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Assessment of Bacterial Contamination of River Avu Amaokwe Item, Bende Local Government Area, Abia State, Nigeria

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ABSTRACT

Water is indispensable for life, yet contaminated water remains a major global health concern. This study assessed the microbiological quality of surface water used for domestic purposes in Amaokwe Item community by determining bacterial and fungal counts, coliform MPN analysis, and microbial identification. Results revealed high microbial loads, with bacterial counts ranging from 1.0×10^3 to 3.6×10^3 CFU/mL, all exceeding WHO and USEPA standards. Coliform densities increased from < 2–9 MPN/100 mL upstream to 33–34 MPN/100 mL, indicating faecal contamination. Water samples contained diverse bacteria including *Staphylococcus*, *Pseudomonas*, *Escherichia coli*, *Enterobacter*, *Bacillus*, *Micrococcus*, and *Serratia* species. The detection of pathogenic and opportunistic organisms highlights contamination from environmental sources, hygiene, and handling practices. The findings confirm that the sampled water sources are microbiologically unsafe for domestic use without proper treatment and improved storage conditions.

Keywords: Surface water, Coliforms, Bacterial load, Fungal contamination, Water quality, Public health.

Introduction

Rivers are vital resources for domestic, agricultural, and industrial use, yet microbial pollution poses serious health risks. Pathogenic bacteria from agricultural runoff, domestic waste, and industrial effluents are linked to diarrheal diseases, typhoid fever, and other infections (Amenu *et al*., 2013; Shield *et al*., 2015; WHO, 2017; Ali *et al*., 2025). Recent studies in Nigerian communities have reported high levels of bacterial contamination in domestic water supplies, underscoring the urgent need for monitoring and intervention (Chukwuemeka *et al*., 2024). River Avu in Amaokwe Item, Abia State, Nigeria sustains local livelihoods but faces contamination from open defecation, waste disposal, and other human activities. Similar Studies in Nigerian rivers have revealed high levels of coliforms and *Escherichia coli*, indicating fecal pollution and unsafe water quality (Aleru-Obogai, *et al*., 2026). This study therefore aims to evaluate the bacterial contamination in River Avu, identifies predominant microbial species present and highlights potential health hazards.

Materials and Methods

Study Area

The study was carried out on water surface of Avu river located in Amaokwe community in Item, Bende Local Government Area, Abia State, Nigeria. Bende is amongst the local governments that make up Abia North senatorial zone. It is in South East geopolitical zone. The Igbo ethnic group is predominant in the area. The people of the area are mostly Christians and traditional worshippers with Igbo and English as the commonly spoken languages

Collection of Surface Water Samples

Water sampling followed APHA (2017) guidelines. Samples were collected from upstream, midstream, and downstream segments during the dry season to reduce rainfall dilution (Adefisoye & Okoh, 2016). At each site, three replicates (A, B, C) were taken in sterile 1000 mL polyethylene bottles, pre-rinsed with sample water. Bottles were submerged ~30 cm below the surface facing upstream.

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Collected samples were secured by corking the bottles properly. Thereafter, each sample was labeled with date, time, location, and environmental observations. The samples were placed in stored cold packs maintained at approximately 4°C and transported to the laboratory. Analyses were conducted within 24 hours to preserve microbial viability.

Cultivation and Enumeration of Total Bacteria and Fungi in the River Water Samples

Standard microbiological techniques were employed to determine the total heterotrophic bacterial count, total coliform count, and total faecal coliform count. One milliliter (1 mL) of each water sample was transferred into 9 mL of sterile physiological saline and mixed thoroughly by swirling to achieve homogenization. Serial dilutions were then prepared.

For cultivation, the spread plate method was used: aliquots (0.1 mL) of the 10−2 dilution were inoculated onto sterilized nutrient agar in Petri dishes for bacteria. Plates inoculated for heterotrophic bacteria were incubated at 37 °C for 24–48 hours. After incubation, colonies were enumerated and expressed as colony-forming units per milliliter (CFU mL^{-1}) of water sample, following the guidelines of APHA (2017) and Aryal (2022).

Enumeration of Total and Faecal Coliform

The multiple tube fermentation technique, also known as the Most Probable Number (MPN) method, was employed to estimate total and faecal coliform populations. This procedure involves three major stages: presumptive, confirmatory, and completed tests. Three sets of five test tubes containing lactose broth were inoculated with graded sample volumes (10 mL, 1.0 mL, and 0.1 mL). Tubes that exhibited acid and gas production were considered positive for coliform organisms.

For further characterization of faecal coliforms, positive tubes from earlier steps were streaked onto Eosine Methylene Blue (EMB) agar plates. The statistical estimation of organism numbers was determined using the MPN Table. Colonies obtained were subjected to Gram staining and biochemical testing for identification, following APHA (2017) guidelines.

Confirmed Test

To confirm the presence of coliforms, inocula from positive presumptive tubes were streaked onto Eosin Methylene Blue (EMB) agar plates and incubated at 35 °C for 24 hours. The methylene blue component of EMB agar suppresses the growth of Gram-positive organisms, thereby allowing Gram-negative coliforms to proliferate. Coliforms typically produce colonies with dark centers. Differentiation between species was based on colony morphology: *Escherichia coli* formed small colonies with a characteristic green metallic sheen, whereas *Enterobacter aerogenes* produced larger, pinkish colonies. These distinctions facilitated the identification of coliform types, in accordance with (APHA, 2017).

Completed test

The completed test was performed using organisms that grew on Eosin Methylene Blue **(**EMB) agar. These isolates were inoculated onto a nutrient agar slant and into a tube of single-strength lactose broth using a sterile wire loop. The cultures were incubated at 35 °C for 24–48 hours. Following incubation, the lactose broth was examined for gas production, while a Gram stain was prepared from organisms on the nutrient agar slant. Microscopic observation confirmed the presence of Gram-negative, non-spore-forming rods that produced gas in lactose broth tubes, thereby verifying coliform contamination in the water samples, in line with APHA (2017) guidelines

Identification of the bacterial isolates

Pure cultures of bacterial isolates were obtained through repeated sub-culturing of distinct colonies on fresh nutrient agar plates until uniform colony morphology was observed. The isolates were identified using a combination of microscopic, cultural, and biochemical methods. Microscopic examination included Gram staining reactions and motility tests. Colony morphology was evaluated based on size, shape, color, elevation, and margin characteristics. Biochemical characterization involved sugar fermentation tests and standard assays such as catalase and oxidase. All procedures followed the Standard Methods for the Examination of Water and Wastewater (APHA, 2017). These approaches are widely recognized and accepted for the identification of environmental bacterial isolates (APHA, 2017).

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Results

Bacteriological analysis of River Avu surface water revealed high levels of heterotrophic bacteria across all sampling points (Table 1). The total heterotrophic bacterial counts (TBC) ranged from 5.4 × 10^3 CFU/mL at the midstream to 8.4 × 10^3 CFU/mL at the downstream, with mean values of 1.80 × 10^3, 1.97 × 10^3, and 2.80 × 10^3 CFU/mL for midstream, upstream, and downstream respectively

Table 1: Total Bacterial (TBC) from Surface Water

Sample Code	Total heterotrophic Bacteria Count		
	Upstream	Midstream	Downstream
A	1.8 x 10^3	1.4 x 10^3	2.6 x 10^3
B	2.0 x 10^3	1.0 x 10^3	2.2 x 10^3
C	2.1 x 10^3	3.0 x 10^3	3.6 x 10^3
Total	5.9 × 10^3	5.4 × 10^3	8.4 × 10^3
Mean	1.97 × 10^3	1.80 × 10^3	2.80 × 10^3
WHO value	1.000 x 10^2		
USEPA	1.000 x 10^2		

Result of the Most Probable Number (MPN) analysis (Table 2) revealed, Coliform levels varied across sampling points. Upstream samples showed the lowest contamination, with MPN values ranging from < 2 to 9 MPN/100 mL Midstream samples recorded higher values (17–27 MPN/100 mL), while downstream samples showed the highest coliform densities (33–34 MPN/100 mL).

Table 2: Coliform count using most probable number (MPN) based on presumptive test tubes

Source	Sample	5.0 ml	1.0 mL	0.1 ml	MPN index/ 100 ml
Upstream	A	0	0	0	<2
	B	1	1	1	6
	C	2	1	1	9
Midstream	A	4	1	0	17
	B	4	2	1	26
	C	4	3	0	27
Downstream	A	4	3	1	33
	B	4	3	1	33
	C	4	4	0	34

Morphological and biochemical characterization of bacterial isolates from water samples revealed the presence of *Staphylococcus* sp., *Pseudomonas* sp., *Escherichia coli*, *Micrococcus* sp., *Enterobacter* sp., *Bacillus* sp., and *Serratia* sp.

Table 3 shows the prevalence of each isolate in surface water. *Staphylococcus* and *Bacillus* were found 100 % at all points (upstream, midstream, downstream) indicating continuous and widespread contamination. *Pseudomonas* sp., and *Enterobacter* sp., had lower prevalence (33.3 %), possibly indicating localized or less consistent pollution sources.

Table 3: Prevalence of each bacterial isolates in Surface Water of River Avu

Isolates	Up-stream	Mid-stream	Down-stream	%
Staphylococcus	+	+	+	100
Bacillus sp	+	+	+	100
Pseudomonas sp.	-	-	+	33.3
E. coli	-	+	+	66.6
Micrococcus sp.	+	+	-	66.6
Enterobacter sp.	-	-	+	33.3
Serretia sp.	+	+	-	66.6

Discussion

River Avu showed heavy bacterial contamination, with loads far above international safety standards. The highest counts downstream reflected cumulative inputs from agricultural runoff, open defecation, and wastewater discharge. This pattern aligns with reports that downstream sections of rivers often exhibit greater microbial loads due to intensified human activity (Adefisoye & Okoh, 2016; Djuikom *et al.,* 2021; Ibe & Okplenye, 2020). Midstream samples showed moderate contamination, though localized spikes suggested point-source discharges.

Overall, the findings confirm progressive pollution along the river course, rendering the water unsafe for direct use without treatment. The consistently high microbial counts across all sampling points highlight the inadequacy of current water management practices in Amaokwe Item.

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The results highlight the urgent need for interventions such as improved sanitation infrastructure, community education on safe water storage, and the introduction of low-cost household water treatment technologies. Without such measures, residents remain at risk of waterborne diseases, including diarrhoeal illnesses and fungal infections, which are major contributors to morbidity in rural communities (WHO, 2017).

Coliform analysis revealed clear variation in River Avu. Upstream samples showed minimal contamination (0–9 MPN/100 mL), likely reflecting reduced human activity. In contrast, midstream values (17–27 MPN/100 mL) indicated moderate faecal inputs from settlements, runoffs, and agricultural effluents. The highest contamination occurred downstream (33–34 MPN/100 mL), consistent with cumulative pollution from open defecation, wastewater discharge, and livestock intrusion. All midstream and downstream values exceeded WHO and USEPA guidelines of 0 coliforms/100 mL, categorizing the water as unsafe for drinking without treatment. Similar upstream-to-downstream contamination gradients have been reported in rivers impacted by human activities (Djuikom *et al*., 2021; Ibe & Okplenye, 2020; Adefisoye & Okoh, 2016). The presence of coliforms, even below 50 MPN/100 mL, signals potential health risks due to possible enteric pathogens (WHO, 2017).

The detection of *E. coli* and *Enterobacter* sp. highlights significant faecal pollution and possible presence of enteric pathogens (WHO, 2017; WHO, 2022; Okoye *et al*., 2023). Their widespread occurrence in River Avu suggests inputs from untreated sewage, indiscriminate defecation, farm runoff, and poor sanitation (Onuoha *et al.,* 2021). Opportunistic pathogens such as *Pseudomonas* sp., known for resistance to disinfectants, point to biofilm formation and poor storage hygiene (Adefisoye & Okoh, 2016).

The presence of *Staphylococcus* sp. and *Micrococcus* sp. indicates contamination from human handling or air exposure (Ibe & Okplenye, 2020), while *Bacillus* sp. and *Serratia* sp. reflect environmental resilience in nutrient-poor or fluctuating conditions (Djuikom *et al*., 2021). Collectively, these organisms highlight both faecal inputs and secondary contamination risks, rendering the water unsafe without treatment.

Prevalence analysis showed *Staphylococcus* and *Bacillus* sp. at 100 % across all sampling points, indicating continuous contamination. Other isolates such as *Pseudomonas*, *Enterobacter*, and *Geotrichum* sp. appeared less frequently (33.3%), suggesting localized pollution sources. These results align with studies in southern Nigeria where rivers often fail to meet WHO safety standards (Nwachukwu & Achi, 2023). According to WHO guidelines, coliforms should be absent in drinking water (WHO, 2022), yet River Avu's bacterial load far exceeded permissible limits. Continued reliance on this water for domestic use exposes residents, especially children, the elderly, and immunocompromised individuals, to significant risks of waterborne disease.

Conclusion

The findings of this study demonstrate that Iyi Avu River was contaminated with bacteria of public health importance. The consistently high bacterial and combined with high coliform MPN values, indicate high faecal contamination and the water is therefore, unsafe for domestic use without adequate treatment. Thus, emphasizes the need for improved sanitation, regular monitoring, community awareness, and adoption of safe household water-treatment and storage methods. Strengthening these measures is essential to reduce contamination, prevent waterborne diseases, and safeguard public health.

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