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Public Health Risk of *Vibrio cholerae* Contamination in Streams of Uli Community

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Abstract

Vibrio cholerae, a prominent species within the *Vibrio* genus, is commonly found in aquatic environments such as streams. This bacterium has garnered significant attention due to its role in causing human infections and its increasing involvement in antibiotic resistance. Notably, a substantial proportion (approximately 80%) of antibiotic-resistant genes in V. cholerae are plasmid-encoded, contributing to the spread of resistance. This cross-sectional study investigated the prevalence of *Vibrio* species in streams within the Uli community. Water samples were collected from various streams and analyzed using standard microbiological techniques. The study significantly ($p \leq 0.05$) identified three strains of *Vibrio cholerae*: VCC6 (*Vibrio cholerae* serovar 01 biovar Eltor strain C6709), VCP2 (*Vibrio cholerae serovar* O1 biovar Eltor strain P27459), and VCE7 (*Vibrio cholerae* strain E7946). The overall prevalence of *Vibrio cholerae* was 44.00%, with VCC6 being the most predominant strain (50.00%), followed by VCE7 (31.82%), and VCP2 (18.18%).The findings highlight the presence of pathogenic *Vibrio cholerae* strains in streams used by the Uli community, posing a significant public health risk. To mitigate this risk, the study recommends promoting personal hygiene, community education, and proper water treatment practices, such as thorough boiling of water before consumption. These measures can help control the transmission of *Vibrio cholerae* and prevent waterborne outbreaks in the community. The study's results underscore the importance of regular water quality monitoring and public health interventions to safeguard the well-being of the community.

Keywords: Vibrio cholerae, stream water, antibiotic resistance, Uli community, waterborne pathogens

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1. Introduction

Vibrio cholerae, a Gram-negative bacillus, is the causative agent of cholera, a severe diarrheal disease with significant global public health implications (Weil *et al*., 2015). The bacterium is primarily transmitted through contaminated water or food, particularly in areas with poor sanitation and hygiene infrastructure. Despite advancements in water treatment and sanitation, cholera remains a persistent threat

in many regions, especially in developing countries with limited access to clean water.

Stream water, a vital component of hydrological cycles and biodiversity conservation, is highly susceptible to microbial contamination due to anthropogenic activities such as industrial waste disposal, sewage effluent discharge, and agricultural runoff (Levantesi *et al.*, 2012; Ouma *et al.*, 2016). These activities contribute to the proliferation of pathogenic microorganisms, including *V. cholerae*, in stream water sources. The microbial quality of stream water is influenced by various physicochemical parameters, such as temperature, pH, biochemical oxygen demand (BOD), and chemical oxygen demand (COD), which affect microbial diversity and abundance (Ethell *et al.*, 2013)

This study aims to evaluate the occurrence and distribution of *V. cholerae* in selected stream waters used in Uli community. The study will provide critical information on the microbiological quality of stream water sources, informing strategies for outbreak prevention and environmental management. By identifying the presence of *V. cholerae* and other indicator organisms, this study will contribute to water safety assessments and public health decision-making. The findings of this study will have implications for public health policy and practice, particularly in rural and peri-urban communities where stream water is used for various purposes. By understanding the distribution and occurrence of *V. cholerae* in stream water sources, this study will inform interventions aimed at reducing the risk of cholera outbreaks and promoting safe water use practices.

2. Materials and Methods

Study Area

The study was conducted in Aluira, Atammiri, Ndiegungwu, Aruorah, and Ubahuadara streams in Uli, Ihiala Local Government Area, Anambra state.The study was conducted at Umuaku, Uli, Ihiala Local Government Area, Anambra State. Uli is a village located between latitudes 5.47°N and 5.783°N and longitude 6.52°E and 6.87°E on the South eastern part of Nigeria. Uli extends westward to the confluence of the rivers of Atammiri and Eyinja, and across Usham lake down to the lower Niger region. Uli has rainforest vegetation with two seasonal climatic conditions: rainy season and dry season, which is characterized by the harmattan between December and February. Uli is characterized by double maxima of rainfall with a light drop in either July or August known as dry spell or August break. The annual total rainfall is about 1,600 mm with a relative humidity of 80 % at dawn.

Sample collection, handling and transportation: The samples used for this study were drawn from the rivers. A total of 100 freshwater samples were collected from five different streams used in Uli community. Samples were taken from twenty different sites, each site in triplicates. The stream samples were collected with sterile containers. The containers were thoroughly washed with detergent, rinsed with water, and then rinsed with 70% ethanol and final 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 ice block, and this transported to the laboratory for immediate analysis.

Isolation of organisms

One milliliter (1.0 ml) water sample was aseptically transferred into a sterile test tube (Pyrex) containing 9.0 ml of the diluent (sterile normal saline) and from this; ten-fold serial dilutions were made up to 10-3. One milliliter of the diluted sample (10-3) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Thiosulfate Citate Bile Sucrose agar medium (TCBS/Biotech) using pour plate method. All the plates in triplicates were incubated inverted at 37±2˚C for 24-48 h.

Characterization and identification of the isolates: The isolates were sub cultured on nutrient agar (Biotech), incubated in 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*. (2018), Iheukwumere *et al*. (2025a) biochemical reactions as described in the study published by Iheukwumere *et al.* (2020), Iheukwumere *et al* (2025b) and molecular characterization as described in the study published by Gabriela *et al.* (2014).

Prevalence and Distribution of the Isolates in the Stream Samples

The number each bacterial isolate in each sampling area were enumerated, and these were calculated in percentage of the occurrences. The bacterial that appeared in each sample location were detected and recorded as described in the study published by Iheukwumere *et al* (2021).

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 (Ekesiobi *et al.*, 2017; Abiodum *et al.*, 2024a; Ekesiobi, 2025; Abiodum *et al.*, 2024b). Pairwise comparison of mean was done by Student "t" test as described in the study published by Iheukwumere *et al* (2018), Iheukwumere *et al*. (2025c), Iheukwumere *et al*. (2025d), Iheukwumere *et al*. (2025e) and Abiodum *et al*. (2024c).

3. Results

The occurrences of the isolates in the Sample are showed in Table 1. The study revealed that 44% of the Samples were Positive for *Vibrio* species. The sample gotten from Location C Showed the highest occurrences of the test organism whereas Sample from location E recorded the lowest occurrences.

The cultural and morphological characteristics of the isolates are shown in Table 2. The study revealed that the isolate Similar appearances on thiosulfate-citrate-bile-Salts-Sucrose-agar, Similar Elevation, Edge and Surface. It also

has similar morphological characterization on string test, Gram reaction, Endospore, Capsule and motile best nature.
The biochemical Characteristics of the isolate revealed that the isolates were hydrogen sulphide production, methyl red, Urease, Arabinose, Dulcitol negative as shown in table 3. The Isolates differ in their variation in utilization of sugars. They were all Catalase, citrate, Gelatin, Oxidase, Glucose, and Galactose positive but differ in their abilities to utilize Inositol, xylose, Sorbitol and lactose.
The nucleic acid extracted from the isolates showed the ratio of their absorbances at wavelength of 260 nm and 280 nm Using Nano drop was at the range of 1.80 -1.90, and This Confirmed that the nucleic acids were DNA as shown in Table 4
The molecular identities of the isolates revealed that Isolate L, M and N were *Vibrio cholerae* 01 biovar ElTor Strain C6709 (VCC6), *Vibrio cholerae* 01 biovar EITor Strain P27459 (VCP2) and *Vibrio cholerae* 01 biovarElTor E7946 (VCE7) as shown in Table 5.
The Study revealed that VCC6 showed highest occurrences in the study whereas VCP2 recorded the least occurrences as Shown in Table 6.

Table 1: Occurrences of the isolates in the samples

Sample Location	Number	P (%)	N (%)
A	20	7(35.00)	13(65.00)
B	20	7(35.00)	13(65.00)
C	20	14(70.00)	6(30.00)
D	20	11(55.00)	9(45.00)
E	20	5(25.00)	15(75.00)
Total	100	44(44.00)	56(67.00)

Table 2: Cultural and morphological characteristics of the isolates

Parameter	L	M	N
Appearance on TCBS	Yellow	Yellow	Yellow
Edge	Smooth	Smooth	Smooth
Elevation	Raised	Raised	Raised
Surface	Smooth	Smooth	Smooth
String Test	+	+	+
Gram Reaction	–	–	–
Shape	Rods/comma	Rods/comma	Rods/comma
Endospore	–	–	–
Capsule	–	–	–
Motility	+	+	+

Table 3: Biochemical characteristics of the isolates

Parameter	L	M	N
Catalase	+	+	+
Citrate	+	+	+
Gelatin	+	+	+
H_2S	–	–	–
Methyl red	–	–	–
Oxidase	+	+	+
Urease	–	–	–
Arabinose	–	–	–
Glucose	+	+	+
Galactose	+	+	+
Inositol	–	+/-	–
Dulcitol	–	–	–
Xylose	+/-	–	+/-
Sorbitol	–	+/-	–
Lactose	+/-	–	+/-

Table 4: DNA quantifications of the nucleic acids from the isolates

Sample ID	Conc (mg/mL)	2600nm	280nm	260/280
L	121.20	3.0120	1.6194	1.86
M	125.70	3.1082	1.6801	1.85
N	132.80	3.2110	1.7643	1.82

Table 5: Molecular identities of the isolates

Parameter	L	M	N
Max Slave	5686	5686	5686
Total Score	7295	7295	7295
Query Cover (1%)	100	100	100
E-valve	0.0	0.0	0.0
Identity (%)	100	100	100
Accession Length	1070357	1073537	1071008
Accession Number	CP047298	CP047300	CPO47304
Description	*Vibrio cholerae* 01 biovar EITor strain C6709 (VCC6)	*Vibrio cholerae* 01 biovar EITor strain P27459 (VCP2)	*Vibrio cholerae* 01 biovar EITor strain E7946 (VCE7)

Table 6: Occurrences of the Isolate in the Sample

Isolates	Number	Percentage (%)
VCC6	22	50.00
VCP2	8	18.18
VCE7	14	31.82
Total	44	100.00

4. Discussion

Waterborne diseases continue to pose significant threats to global health, despite advancements in disinfection, sanitation, and water purification (Fanique & Nair, 2008). Approximately one billion people lack access to safe drinking water, and 2.5 billion lack adequate sanitation, leading to millions of cholera cases annually, particularly in developing countries (Sonu et al., 2014). This study investigated *Vibrio cholerae* in major streams used by the Uli community in Nigeria. Out of 100 samples analyzed, 84 (46.47%) showed significant growth of *Vibrio cholerae*, with higher counts observed during the wet season compared to the dry season. The EITOR biotype was more prevalent than the classical biotype in both seasons.

The higher occurrence of *Vibrio cholerae* during the wet season may be attributed to runoff from vegetation decay, municipal sewage, and domestic waste, contaminating the streams (Fanique & Nair, 2008; Sonu *et al*., 2014). The counts ranged from (0.330-0.630) x 10^2 CFU/ml in the wet season and (0.130-0.380) x 10^2 CFU/ml in the dry season, exceeding the WHO's guideline value of zero *Vibrio cholerae* per 100 ml (WHO, 2011).

Significant correlations were observed between *Vibrio cholerae* growth and pH, temperature, and sodium chloride concentration. Complete death of the organisms was observed at 60°C and above. Similar findings were reported by Johnston and Brown (2002), Ye *et al*. (2012), Elgaml *et al*. (2014), and Green *et al*. (2019).

The study highlights the importance of environmental factors in influencing *Vibrio cholerae* growth and the need for proper sanitation and water treatment to prevent waterborne diseases. The data suggests that temperature, pH, and sodium chloride concentrations are crucial factors affecting *Vibrio cholerae* densities, consistent with findings by Mishra *et al*.

(2012) and Green *et al*. (2019). The higher prevalence of the EITOR biotype may be due to its adaptability in these environments.

5. Conclusion

The findings highlight the presence of pathogenic *Vibrio cholerae* strains (ELTOR C6709, ELTOR P27459 and E7946) in streams used by the Uli community, posing a significant public health risk. To mitigate this risk, the study recommends promoting personal hygiene, community education, and proper water treatment practices, such as thorough boiling of water before consumption. These measures can help control the transmission of *Vibrio cholerae* and prevent waterborne outbreaks in the community.

Conflicts of Interest

Authors declare that there is no conflict of interest

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