

Lac-Positive Enteric Bacteria in Stream Water: Antibiotic Resistance and Public Health Implications

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ABSTRACT

Received: 28 Dec 2025

Accepted: 30 Jan 2026

Published: 10 Feb 2026

Stream water contaminated with antibiotic-resistant enteric bacteria poses a significant threat to public health, serving as a reservoir for pathogens that can cause waterborne disease and complicate treatment. This study aimed to characterize enteric bacterial isolates from stream water samples and determine their antibiotic resistance patterns. Bacterial isolates were obtained from environmental samples and characterized using cultural, morphological, and biochemical tests. Molecular identification was performed using 16S rRNA gene sequencing. Antibiotic susceptibility testing was conducted using the disc diffusion method. Four bacterial species were identified: *Escherichia coli* strains NE1127 and JKHS016 (ECNE11 and ECJ6), *Klebsiella pneumoniae* strains 2014C06-125 and Kp2092 (KP2 and KPK2). The isolates exhibited high levels of resistance to antibiotics, including streptomycin (S), penicillin (PN), sulphamethoxazole/trimethoprim (SXT), augmentin (AU), and ciprofloxacin (CN). The overall prevalence of antibiotic resistance was 63.33%, with 78.95% of isolates exhibiting multidrug resistance. Statistical analysis showed significant differences in resistance patterns among the isolates ($p = 0.012$). The study highlights the presence of multidrug-resistant enteric bacteria in the environment, posing a risk to public health. There is a need for regular monitoring of antibiotic resistance patterns and implementation of effective control measures. This study provides valuable data on the prevalence of antibiotic-resistant enteric bacteria in environmental samples, emphasizing the need for judicious use of antibiotics and proper waste management practices.

How to cite this article

Anagor, I. S., Iheukwumere, I. H., Iheukwumere, C. M., Ike, V. E., Ezendianefo, J. N., Abba, O., Unaeze, B. C., Ofuani, A. G., Ochibulu, S. C., Nnagbo, P. A., & Mere, C. A. (2026). Lac-positive enteric bacteria in stream water: Antibiotic resistance and public health implications. *Journal of Public Health, Policy, and Society*, 3(1), 51–61.
<https://doi.org/10.54117/9w36yv48>

Keywords

Antibiotic resistance, enteric bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, multidrug resistance, environmental samples.

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INTRODUCTION

The global rise of antimicrobial resistance (AMR) among enteric bacteria represents a critical public health crisis, significantly increasing morbidity, mortality, and the burden on healthcare systems, particularly in resource-limited settings (Neunhoeffer *et al.*, 2017; Okeke *et al.*, 2017; Dim *et al.*, 2025a). Enteric bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae*, are Gram-negative, facultative anaerobes native to the gastrointestinal tracts of humans and animals. While many strains are commensal, pathogenic variants are a leading cause of gastrointestinal and extra-intestinal infections, including urinary tract infections, sepsis, and pneumonia. The effectiveness of treatment for these infections is increasingly compromised by the emergence of multidrug-resistant (MDR) strains, which can harbor resistance to multiple first-line antibiotics (Patolla *et al.*, 2019; Amadi *et al.*, 2017; Dim *et al.*, 2025b).

Aquatic environments, particularly freshwater streams, are major receptacles for fecal contamination from agricultural runoff, wastewater discharge, and surface wash-off. These water bodies can thus become significant reservoirs and dissemination routes for antibiotic-resistant enteric bacteria (Mesele *et al.*, 2023; Dim *et al.*, 2025c; Chude *et al.*, 2020). Streams, often characterized by low flow rates, provide a conducive environment for the persistence and potential proliferation of these pathogens. The presence of MDR enteric bacteria in water sources used for domestic purposes—such as drinking, bathing, and irrigation—poses a direct and severe risk to community health, facilitating the transmission of resistant infections beyond clinical settings (Abera *et al.*, 2016).

Lactose-positive enteric bacteria, notably *E. coli*, are commonly used as fecal indicator organisms (FIOs) to assess water quality. However, their role extends beyond mere indicators; they can themselves be pathogenic carriers of resistance genes.

While previous studies have documented the isolation of enteric bacteria from stream water (Abera *et al.*, 2016; Wognin *et al.*, 2024), comprehensive data on the specific antibiotic resistance profiles of these environmental isolates, particularly lactose-fermenting strains, remain limited in many local contexts. Therefore, this study aims to survey the degree and patterns of antibiotic resistance among lactose-positive enteric bacterial species isolated from stream water.

MATERIALS AND METHODS

Study Area

Uli is a town located at the end southeast angle of Ihiala local government area of Anambra state in Nigeria. Its closest neighboring towns are Ohakpu, Ihiala, Amorka, Ubulu, Ozara, and Egbuoma. Uli communities stretch westward over Usham Lake to the lower Niger region and to the confluence of the Atamiri and Enyinja rivers. Its coordinates are 5.783°N 6.687°E and 5°47'N 6°52'E. It occupies a landmass of 99 square miles (256-kilometer square). The people of Uli are basically traders and farmers. The climate of the town is typically an equatorial rainforest type characterized by two main seasons; the rainy, which lasts between April and October and the dry season which lasts between November and March, with temperature which is usually high throughout the year and average minimum temperature at about 32°C and 25°C respectively.

Sample Collection, Handling and Transportation

The samples used for this study were drawn from different streams at Uli community. The samples were collected using sterile containers. The containers were thoroughly washed using detergent, rinsed with water, then rinsed with 70% ethanol and finally rinsed three times with distilled water. The containers were placed inverted in order to drain the water inside them. The container was inverted and lowered 5 cm below the river water sample, then placed vertically for the water sample to refill the sample container. This sample was covered immediately and kept in a cooler containing an ice block, and this was transported to the laboratory for immediate analysis. This was done using the method described in work published by Iheukwumere *et al.* (2025a), Iheukwumere *et al.* (2025b), Iheukwumere *et al.* (2025c), Egbe *et al.* (2025a).

Culture and Isolation of Enteric Bacteria

This was carried out using the modified method of Cheesbrough. The swab sticks were streaked on Petri dishes (60 mm OD × 55 mm ID × 13 mm high) containing MacConkey agar medium (MA/Biotech). All the plates in triplicates were incubated in inverted at 37±2°C for 24-48 h. (Egbe *et al.*, 2025b; Egbe *et al.*, 2025c; Iheukwumere *et al.*, 2025d; Iheukwumere *et al.*, 2025e).

Characterization and identification of the isolates

The isolates were subcultured on nutrient agar (Biotech), incubated in an inverted position at 37±2°C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al.* (2018b), Iheukwumere *et al.* (2025f), biochemical reactions as described in the study published by Iheukwumere *et al.* (2020a), Iheukwumere *et al.* (2025g) and molecular characterization as described in the study published by Gabriela *et al.* (2014), Ekésiobi *et al.* (2025), Ekechukwu *et al.* (2025a), Ekechukwu *et al.* (2025b), Ezedianafu *et al.* (2025a), and Ezedianafu *et al.* (2025b).

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al.* (2020b), Idigo *et al.* (2025a), Idigo *et al.* (2025b), Idigo *et al.* (2025c), Idigo *et al.* (2025d), and Ezedianafu *et al.* (2025c).

Gram staining technique: A thin smear was made on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed (Ejike *et al.*, 2017; Iheukwumere *et al.*, 2017a; Iheukwumere *et al.*, 2017b; Iheukwumere *et al.*, 2023a; Iheukwumere *et al.*, 2023b). The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with clean water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with clean water. This was followed by decolorizing the slide content with 95% w/v ethyl alcohol for 10 seconds and then rinsing with clean water. The smear was then counterstained with safranin solution (0.025%) for 60 seconds, rinsed with clean water, blot drained, and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al.* (2017c), Iheukwumere *et al.* (2018c) Ike *et al.* (2025a), Iheukwumere *et al.* (2024).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35±2°C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al.* (2017d), Iheukwumere *et al.* (2022b), Iheukwumere *et al.* (2022c), Iheukwumere and Iheukwumere (2022a), Iheukwumere and Iheukwumere (2022b), Iheukwumere and Iheukwumere (2022c).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Iheukwumere and Iheukwumere (2022e) Ike *et al.* (2025b) Ike *et al.* (2025c) Iheukwumere *et al.* (2022d), Idigo *et al.* (2025e), Obiefuna *et al.* (2025a).

Indole test: The test was carried out as described by Cheesbrough (2010), Nwikei *et al.* (2017), Obianom *et al.* (2024), Ekechukwu *et al.* (2025c), Obiefuna *et al.* (2025b), Iheukwumere and Iheukwumere (2022g), and Iheukwumere *et al.* (2022f). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37°C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al.* (2025h), Ike *et al.* (2025d), Idigo *et al.* (2025e), Ezedianafu *et al.* (2025d), Ezedianafu *et al.* (2025e) and Iheukwumere *et al.* (2025i). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37°C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al.* (2025d), Ike *et al.* (2025e), Idigo *et al.* (2025f), Idigo *et al.* (2025g) and Obiefuna *et al.* (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45°C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37°C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production

Urease test: The test was carried out as described by Cheesbrough (2010), Ejike *et al.* (2017), Iheukwumere *et al.* (2025j), Iheukwumere *et al.* (2025k), and Idigo *et al.* (2025g). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37°C for 48 h. The presence pink/red colouration indicated positive urease test

Methyl red test: The test was carried out as described by Cheesbrough (2010), Idigo *et al.* (2025h), Idigo *et al.* (2025i), Iheukwumere *et al.* (2025j) and Idigo *et al.* (2025j). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37°C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al.* (2025j), Iheukwumere *et al.* (2025k), Idigo *et al.* (2025k), Idigo *et al.* (2025i). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37°C for 48 h. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of α -naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al.* (2025c), and Idigo *et al.* (2025m). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37°C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al.* (2025i), Iheukwumere *et al.* (2025m). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H₂O₂) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al.* (2025c) Iheukwumere *et al.* (2025n), and Iheukwumere *et al.* (2025o). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of

extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al.*, 2025p; Iheukwumere *et al.*, 2025q; Chude *et al.*, 2020),

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72°C for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al.*, 2025r; Iheukwumere *et al.*, 2025s; Ejike *et al.*, 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with $\geq 95\%$ similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al.*, 2017; Iheukwumere *et al.*, 2025t; Nwike *et al.*, 2017).

Prevalence and Distribution of the Isolates in the Frozen Meat Samples

The number of each bacterial isolate in each sampling area was enumerated, and these were calculated as a percentage of the occurrences. The bacteria that appeared in each sample location were detected and recorded as described in the study published by Iheukwumere *et al.* (2025u).

Susceptibility Patterns of the Pathogenic Bacterial Isolates against Conventional Antibiotics

Preparation of test isolate: The test isolates were prepared using the method described by Cheesbrough (2010), Iheukwumere *et al.* (2025u). The isolates were aseptically subcultured into a broth culture and incubated at $35 \pm 2^\circ\text{C}$ for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.5 mL of 1.175% $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ and 99.5 mL of 1% Conc. H_2SO_4 . The prepared isolates were standardized by comparing the absorbance with that of 0.5 McFarland standards at 640 nm using UV/visible spectrophotometer.

In vitro antibacterial susceptibility test: This was carried out using the method described in the study published by Iheukwumere *et al.* (2025v). Each labeled plate was uniformly inoculated with the test organism using pour plate method. An antibiotic sensitive disk (MAXI Disk) was aseptically placed on the surface of the seeded plate, labeled and then incubated at $37 \pm 2^\circ\text{C}$ for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation.

Statistical Analysis

The results of the data generated were expressed as mean, percentage and Table, Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the main effects and interactions at 95 % confidence level. Pair wise comparison of mean was done by Student "t" test as described in the study published by Iheukwumere *et al.* (2017e), Manasseh *et al.* (2025), Idigo *et al.* (2025n), Idigo *et al.* (2025o), Idigo *et al.* (2025p), Idigo *et al.* (2025q), Idigo *et al.* (2025r), Idigo *et al.* (2025s), Idigo *et al.* (2025t), Ugwu *et al.* (2025a) and Ugwu *et al.* (2025b).

RESULTS

The enteric bacterial isolates (A, B, C, D) were characterized based on biochemical and molecular tests. Isolates A and B were identified as *Escherichia coli*, while C and D were identified as *Klebsiella pneumoniae* (Table 2). The isolates showed varying biochemical reactions (Table 1), with significant differences in parameters like citrate utilization, indole production, and sugar fermentation patterns

The isolates' antibiotic susceptibility patterns were assessed (Table 3). The results showed that 63.33% of the isolates were resistant to conventional antibiotics, with *E. coli* isolates (A and B) showing high resistance rates (65.22% and 76.47%, respectively). The implicated antibiotics included amoxicillin, sulphonamide, and tetracycline, among others. Statistical analysis revealed that the differences in resistance patterns were significant ($p < 0.05$).

Further analysis of the degree of resistance (Table 4) showed that 78.95% of the isolates were multiple antibiotic-resistant, with *K. pneumoniae* isolates (C and D) showing 80% and 100% multiple resistance, respectively. The p-values for the antibiotic resistance patterns were < 0.05 , indicating statistical significance. Overall, the isolates showed high levels of antibiotic resistance, posing a public health concern.

Table 1: Characteristics of the enteric bacterial isolates

Parameter	A	B	C	D
Appearance on MacConkey agar	Pink	Red	Red and Muroid	Red and Muroid
Elevation	Convex	Convex	Slightly raised	Slightly raised
Motility	+	+	-	-
Gram reaction	-	-	-	-
Cell morphology	Rods	Rods	Rods	Rods
Catalase	+	+	+	+
Citrate	-	-	+	+
Indole	+	+	-	-
MR	+	+	-	-
VP	-	-	+	+
Glucose	+	+	+	+
Maltose	+	+	+	+
Xylose	+	+	+/-	+/-
Sorbitol	-	+	+/-	-
Inositol	+/-	+/-	+	+/-
Dulcitol	+/-	+	+/-	+/-

Table 2: Molecular characteristics of the enteric bacterial isolates

Isolate code	Max score	Total score	Query cover (%)	E-value	Percent identity (%)	Accession Number	Description
A	1681	1681	100	0.0	100	CP038321.1	<i>Escherichia coli</i> 0157:H7 strain NE1127 chromosome complete genome (ECNE11)
B	1936	1936	100	0.0	100	CP147059.1	<i>Escherichia coli</i> strain JKHS016 (ECJ6)
C	1552	1552	100	0.0	100	CP170972.1	<i>Klebsiella pneumoniae</i> strain 2014C06-125 (KP2)
D	1552	1552	100	0.0	100	CP141801.1	<i>Klebsiella pneumoniae</i> strain Kp2092 (KPK2)

Table 3: Susceptibility of the bacterial isolates to conventional antibiotics

Isolate	N	Susceptible Strain (%)	Resistance Strain (%)	Implicated antibiotics
A	23	8 (34.78)	15 (65.22)	S, PN, SP, SXT, AU, CH, PER
B	17	4 (23.53)	13 (76.47)	AMX, AU, S, PN, SXT, CN
C	9	2 (28.57)	5 (71.43)	PN, S, SXT, AU
D	11	8 (72.73)	3 (27.27)	AU, PN, S, SXT, CN, CH
Total	60	22 (36.67)	38 (63.33)	

Table 4: Degree of resistance among the isolates

Isolates	NR	Single resistant strain (%)	Multiple resistant strain (%)
C1	15	3 (20.00)	12 (80.00)
C2	13	5 (38.46)	8 (61.54)
D1	5	0 (0.00)	5 (100.00)
D2	3	0 (0.00)	3 (100.00)
Total	38	8 (21.05)	30 (78.95)

DISCUSSION

This study confirms that stream water serves as a significant environmental reservoir for lactose-positive enteric bacteria exhibiting substantial antibiotic resistance, posing a direct conduit for multidrug-resistant (MDR) pathogens into the community. The isolation of high-risk strains, including *Escherichia coli* O157:H7 and *Klebsiella pneumoniae*, aligns with global research identifying freshwater systems as critical amplifiers of antimicrobial resistance (AMR) (Abera *et al.*, 2016; Mesele *et al.*, 2023). These findings highlight a One Health crisis, where contamination from human and animal sources converges in aquatic ecosystems, facilitating the persistence and dissemination of resistant bacteria (Wellington *et al.*, 2013). The observed resistance profiles are particularly alarming. The high prevalence of resistance to a broad panel of antibiotics, including critically important agents like Ciprofloxacin and combination drugs like Augmentin, indicates a loss of efficacy for first- and second-line therapeutic options. This resistance pattern, resulting in a 65.52% multiple antibiotic resistance (MAR) rate among resistant isolates, corroborates trends reported in other environmental and clinical studies (Elshebrawy *et al.*, 2022; Hossain *et al.*, 2022). This multidrug-resistant phenotype is primarily driven by the horizontal gene transfer of plasmid-borne resistance determinants, which can be readily exchanged among bacterial populations in nutrient-rich aquatic environments (Partridge *et al.*, 2018).

Variations in the specific strains and resistance patterns compared to other studies (e.g., Enayat *et al.*, 2012; Murad *et al.*, 2014) are expected and reflect localized epidemiological factors. These differences can be attributed to variable sources of fecal pollution (human vs. agricultural), distinct regional antibiotic usage practices in human medicine and livestock, and the unique hydrological and climatic conditions influencing the microbial ecology of each water body (Bürgmann *et al.*, 2018). The presence of these resistant, lactose-fermenting bacteria—common fecal indicators—transforms a standard water quality parameter into a direct warning signal for the potential presence of untreatable pathogens.

CONCLUSION

This investigation demonstrates that stream water is a critical and under-addressed hotspot for antibiotic-resistant, lactose-positive enteric bacteria. The convergence of fecal contamination and high levels of multidrug resistance creates a significant public health hazard for communities relying on these water sources for domestic use. The findings necessitate urgent, integrated interventions. These must include enhanced surveillance of AMR in environmental waters, stringent controls on antibiotic use and waste discharge, and robust public health education on water treatment and safe use. Protecting water resources is not only essential for preventing waterborne disease outbreaks but is also a fundamental strategy for containing the global spread of antimicrobial resistance.

Acknowledgment

We are grateful to all our study participants who join the study voluntarily. We are grateful to ZAHARM Analytical and Research Laboratory, Amawbia, Awka Anambra State, Nigeria for providing enabling environment, resources and techniques for this study. We really salute their wonderful efforts.

Conflict of interests: The authors declare that they have no conflict of interests.

Funding: This research did not receive specific grant from any funding agencies.

Ethical approval: Not applicable

Authors Contributions: All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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