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**RESEARCH ARTICLE**

# IDENTIFICATION OF SALMONELLA AND VIBRIO IN WATER AND *OREOCHROMIS NILOTICUS* IN MWANZA GULF, LAKE VICTORIA, TANZANIA

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**ABSTRACT**

A cross sectional study to identify Salmonella and Vibrio species in water and fish in Mwanza Gulf, Lake Victoria was conducted during April to May 2012. A total of 30 water and 82 fish samples were collected and analyzed for *Salmonella*, *Vibrio cholera* and *Vibrio parahaemolyticus.* In water, the overall prevalence of *Salmonella spp*, *Vibrio cholerae* and *Vibrio parahaemolyticus* was 13.3%, 20% and 23.3%, respectively. Out of 15 water samples collected in beaches, contamination by *Salmonella spp*, *V. cholerae* and *V. paraheamolyticius* was 6.7%, 20% and 20%, respectively. In ponds, only *V. parahaemolyticus* was isolated in two out of three water samples. The prevalence of *Salmonella spp* and *V. cholerae* was 25% and 16.7% for *V. parahaemolyticus* in 12 river water samples. In fish, prevalence of *Salmonella spp.* on the surface, gills and intestines of fish was 19.5%, 4.9% and 2.2% (n=41); *V. cholerae* on the surface, gills and intestines of fish was 53.7%, 17.1% and 4.9% (n=41); and for *V. parahaemolyticus* it was 14.6%, 2.4% and 22% (n=41) respectively. Based on findings from this study, it was evident that there are some enteric pathogens of public health importance in fish and water in Lake Victoria and rivers emptying water into the lake. Detection of *Salmonella spp*, *V. cholerae* and *V. parahaemolyticus* in upstream water samples and fish before processing suggests that fish and water may be unsafe for human consumption. Thus it implies the necessity for application of HACCP system along the fish supply chain from fishing to processing establishments.



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## INTRODUCTION

Lake Victoria is the second largest freshwater lake in the world, which is central to the socio-economic development in the East African region. Due to rapid increase of human population and urbanization in the region, the lake is under considerable pollution pressure resulting from a variety of interlinked human activities. The majority of the human populations in urban centers in the Lake Victoria region live in un-planned settlements with insufficient measures for management of wastewater and other wastes. Thus, despite several efforts that are made, the wastes generated are poorly handled and are often discharged into the lake leading to contamination of water and fish. The contamination of water and fish with faecal matter, and enteric pathogens is therefore considered to be high (Nganga *et al*., 2011). Although there are limited studies on enteric pathogens in humans in the Lake Victoria basin, several cases of gastroenteritis, cholera and typhoid were reported in previous studies (Shapiro *et al*., 1999; Kagiko *et al*., 2001; and Onyango *et al*., 2009). Such studies demonstrated a high prevalence of enteric pathogens in particular *S. typhimurium (49.6%)*, *V. cholerae* (3.8%) and *E. coli* (46.6%) in water and fish (Onyuka *et al*., 2011). Contamination of fish and water sources with microbial pathogens may pose a serious public health risk to people living in the Lake Victoria region and beyond. In addition, contamination of fish and fish products with such pathogens leads to significant economic losses as a result of export ban, costs of treatment, time lost by sick individuals and the attendants and costs for containing the disease outbreaks. For instance, in 1998, the European Union (EU) banned fish exports from East Africa and Mozambique due to cholera outbreak and detection of Salmonella and Vibrio in exported fish products. Although the export ban of fish and fish products to European countries was uplifted, still during 1998 to 2012, there were a number of Rapid Alerts for Food and Feeds

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(RASFF) notifications that resulted in border rejections of fish products to various EU countries. Whereas eight notifications were due to *Salmonella* spp. in Nile perch exported to EU countries, three were due to high counts of *Enterobacteriae,* one due to high counts of Thermotolerant coliform and two due to bad hygiene (ECFVO 2006; 2011). Given the magnitude of exported products and the current fishing and handling practices together with the poor hygienic environment of the beaches, reported alerts are considered few. This is as a result of a well established quality control system focusing on handling and processing of fish for the export markets based on the European Union Directive on Hygiene (91/493/EEC). On the other hand, a well established quality control system does not exist in the Lake Victoria basin for fish and fish products that are consumed locally or regionally and this poses a danger to the health of consumers (Ogwan'g *et al*., 2005). Despite that, limited studies have been conducted to concurrently determine the microbial contamination in fish and water in Mwanza gulf. The fact that fish and fish products from Lake Victoria attract both local and International markets; and that inhabitants relay on this type of water for drinking and other domestic purposes, identification of hazards and eventually risk assessments associated with these products is important. This study was therefore designed to determine the extent and magnitude of enteric pathogens contamination in rivers, beaches and lake water as well as in captured fish before being processed in an attempt to generate knowledge for improvement of risk assessment models and their management.

## MATERIALS AND METHODS

### *Collection of water and fish samples*

Water and fish samples were collected in May 2012 during the end of rain season. Water samples were collected from Lake Victoria, Mwanza Gulf and rivers entering the lake and aquaculture fish farm. Four rivers (Nyashishi, Butimba, Nyakurunduma and Mirongo near Kirumba fish market; five beaches or landing sites (Shede-Fish factory area at igogo industrial area, Mwanza urban water and sewerage authority (MWAUWASA) - Butuja wastewater stabilization ponds at ilemela industrial area, Old Igombe fish landing site, Kigongo ferry and Maganga beach), (Mdegela *et al*., 2014); and one aquaculture farm own by Fisheries Education and Training Agency (FETA) (Figure 1). A total of 30 water samples from these sites were collected. Out of the 30 samples, a total of 12 samples (1 at 100m before inshore, 1 at inshore (0m), and 1 at 500m offshore) were collected from the 4 rivers. A total of 15 water samples (1 at inshore (0m), 1 at 100m and 1 at 500m offshore) from five beaches (fish landing sites) were collected. The remaining three (3) samples were collected from the aquaculture farm. Water samples were aseptically collected by using sterile autoclaved 250 ml bottles from the water surface. Collected water samples were placed in a cool box with ice and delivered to the laboratory within 6 hours of collection for analysis. Fish samples were fished from Nyamiruguyu, Nyamisi and all were procured from Maganga beach at Nyegezi bay.

A total of 82 live fish (Nile tilapia) ranging from 200g to 1000g were collected and used in the study. From each fish; gills, intestine and kidney were collected for laboratory analysis. Samples were examined for *Salmonella* spp (41 fish samples) and pathogenic *Vibrio* spp (41 fish samples). Samples of gills, intestines and swabs from the kidney were collected and placed in enrichment/transport media (Buffered Peptone Water (BPW – CM0509) for Salmonella and Alkaline Peptone Water (APW - CM1028) for Vibrio spp.).

### Detection of Salmonella

Detection of *Salmonella* was done according to TZS122:2007-ISO6579:2002/Amd.1:2007. 100mL of BPW surface washing and swabs of kidney placed in 10ml of BPW were incubated at 37$^{o}$C for 24 hours. Weight of intestines, gills and equivalent volume of BPW was added; and water samples (25mL equivalent to 25g was weighed and added 225mL BPW) to make 1/10 dilution, then mixed and incubated at 37$^{o}$C for 24 hours. Subsequent steps described in the test procedure (TZS122:2007-ISO6579:2002/Amd.1:2007) were followed. Rappaport Vassiliadis medium (RV-CMCM0866), Xylose Lysine Desoxycholate (XLD) Agar (CM0469) and Bismuth Sulfite Agar (BSA-CM0201) selective plating media , Nutrient agar (NA-CM) and Triple Sugar Iron Agar (TSI-CM0277) were used. Suspected positive isolates were serotyped confirmed by slide agglutination with homologous Salmonella O and H group antisera (Statens Serum Institute, Copenhagen, Denmark).

### *Detection of Vibrio cholerae and Vibrio parahaemolyticus*

Detection of pathogenic *Vibrio* spp. was done in accordance to TZS733:2002: Examination for *V. cholerae* in food stuffs and TZS 127:2006 for detection and isolation of *V. parahaemolyticus*. One hundred mls of surface washing (APW), Intestines and gills were weighed and added with equivalent volume of APW, swabs of kidney were put in 10 mL BPW; (25mL equivalent to 25g was weighed and added 225mL BPW) to make 1/10 dilution, all were incubated at 37$^{o}$C for 24 hours for enrichment. The enrichment culture was streaked on to selective Thiosulfate Citrate Bile Salts Sucrose (TCBS Agar -CM0333) plates and incubated overnight at 37$^{o}$C for 18 hours.

Purification of suspected yellow colonies for *V. cholera* in TCBS was done by streaking onto Saline TSI Plates then incubated at 37$^{o}$C for 24 hours. Well isolated colonies were inoculated in Saline TSI slopes. Yellow colour in Saline TSI slopes without gas production was reported as presumptive positive *V. cholera* per 25ml. The suspected green colonies of *V. parahaemolyticus* were streaked onto Saline NA plates and incubate at 37$^{o}$C for 18- 24 hours. After incubation, colonies were examined using Gram staining and subjected to further tests that included oxidase, indole and biochemical (Saline TSI) tests. The presumptive positive results for *V. parahaemolyticus* were based on the appearance of biochemical reaction characteristic colour on saline TSI that appeared yellow butt and red slant without gas production.

### Data analysis

Data were analyzed using the SPSS; PASW Statistics 18 (2009) statistics software (IBM SPSS). Proportions of samples

**Table 1. Distribution of pathogens stratified by type of water body**

| Pathogen | Overall (N=10) | | Beach* (N=5) | | Rivers** (N=4) | | Pond (N=1) | |
|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % |
| *Salmonella spp.* | 4 | 40 | 1 | 20 | 3 | 75 | 0 | 0 |
| *V. cholerae* | 6 | 60 | 3 | 60 | 3 | 75 | 0 | 0 |
| *V. parahaemolyticus* | 5 | 50 | 3 | 60 | 1 | 25 | 1 | 100 |

**Key:**
Beach* = Entails 0m inshore
River** = Entails 100m before entrance into the lake (before inshore)
N = Total number of the type of water body sampled
n = Total number of positive samples
% = Percentage positive

**Table 2. Overall prevalence of pathogens stratified by distance from inshore**

| Distance of sampling site from inshore | 100m before Inshore | Inshore (0m) | 100m offshore | 500m offshore | Pond*** | Total | % |
|---|---|---|---|---|---|---|---|
| Number of samples | 4 | 9 | 5 | 9 | 3 | 30 | |
| *Salmonella spp* | 2 | 2 | 0 | 0 | 0 | 4 | 13.3 |
| *Vibrio cholera* | 1 | 2 | 1 | 2 | 0 | 6 | 20 |
| *V. parahaemolyticus* | 1 | 3 | 0 | 0 | 1 | 5 | 16.6 |

*** Undefined distance

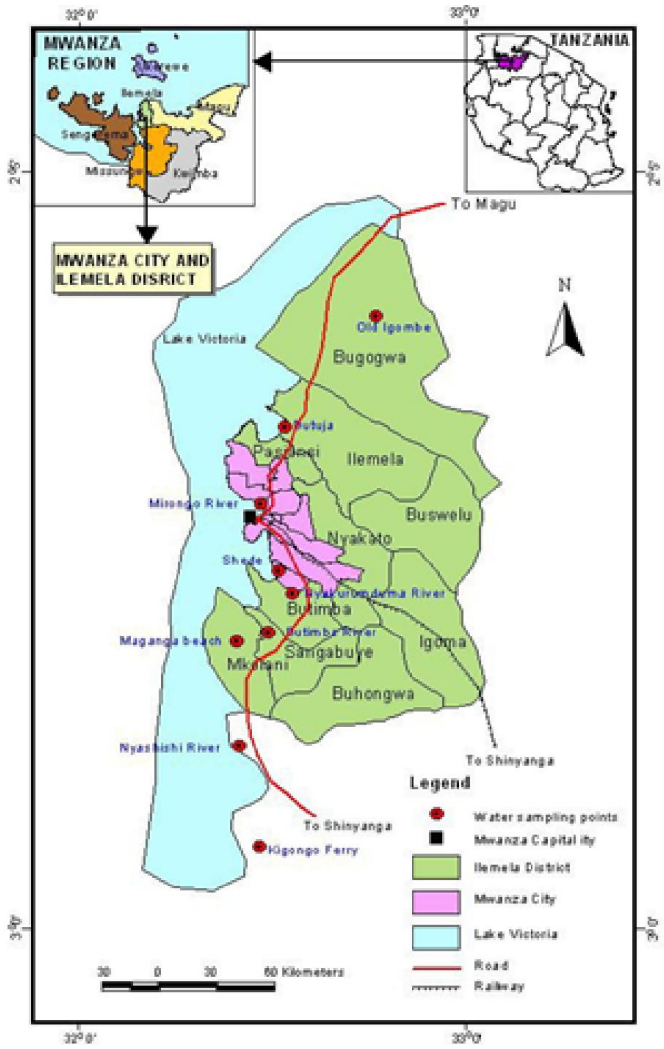


**Fig.1. Map showing water sampling sites for identification of Salmonella and Vibrio spp in Mwanza Gulf, in Mwanza City**

contaminated with microbial pathogens were reported in % and further analysed using $\chi^2$ test. An alpha level of significance of 0.05 was used through out the analysis for testing levels of significance.

## RESULTS

Thirty water samples from 10 different sampling sites were analysed for the presence of Salmonella and pathogenic Vibrio spp. of public health importance (Table 1). Among of 10 sites contamination by *Salmonella spp.* was observed at 40%, *V. cholerae* at 60% and *V. parahaemolyticus* at 50%. Contamination of water bodies by *Salmonella spp.* and *V. cholerae* was significantly higher in rivers *(P < 0.05)* than in beaches (Figure 2). However, the proportion of contamination of water bodies by *V. parahaemolyticus* was comparable (*P > 0.05*) for these water bodies (Figure 2). A total of 4/30 (13.3%) *Salmonella spp.*, 6/30 (20%) of *V. cholerae* and 5/30 (16.6%) of *V. parahaemolyticus* were isolated from the water samples. *Salmonella spp.* was isolated from two rivers and inshore water samples. *Vibrio cholera* was isolated from beaches, rivers and lake water samples except in the ponds.

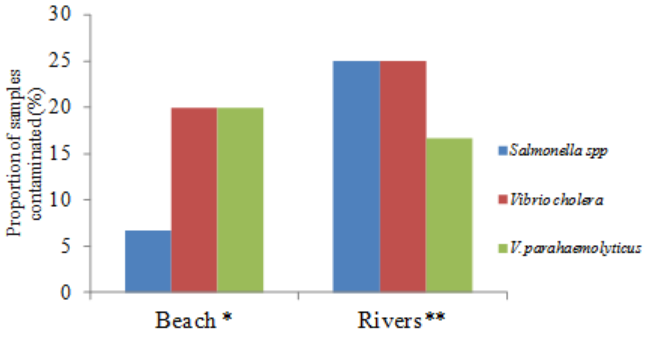


**Fig.2. Contamination of water samples from different type of water bodies. Beach* = Entails the 0m inshore, 100 offshore and 500m inshore. River** = Entails the 100m inshore, 0m inshore, 100 offshore and 500m inshore. Contamination of water bodies by *Salmonella spp.* and *V. cholerae* was significantly higher in rivers *(P < 0.05)* than in beaches. The proportion of contamination of water bodies by *V. parahaemolyticus* was comparable (*P > 0.05*).**

*Vibrio parahaemolyticus* was isolated from all types of sites except from the lake at 100m and 500m offshore (Table 2, Figure 3). Water samples collected 100m before inshore (rivers) were heavily contaminated by all the three pathogens, with the highest proportion of contamination being for

*Salmonella spp*. Equally, water samples collected at 0m were positive for all three types of pathogens at equal proportions. The 100m and 500m offshore water samples were only positive for *V. cholera* (Figure 3). Only three sampling sites (Nyashishi River; Kigongo ferry and Shede) were not contaminated with at least one of the pathogens detected.

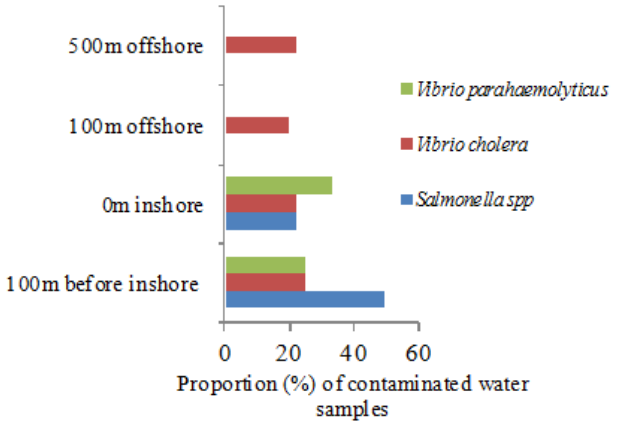


**Fig.3. Prevalence of *Salmonella spp. V. cholerae* and *V. parahaemolyticus* in different sources of water. *Salmonella spp.* was isolated from two rivers and inshore water samples. *Vibrio cholera* was isolated from beaches, rivers and lake water samples except in the ponds. *Vibrio parahaemolyticus* was isolated from all types of sites except from the lake at 100m and 500m offshore.**

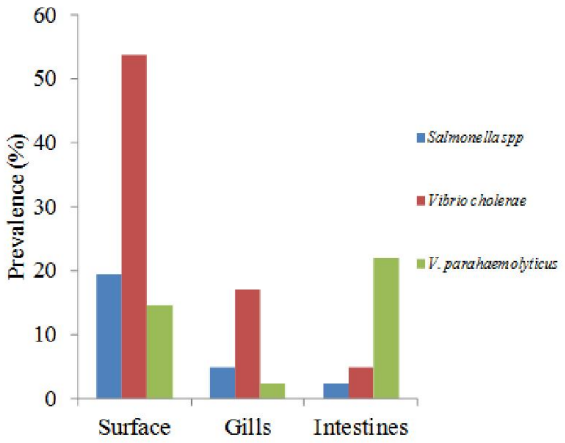


**Fig.4. Prevalence of *Salmonella spp.*, *Vibrio cholerae* and *V. parahaemolyticus* in different organs of fish. *Salmonella Salmonella spp.*, *V. cholerae* and *V. parahaemolyticus* were isolated from the fish surface, gills and intestines. There was a significant difference ($P < 0.05$) of infections by *Salmonella spp.* between these organs. For *V. parahaemolyticus*, there was a significant difference between groups ($P < 0.05$), being highest in the intestines, followed by the surface and finally by the gills**

Similarly, *Salmonella spp.*, *V. cholerae* and *V. parahaemolyticus* were also isolated from the fish surface, gills and intestines. There was a significant difference ($P < 0.05$) of infections by *Salmonella spp.*between these organs. It was highest on the surface, followed by the gills and lowest in the intestines (Figure 4). The same trend was observed for *V. cholerae*. For *V. parahaemolyticus*, there was a significant difference between groups ($P < 0.05$), being highest in the intestines, followed by the surface and lowest in the gills (Figure 4).

## DISCUSSION

Findings in this study have shown a high prevalence of enteric pathogens of public health importance in water and fish in Mwanza Gulf of the Lake Victoria. Generally, the Lake Victoria receives large quantities of pollutants from human and industrial sources (Onyango *et al*., 2009). The major routes of such pollutants are rivers that drain from residential areas and industrial sources as well as from rainfall runoff. Rivers that drain from residential and industrial areas are contaminated by pathogens and may serve as point sources of pollution into the lake. The discharge of untreated effluents into the rivers and eventually into the lake or into the lake directly, compounded by lack of awareness of good hygiene practices, can also directly contribute to high load of pollutants (South *et al*., 2004), with potential to introduce enteric pathogens in the lake (Chaggu, 2011). Human infections caused by pathogens transmitted from contaminated fish and fish products or the aquatic environments are common. The extent of infections depends on the season, contact of people with fish and related environment, dietary habits and the immune status of the exposed individuals. *Salmonella spp, E. coli, V. cholera*, *V. parahaemolyticus* and Enterococci are among known pathogens of human and animal origin that can result from different activities along the Lake Victoria basin. These pathogens have been reported in the Lake Victoria basin and some of them being implicated in the export ban of fish products from the region (ECFVO, 2011; and Onyuka *et al*., 2011).

Based on findings from this study, most of the lake contaminations by microbial pathogens in Mwanza originate from rivers and to some extent from beaches related activities (Figure 3 and Table 2). Contamination of water by *Salmonella spp.*, *V. cholera* and *V. parahaemolyticus* was evident in rivers 100m before entry into the lake and 0m at inshore. It is apparent that *Salmonella spp.* was only prevalent in rivers 100m before entry into the lake at about 50%. At 0m (inshore) its prevalence decreased to about 20% and no Salmonella was detected in subsequent water samples collected at 100m and 500m offshore. Similar observations were evident for *V. parahaemolyticus*. These findings indicate that if measures are taken to contain *Salmonella spp.* and *V. parahaemolyticus* in rivers before entering into the lake, it will minimize the risk of contamination of fish significantly. The risk will be decreased further if good hygiene of practice will be observed.

The prevalence of *V. cholerae* was high and almost the same for water samples collected in rivers and beaches, very high in water samples collected in the ponds, and highest on the fish surface samples than in the intestine and gills (Fig. 2, 3 & 4). This is due to the fact that *V. cholerae* is not only able to survive but is also an indigenous pathogen in water with ability to grow in fresh water habitats (Vital *et al*., 2007). *Vibrio cholerae* found in the waters of cholera endemic areas exists in biofilm-like aggregates in which cells are in conditional viable state (Islam *et al*., 1993). They are metabolically impended cells which can regain its metabolic activity under specific *in vitro* conditions. Such vibrio cells might play a critical role in the transmission of pathogens (Watnick and Kolter 1999). In view of that, the likelihood of this pathogen to contaminate

deep water fishes is high. This is evidenced by findings presented in figure 3 where the same prevalence of about 20% was pragmatic in all types of water including the rivers, beaches and the lake regardless of the distance from the inshore.

*Vibrio cholerae* is often transmitted by water but fish or fish products that have been in contact with contaminated water or faeces from infected persons also frequently serve as a source of infection (Kam *et al*., 1995; Colwell, 1996; and Rabbani G. H. and Greenough, 1999). Grey water is another source of pathogens of public health importance with potential to contaminate Lake Victoria water (Rabbani G. H. and Greenough, 1999). Since *V. cholerae* is a normal inhabitant in water bodies, implies that even if the disease can be eliminated from human and animal populations, the vibrio will continue to survive independently in the environment. This calls for measures with capacity to eliminate the pathogens in fish and fish products processed for export and local markets. The results from this study have shown that *Salmonella spp.* was the most prevalent pathogen in rivers 100m before entry into the lake and at inshore which could be the reason for its high prevalence on the surface, gills and intestines of fish. Since no *Salmonella spp.* was isolated in offshore water samples where fishing is done, the pathogens found on the fish surface and gills could be due poor handling and contamination during fishing, inshore water and handling containers. At the beach, live fish were transferred into the containers filled with water from the inshore or beaches. Thus, it is likely that water from the inshore was the source of *Salmonella spp.*isolated on the fish surfaces and gills at significantly higher proportion ($P < 0.05$) than in the intestines (Figure 4).

*Vibrio parahaemolyticus* that cause self limiting and rarely fatal, mild to moderate gastrointestinal infections in humans is often associated with consumption of sea food. It is a normal microflora of coastal and estuarine waters and an obligate halophile that grows at salt concentration of at least 0.5%. In Tanzania, the occurrence of *V. parahaemolyticus* at high proportions has been reported in coastal waters and fish (Ijumba (1984). It is rarely isolated in freshwater environment (Sarker *et al*., 1985) and fish. This is supported by findings from previous studies that reported prevalence of *V. parahaemolyticus* at 1% and 0.3% in fish in Lake Naivasha and Lake Victoria respectively (Kagiko *et al*., 2001). Previous detailed studies of *V. parahaemolyticus* in Nile perch and in deep waters and sediments in Lake Victoria demonstrated a prevalence of 0% (Mhongole, 2009). Since during this study, none of water samples collected at offshore in the lake was positive for *V. parahaemolyticus*, indicates that the isolates found in Water Rivers and beach (inshore water) samples may be results from humans and their related activities.

**Conclusion**

Based on findings from this study, it is evident that rivers are the main point sources that have a significant contribution to the total microbial pollutants in water and fish in the lake. Detection of high prevalence of *Salmonella spp.*, *V. cholerae* and *V. parahaemolyticus* is an indication that these pathogens are responsible for making fish and water unsafe for human consumption. Further, the pathogens may also be responsible for endemic state of enteric diseases in humans in Mwanza city. The effect of these pathogens on consumers, fishermen, processors and traders among others, can be controlled by HACCP which if stringently implemented, can ensure the safety of fish and fish products intended for export and local markets.

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