**An Analysis of Fresh Fish and Tank Water Sold at Ekpoma for *Vibrio cholerae***

**ABSTRACT**

Microbial contamination of fresh fish and water, particularly caused by *Vibrio cholerae,* is a vital nutritional and public health concern in semi-urban and rural settlements with poor hygiene practices. Thus, this study has investigated the prevalence of *V. cholerae* in fresh fish and water samples sold in Ekpoma. Using standard cultural techniques, microbiological screening was conducted to determine the *V. cholerae* load of fresh fish and tank water samples. Fifteen (15) fresh fish samples were collected from three markets in Ekpoma: Poultry Road, Ekpoma Market, and Iruekpen markets, and nine (9) tank water samples were collected from three locations in Ekpoma: locations Iruekpen, Eguare and Ujemen. All fresh fish and tank water samples tested positive for *V. cholerae*. In the fresh fishsamples, vibriocountranged from 1.1 × 10³ to 3.0 × 10³ CFU/g, with the highest load at Ekpoma Market and the lowest load at Poultry Road Market. In the tank water, vibriocount ranged from 1.0 × 10² to 2.8 × 10² CFU/mL, with the highest load at Ujemen and the lowest load at Iruekpen. The difference in the vibrio counts for water tank samples was not statistically significant (p>0.05). However, the difference in the vibrio counts for fresh fish samples was statistically significant (p<0.05) between Ekpoma Market and the other two locations. This study confirms the widespread presence of *V. cholerae* in both fresh fish and tank water sold in Ekpoma. Proper preparation (cooking or roasting) of fresh fish and treatment of tank water is advised before eating and drinking.

**Keywords**: Fresh fish, poor hygiene, public health, tank water, *Vibrio cholerae*

**1. INTRODUCTION**

*Vibrio cholerae*, the causative agent of cholera, is a Gram-negative facultative anaerobe typically found in aquatic environments and primarily transmitted through contaminated drinking water. However, emerging evidence indicates that fresh fish and tank water also serve as environmental reservoirs and significant sources of exposure, offering favourable conditions for their survival [1-3]. Furthermore, fish can act as carriers, mechanically spreading the pathogen on their surfaces or biologically harbouring it within their intestines [3]. Additionally, faecal matter, decaying organic material, and biofilms in tank water create an ideal environment for this pathogen to thrive [3-5].

In Nigeria, cholera continues to pose a serious public health challenge, with over 10,000 cases reported between January and September 2024, resulting in 359 deaths and a case fatality rate of 3.3%, primarily attributed to contaminated water and food sources [6]. In Ekpoma, the risk of transmission is particularly high due to the consumption of potentially contaminated tank water and poorly cooked fresh fish [7]. These factors present significant public health concerns, especially in regions characterized by inadequate water sanitation practices and a high consumption rate of poorly or undercooked food.

Transmission of the pathogen through tank water and food is becoming of great concern, as underdeveloped regions face a heightened risk of cholera outbreaks through these sources, especially in areas with poor sanitation and hygiene [8]. These settings are exposed to multiple exposure pathways through unhygienic food handling, open defecation, and contaminated surface water [9-10].

The presence of pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., and *Vibrio cholerae* in fish, river water and tank-stored water used for domestic applications [7,11,12]. Faecal, organic, and microbial contamination of fresh fish and tank water poses a great public health concern. This paper aimed to evaluate the microbial risk associated with pathogenic *V. cholerae* found in fresh fish and tank water sold in Ekpoma, Edo State.

**2. MATERIALS AND METHODS**

**2.1 Study Area**

This study was carried out in Ekpoma, located between longitudes 6.08° to 6.13°-E longitude and 6.45° to 6.75°-N latitude, with altitude of 332m above sea level in Esan West Local Government Area, Edo State of Nigeria [13], at the Microbiology Laboratory in Ambrose Alli University, Esan West Local Government Area in Edo State.

Ekpoma, lies between 6.08° to 6.13°-E longitude and 6.45° to 6.75°-N latitude, with altitude of 332m above sea level [13], and a total population of 127,718 [14] as of 2015, is predominantly constituted of transporters, businessmen/women, farmers, teachers/lecturers and students.

**2.2 Sample Collection**

Fresh fish samples were randomly collected from three locations in Ekpoma: Poultry Road market, Iruekpen market, and Ekpoma Market in sterile polyethylene bags. A sterilized glass bottle was also used to randomly collect fifteen tank water samples from three locations (Eguare, Iruekpen and Ujemen) in for microbial examination. The bottles were not filled; 20mm of space was left to allow for effective shaking. All samples were labelled accordingly, then transported in an icebox and delivered to the Microbiology Laboratory at Ambrose Alli University for analysis within 2 hours.

**2.3 Isolation of Bacteria**

Five grams (5g) of fish muscle was homogenized in 225 ml of buffered peptone, serially diluted and 0.1 ml of homogenized solution from 10-4 dilution was spread on thiosulfate-citrate-bile-salt-sucrose (TCBS) agar using a sterile glass. Similarly, a 0.1 mL aliquot of suspension from the water samples (10-4 dilution) was plated on the TCBS agar and incubated at 37℃ for 24 hours. Isolates were then subcultured into a slant for further analysis. Isolates were enumerated as total viable counts in CFU/g and CFU/mL for the fish and water samples respectively.

**2.4 Characterization and identification of Isolates**

All presumptive isolates were identified based on their morphology, Gram staining properties, and biochemical properties, through oxidase, citrate, motility, sugar fermentation, urease, and catalase tests [15].

**2.5 Statistical Analysis**

Data were analysed by descriptive statistics using frequency tables, means and percentages in Microsoft Excel. Inferential statistics, analysis of variance (ANOVA) was used to ascertain significant difference in viable count at p<0.05.

**3. RESULTS**

**3.1 Biochemical Characterization and Identity of Isolates**

Table 1 shows the morphological and biochemical characteristics of a bacterial isolate from water and tank samples. All presumptive isolates were identified as *V. cholerae.*

Table 1: Morphological and biochemical characterization of bacterial isolates

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | Cultural characteristics | Gram reaction | Spore | Ur | Cat | Ind | Oxi | Cit | MO | Glu | Gal | Suc | Organism |
| Fish | Greenish or yellowish on TCSA | + | - | - | + | + | + | - | + | *+* | + | *+* | *Vibro cholerae* |
| Tank water | Greenish or yellowish on TCSA | + | - | - | + | + | + | - | + | *+* | + | *+* | *Vibro cholerae* |

**Keys**: Cat= Catalase, Ind= Indole, Ur= Urease, Oxi= Oxidase, MO= Motility, CT=Citrate, Glu=glucose, Gal=galactose, Suc=sucrose, + = Positive, - = Negative

**3.2 Mean total viable count of *Vibrio cholerae* isolates**

Table 2 shows the mean total viable count of *V. cholerae* obtained from the nine (9) tank water sample point from the three sample locations in Ekpoma, the count ranged from 1.0 x 102 to 2.8 x 102 (CFU/mL).

Table 3 shows the mean total viable count of *V. cholerae* obtained from the three (3) sample locations which ranged from 1.1 x 103to 3.0 x 103 (CFU/g)

Table 2: The mean total viable count (CFU/mL) of *Vibrio cholerae* isolates from the tank water sample in Ekpoma

|  |  |  |
| --- | --- | --- |
| Location | Sample points | Count (CFU/mL) |
| Iruekpen | A1 | 1.5 x 102 |
|  | A2 | 1.6 x 102 |
|  | A3 | 1.0 x 102 |
| Eguare | B1 | 2.2 x 102 |
|  | B2 | 1.8 x 102 |
|  | B3 | 2.4 x 102 |
| Ujemen | C1 | 1.7 x 102 |
|  | C2 | 2.6 x 102 |
|  | C3 | 2.8 x 102 |

Table 3. The mean total viable count (CFU/g) of *Vibrio cholerae* isolated from fresh fish samples sold at Ekpoma

|  |  |  |
| --- | --- | --- |
| Location | Sample | Count (CFU/g) |
| Poultry Road Market | P1 | 1.5 x 103 |
|  | P2 | 1.2 x 103 |
|  | P3 | 1.4 x 103 |
|  | P4 | 1.9 x 103 |
|  | P5 | 1.1 x 103 |
| Iruekpen Market | I1 | 1.7 x 103 |
|  | I2 | 1.7 x 103 |
|  | I3 | 2.2 x 103 |
|  | I4 | 2.0 x 103 |
|  | I5 | 1.7 x 103 |
| Ekpoma Market | U1 | 2.9 x 103 |
|  | U2 | 2.4 x 103 |
|  | U3 | 2.5 x 103 |
|  | U4 | 2.8 x 103 |
|  | U5 | 3.0 x 103 |

**DISCUSSION**

This work investigated the presence of *Vibrio Cholerae* in tank water and fresh fish sold at Ekpoma, as both tank water and fresh fish have become a major concern as potential reservoirs the pathogen in the recurrent outbreaks of vibriosis.

*Vibro cholerae* was identified in the samples by their diagnostic characteristics with include greenish and yellowish colonies on TCBS agar, positive Gram reaction, and other biochemical properties consistent with *Vibrio* spp. commonly found in aquatic environment as was also reported in a study on the stream surface water [16-18].

All fresh fish and tank water samples tested positive for *V. cholerae*. In the fresh fishsamples, vibriocountranged from 1.1 × 10³ to 3.0 × 10³ CFU/g, with the highest load at Ekpoma Market and the lowest load at Poultry Road Market. *Vibrio* spp. is reported to be the most severe and wide spread pathogen in fish and seafood [19,20]. However, the high microbial contamination of fresh fish as observed in this study, may be a result of unhygienic cutting utensils, handling techniques, and contaminated tables.

In the tank water, vibriocount ranged from 1.0 × 10² to 2.8 × 10² CFU/mL, with the highest load at Ujemen and the lowest load at Iruekpen. The difference in the vibrio counts for water tank samples was not statistically significant (p>0.05). However, the difference in the vibrio counts for fresh fish samples was statistically significant (p<0.05) between Ekpoma Market and the other two locations. Water can become contaminated with the pathogen during the process of buying or storage of water [21] and cross-contamination through the oral faecal route [22].

The finding of this study aligns with previous studies, Elhadi *et al*. [19] and Noorlis *et al*. [23]. Emerging evidences supports the transmission of *V. cholera* from these sources, with the risk of vibriosis heightened in rural and underdeveloped region with poor hygiene and sanitation practice [24].

The recent outbreak of cholera in Nigeria was reported in 34 out of 36 states of the country resulting in serious fatality [25,26]. Food and water-related illness and death associated with *V. cholera* is preventable with improve access to clean water, sanitation and proper hygiene.

**5. CONCLUSION**

This study focused on isolating and identifying *Vibrio cholerae* from fresh fish and tank water sources and estimating the health risk posed by this organism by estimating its concentration in these sources in the study area, Ekpoma. This study confirm the widespread presence of *V. cholerae* and high number of the pathogen in both fresh fish and tank water available in Ekpoma. The high contamination rate of tank water and fresh fish sold in Ekpoma presents a significant public health threat in the region. This highlights the necessity for stricter hygiene practices, water treatment, and proper cooking of fish to reduce cholera outbreaks and ensure food and water safety in the area.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, manuscript.

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