance determinants across sources, ideally using whole-genome sequencing and characterization of mobile genetic elements [12, 13]. The present findings instead identify ecological compartments in which resistant organisms coexist and from which potential transmission pathways can be investigated in future studies.

# Spatial and seasonal heterogeneity in bacterial contamination

Environmental coliform concentrations varied markedly among sampling locations, demonstrating substantial spatial heterogeneity in bacterial contamination. Several urban sites had particularly high concentrations, suggesting that microbial inputs were not uniformly distributed across the sampled environment. This is consistent with previous work in the Winam Gulf demonstrating substantial spatial variation in microbial and anthropogenic pollution [6]. More recent investigations have identified diverse ARGs and clinically relevant bacteria within Lake Victoria and connected water and wastewater systems [4, 5].

Rather than simply reflecting a single contamination source, the observed spatial variation is likely to represent differences in local environmental conditions and anthropogenic inputs. Kenyan studies have documented antimicrobial residues in wastewater, surface waters, and sediments [9] and pharmaceutical contamination within the Nairobi River Basin [10]. Such findings demonstrate that aquatic environments can receive both microbial contaminants and chemical selective pressures. However, because the present study did not quantify wastewater inputs, antimicrobial residues, land use, or other site-specific exposures, the drivers of differences between individual sampling sites cannot be determined from these data.

Coliform concentrations tended to be higher during the dry season than during the rainy season, but the difference was not statistically significant ($p = 0.059$). This should therefore be interpreted as a possible seasonal signal rather than evidence of a definitive seasonal effect. Seasonal patterns in surface-water bacterial contamination can reflect competing processes: rainfall may increase runoff and transport of faecal material, whereas reduced flow and dilution during dry periods may concentrate existing contamination. The limited number of water samples in the present study restricts inference about these processes, and repeated sampling across multiple wet and dry seasons would be needed to determine whether the observed pattern is reproducible.

# Coliform burden did not predict antimicrobial resistance burden

A particularly important finding was the absence of evidence for an association between site-level coliform concentration and mean MAR index (Spearman's ρ = 0.106, $p$ = 0.731). Despite substantial differences in bacterial concentrations among sampling sites, sites with higher coliform burdens did not consistently have higher resistance indices. This finding indicates that bacterial abundance and antimicrobial resistance burden captured different characteristics of the sampled environments.

This distinction is important because coliform concentrations are commonly used to characterize microbiological water quality, whereas MAR directly summarizes the resistance phenotype of recovered isolates. MAR indexing was originally developed to characterize resistance across bacterial isolates and identify populations associated with differing antimicrobial exposure histories [2]. The weak relationship observed in the present study suggests that high bacterial contamination alone cannot be assumed to identify sites with the greatest AMR burden.

Several mechanisms could contribute to this disconnect. Environmental AMR is shaped not only by the abundance of bacteria entering an aquatic system but also by antimicrobial residues, wastewater inputs, agricultural activities, microbial community composition, horizontal gene transfer, and other ecological or co-selective pressures [7–11]. In Kenya, antimicrobials have been detected in wastewater, receiving surface waters, and sediments at concentrations of potential relevance to resistance selection [9]. Meanwhile, metagenomic studies from Lake Victoria demonstrate diverse ARGs whose distribution does not necessarily correspond directly to conventional indicators of bacterial contamination [4–6]. These findings support assessment of AMR as a distinct environmental surveillance endpoint rather than inferring resistance risk solely from bacterial counts.

The site-level heterogeneity in MDR and MAR further supports this interpretation. From a surveillance perspective, combining conventional bacterial water-quality indicators with antimicrobial susceptibility testing may therefore identify risks that would be missed if either measure were used alone. Where resources permit, genomic or metagenomic surveillance could additionally characterize ARGs, mobile genetic elements, and bacterial lineages circulating across environmental and animal-associated sources.

# Strengths and limitations

A major strength of this study was the integration of water, animal feed, and animal waste within a common One Health analytical framework. This enabled direct comparison of phenotypic resistance across ecological compartments rather than examining environmental water in isolation. The simultaneous assessment of antimicrobial-specific resistance, MDR, MAR index, and coliform burden also enabled examination of whether bacterial contamination corresponded with resistance burden. The inclusion of environmentally diverse sampling sites further provided an opportunity to examine spatial and seasonal variation within the Lake Victoria basin.

Several limitations should be considered. First, the overall number of isolates was modest, particularly for animal feed and some individual sampling sites, resulting in relatively wide confidence intervals

around source-specific MDR estimates. The large odds ratio observed for animal feed should consequently be interpreted as a strong signal requiring confirmation rather than a precise estimate of effect size. Second, the cross-sectional design cannot establish temporal relationships or transmission pathways among animal and environmental compartments. Third, phenotypic susceptibility testing does not identify the genetic mechanisms underlying resistance or determine whether resistance determinants are shared among isolates from different sources.

Fourth, the susceptibility panel comprised seven antimicrobial agents and did not encompass all antimicrobial categories relevant to Enterobacterales. MDR should therefore be interpreted according to the operational definition and antimicrobial categories represented in the study panel, rather than as comprehensive resistance profiling across all categories [1]. Fifth, only 26 water samples were collected across 13 sites, limiting statistical power for seasonal and site-level comparisons. The correlation between coliform concentration and MAR index was based on only 13 site-level observations and should therefore be regarded as exploratory. Finally, antimicrobial-use practices, antimicrobial residues, wastewater exposure, livestock-management practices, and other potential environmental drivers were not measured systematically, limiting causal interpretation of the observed source- and site-specific differences.

# Implications for One Health surveillance

The findings have several implications for environmental AMR surveillance. Most importantly, the substantially higher MDR burden among animal-associated isolates indicates that surveillance focused solely on surface water may overlook important reservoirs of resistance within the same ecological system. Animal feed and animal waste should therefore be considered alongside water and, where feasible, human and livestock samples when designing integrated One Health surveillance.

The absence of a relationship between coliform concentration and MAR index provides a second practical implication. Conventional bacterial water-quality monitoring remains important for characterizing microbiological contamination, but these data suggest that it should not be assumed to provide an indirect measure of AMR burden. Incorporating phenotypic susceptibility testing into environmental monitoring could therefore provide complementary information about resistance patterns.

Future studies should expand the number and temporal frequency of samples, incorporate antimicrobial-use and livestock-management information, and quantify antimicrobial residues and other environmental selective pressures. Whole-genome sequencing and metagenomic approaches could then determine whether resistant organisms identified in water, animal feed, and animal waste share bacterial lineages, resistance genes, or mobile genetic elements. Such integration would move beyond documenting the presence of AMR towards understanding the ecological processes and potential transmission pathways that sustain resistance within the Lake Victoria basin.

## Conclusion

Antimicrobial-resistant and multidrug-resistant bacteria were detected across environmental water, animal feed, and animal waste within the Lake Victoria basin. *K. pneumoniae* and *E. coli* predominated among recovered isolates, resistance to commonly used β-lactam antimicrobials was frequent, and MDR was substantially more common among animal-associated isolates than among water isolates. Environmental bacterial contamination varied markedly across sampling locations, but coliform burden did not predict site-level MAR. These findings demonstrate that bacterial contamination and antimicrobial resistance provide complementary rather than interchangeable measures of environmental microbiological risk. Integrating animal-associated matrices and direct AMR measurements into environmental surveillance could therefore strengthen One Health approaches to monitoring antimicrobial resistance within the Lake Victoria basin.

## Declarations

# Funding

declaration:

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# Author Contribution

A.A.: conceptualization, data curation, formal analysis, methodology, writing—original draft; Y.B.: data curation, formal analysis, investigation, methodology, supervision, visualization, writing—original draft, writing—review and editing; C.S: investigation, writing—review and editing; P.N.: resources, writing—review and editing; M.H.: resources,review and editing; L.V.: writing—review and editing.

# Data Availability

This work was supported by the VLIR-UOS (Grant Number: KE2025SIN466B140)

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# Figures

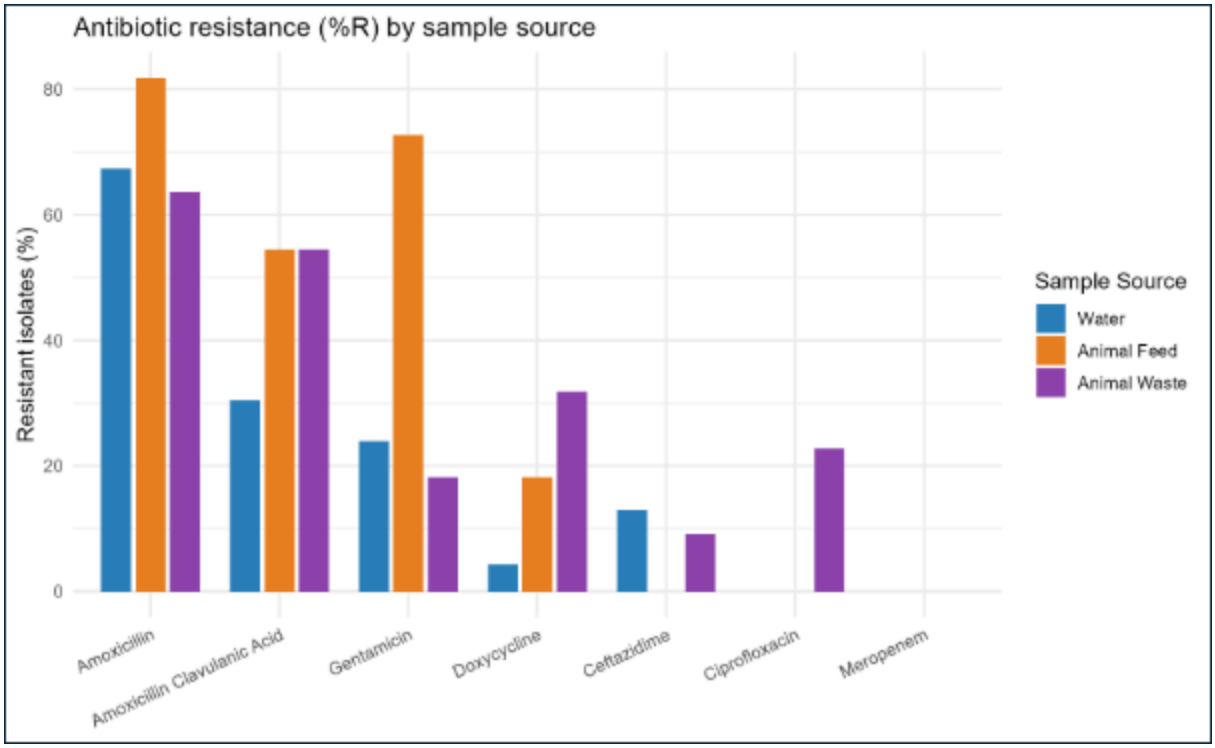



**Figure 1**

**Antimicrobial resistance profiles of bacterial isolates by sample source.**
Percentage of isolates resistant to each antimicrobial agent among isolates recovered from water (n=46), animal feed (n=11), and animal waste (n=22). AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; CAZ, ceftazidime; CIP, ciprofloxacin; DOX, doxycycline; GEN, gentamicin; MEM, meropenem.

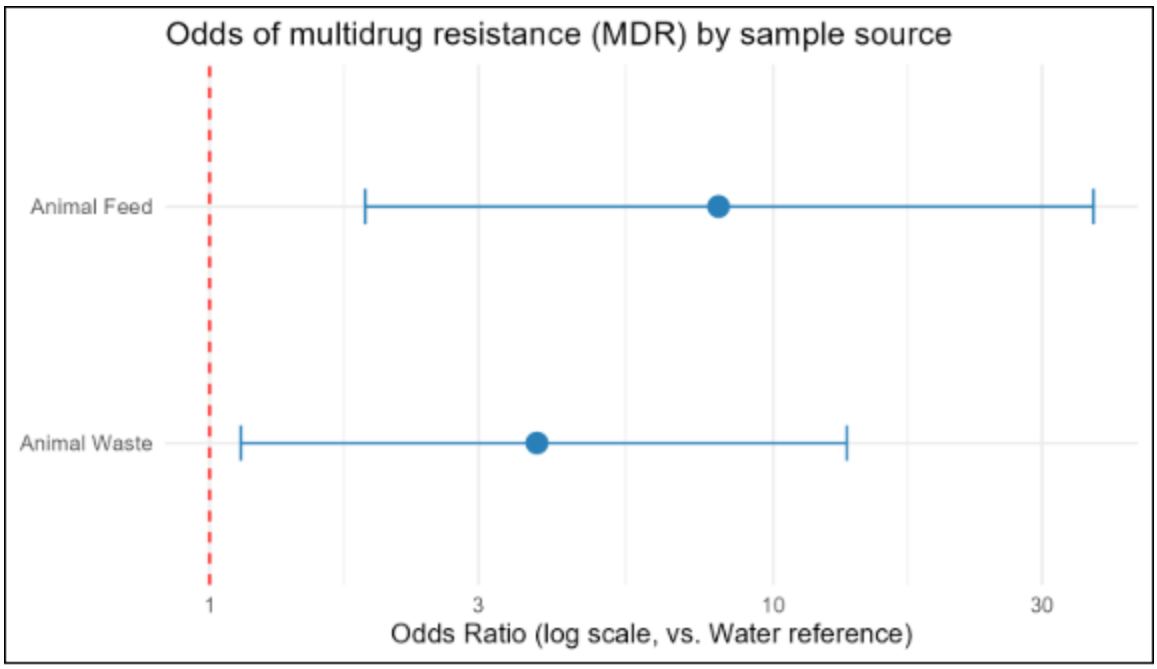



**Figure 2**

**Association between sample source and multidrug resistance.**
Forest plot showing odds ratios (ORs) and 95% confidence intervals (CIs) from univariable logistic regression examining the association between sample source and MDR. Water isolates were used as the reference category.

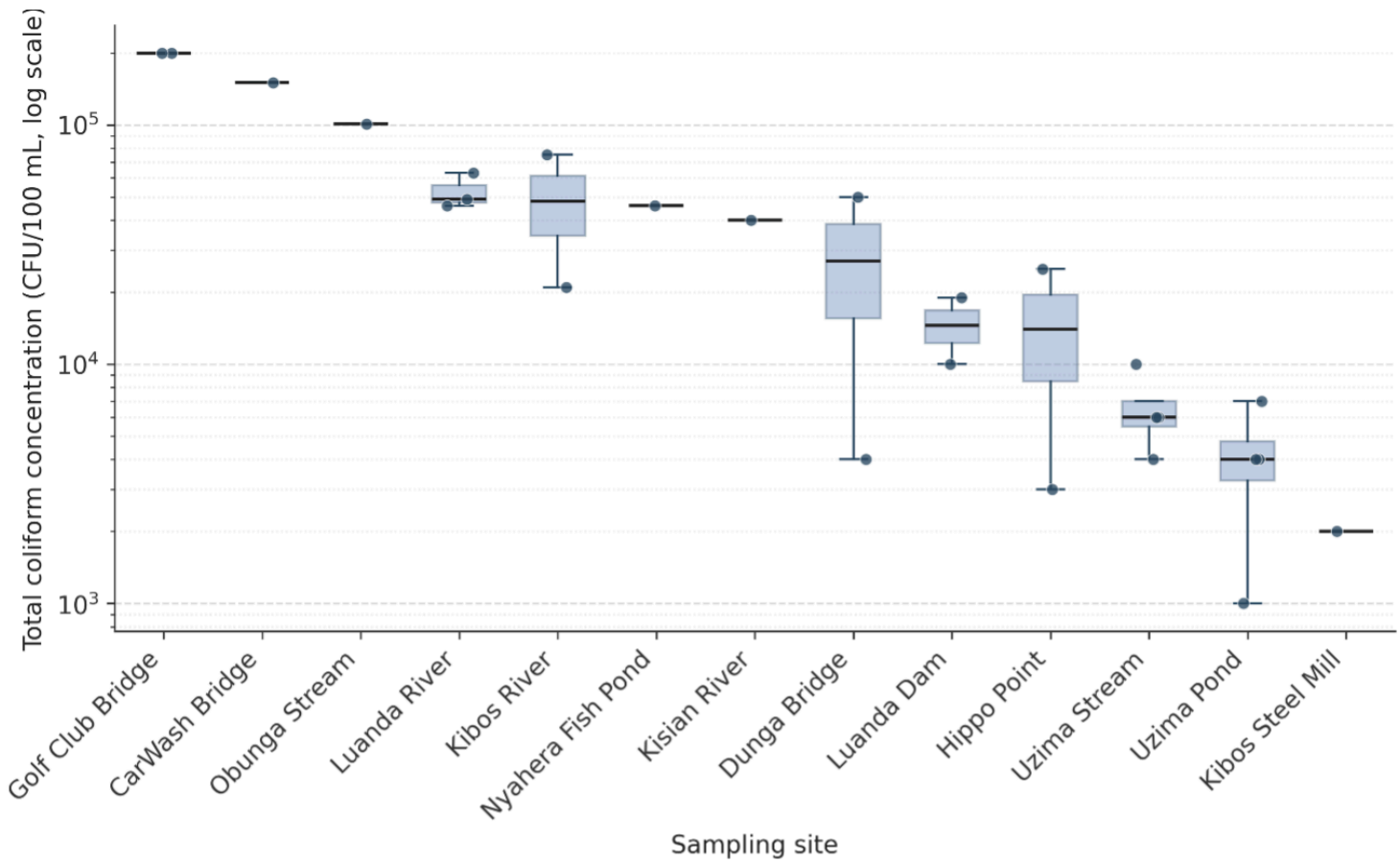


**Figure 3**

**Distribution of total coliform concentrations across environmental sampling sites.**
Total coliform concentrations (CFU/100 mL) across the 13 environmental sampling sites. The logarithmic scale is used to accommodate the highly skewed distribution of coliform concentrations.

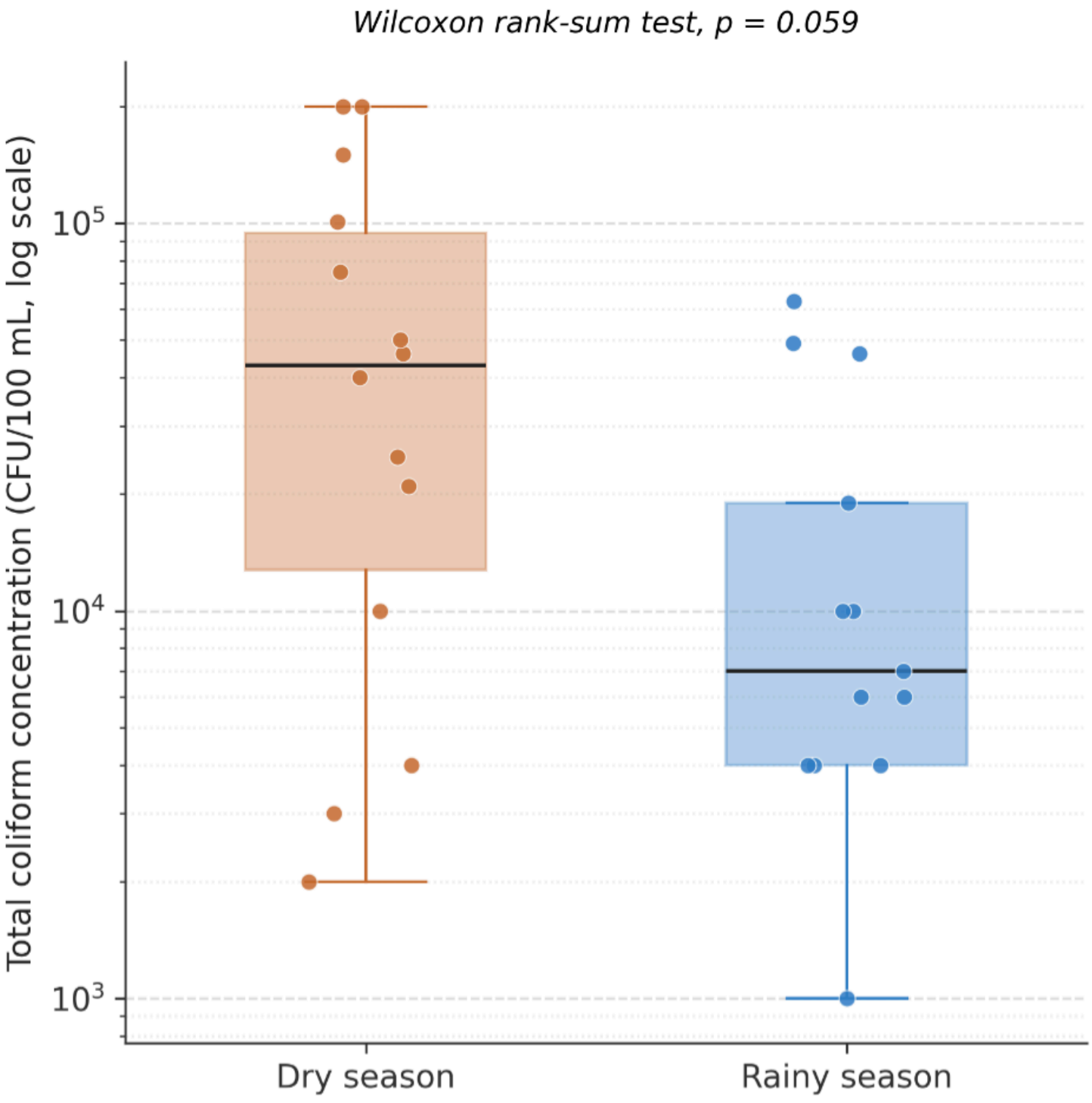



**Figure 4**

**Total coliform concentrations by sampling season.**
Distribution of total coliform concentrations (CFU/100 mL) during the dry and rainy seasons. Differences between seasons were evaluated using the Wilcoxon rank-sum test ($p$=0.059). Coliform concentrations are presented on a logarithmic scale.

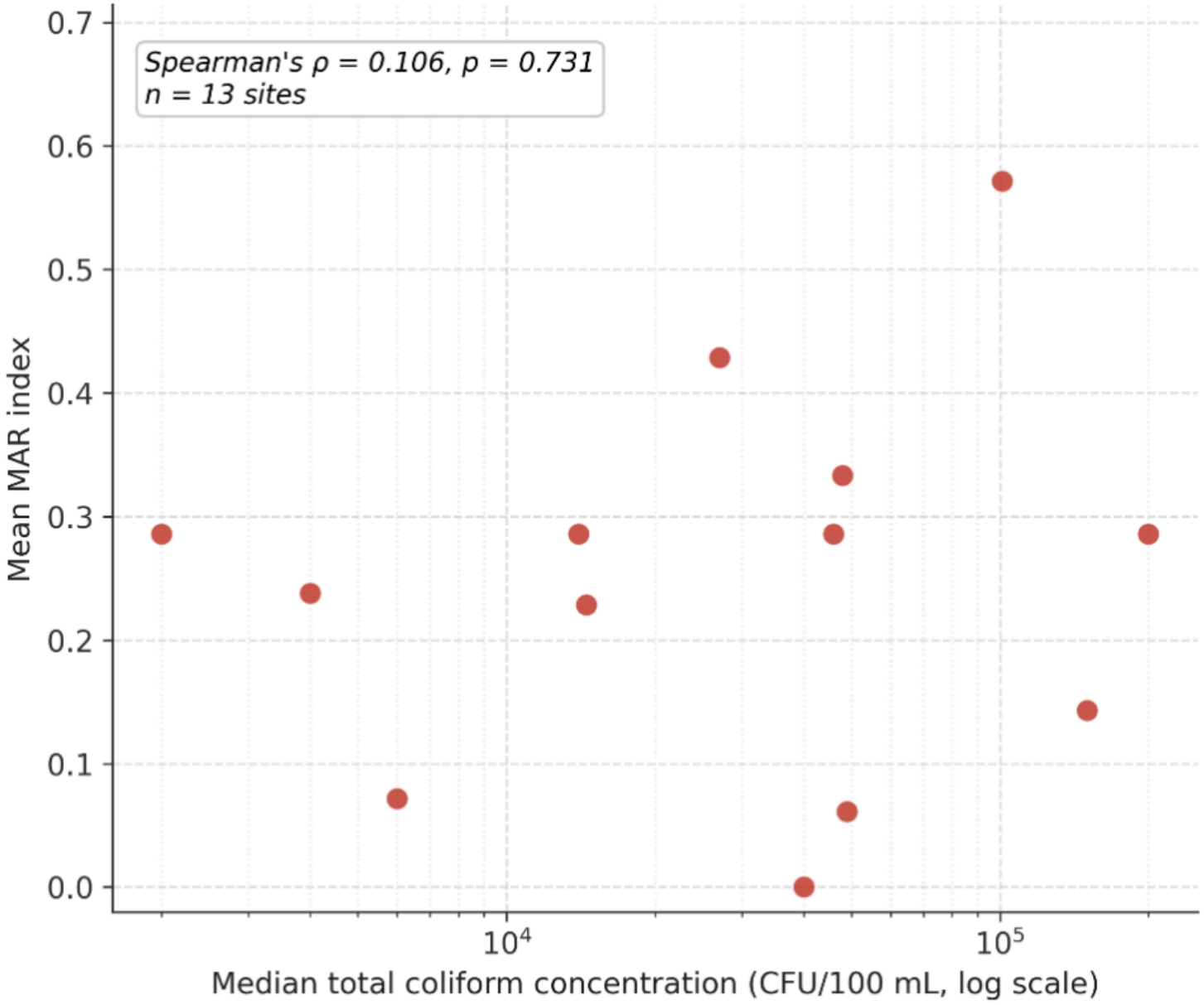



**Figure 5**

**Relationship between site-level coliform concentration and multiple antibiotic resistance index.**

Scatter plot showing the relationship between median total coliform concentration (CFU/100 mL) and mean MAR index across 13 sampling sites. The association was assessed using Spearman's rank correlation (ρ=0.106, $p$=0.731).

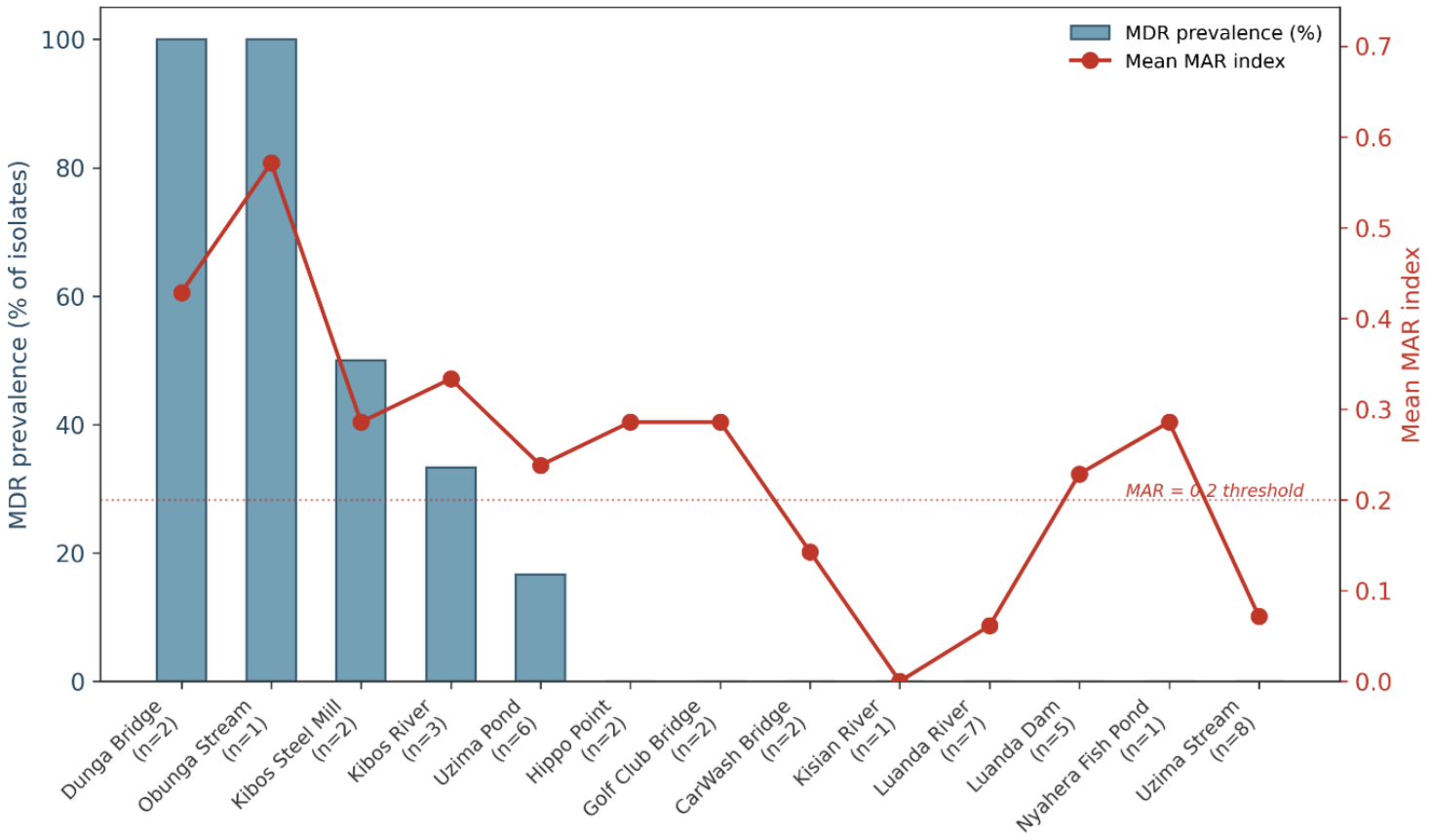



**Figure 6**

**Spatial variation in antimicrobial resistance burden across environmental sampling sites.** Distribution of antimicrobial resistance across sampling sites, presented using site-specific MDR prevalence and/or MAR index. MDR was defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories. MAR, multiple antibiotic resistance index.