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CONTAMINATION OF THE MINNOW *RASTRINEOBOLA ARGENTEA*, THROUGH HANDLING AT LANDING SITES AND RETAIL MARKETS AROUND LAKE VICTORIA

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ABSTRACT

Background: The quality of fish could be a challenge both locally and internationally as microbial contaminants may result in infections of humans when seafood is handled unhygienically. This is because enteric bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp*. and *Vibrio cholerae* are likely to accumulate in fish where low hygienic standards are maintained. Therefore, studies on fish contamination are critical for the acceptance of fish food locally and in international markets.
Objective: To evaluate and compare the microbiological quality of *R. argentea* at the fish landing sites and local retail markets in Homa Bay County.
Design: Purposive, cross-sectional study.
Setting: Fish landing sites and retail markets in Homa Bay County
Subject: Studies on fish contamination are critical for the acceptance of fish food locally and in international markets. In this study, *Rastrineobola argentea* were examined for microbial loads.
Results: Samples indicated high levels of coliforms and *Escherichia coli* in fish. At the landing sites, coliforms ranged from 1.6×10^3 to 4.0×10^3 while *E. coli* was between 0.8×10^1 and 4.8×10^2. In water samples, coliforms ranged between 1.3×10^3 and 1.8×10^6 / ml whereas *E. coli* was 1.1×10^2 to 4.6×10^1. Personnel hands were found to be contaminated with *Salmonella typhimurium* and *Shigella dysenteriae*. The samples from markets had coliform and *E. coli* at 5.0×10^4 to 9.0×10^5 and 2.0×10^2 to 4.0×10^2 respectively.
Conclusion: The findings demonstrate that precaution should be taken to improve the hygiene levels along the value chains to ensure that the quality of fish is not compromised.

INTRODUCTION

Over the years, the Kenyan part of Lake Victoria has experienced tremendous changes in fish species with *Rastrineobola argentea* overtaking *Lates niloticus* (Nile perch) in total annual fish landings. Economically, it supports more families in terms of employment than other fisheries since more rural poor women are involved in the *R. argentea* trade than any other fishery in the Lake Victoria (1). Generally, Homa Bay County is an important fish producing region along Lake Victoria producing over 64% of total fish landings. The *R. argentea* has continued to attract huge demand as human food and animal feed production.

Microbial composition of fish depend on environmental conditions, species of fish, storage conditions and species of bacteria (2). Bacteria can be present in food when it is eaten especially in fish that get into the body and then release endotoxins which irritate the stomach and the bowels. The quality of fish could be a problem both locally and internationally as microbial contaminants may result in infections of humans when seafood is handled unhygienically. This is because enteric bacteria such as *Escherichia*

coli, Staphylococcus aureus, Salmonella spp and *Vibrio cholerae* are likely to accumulate in fish where low hygienic standards have been maintained. These bacteria could survive for considerable lengths of time in contaminated fish. Furthermore, fish with high bacterial content have usually a disagreeable smell for consumption as some bacteria have abilities to produce metabolites responsible for off-oduors and off-flavours (3).

Bacterial pathogens that infect humans and are non-indigenous in seafood only get contaminated with seafood during handling and processing (3). Microbial evaluation of on-line and finished products indicate that bacterial contamination occurs primarily through exposure to polluted environments or cross-contamination during landing, transporting, processing, storage or during preparation for consumption (4). At industrial level in Kenya, incidents of *Salmonella spp* and *Vibrio cholerae* have been reported in fish samples collected in two establishments as 1 and 1.4% respectively (5). Additionally, (6) reported the presence of *V. cholerae* and *S. aureus* in smoked tilapiine species, *S. aureus* and *E. coli* in smoked Nile perch.

Locally studies to understand the quality of *R. argentea* have focused on either products at the market or fish landing sites distinctively. No effort has been made to understand the microbiological quality of *R. argentea* along the value chain. Therefore in this study, we attempt to evaluate and compare the microbiological quality of *R. argentea* at the fish landing sites and local retail markets in Homa Bay County. The study used coliforms and *Escherichia coli* as indicator organisms for faecal contamination.

MATERIALS AND METHODS

Study site: Homa Bay County is in Western Kenya and borders Migori, Kisumu and Siaya counties around Lake Victoria. A cross-sectional study approach was adopted based on random sampling.

Sample collection and handling: Fresh *R. argentea* was collected from three landing sites on every sampling occasion for a period of three months from April to June, 2015. At landing sites, surfaces that had come into contact with fish and personnel hands were sampled by swabbing 100 cm² of various surfaces. If the surface was dry, the swab was first moistened in sterile 0.1% peptone water and squeezed to remove excess water before taking the swab. Besides, water used by fishermen and fish mongers to clean landed fish was aseptically collected. The collected water (50 ml) was poured into 100 ml Bijou bottles containing 50 ml Trypticase Soy Broth and labelled using adhesive taps. All samples were then put in a cooler box filled with ice and transferred to National Microbiology Reference Laboratory in Nairobi for analysis. Using a sterile scalpel and forceps, fish samples (25g) were aseptically transferred to sterile flasks. Then 225 ml of 0.1% buffered peptone water was added to make a 1:10 M/V dilution (stock solution/ homogenate). The homogenate was thoroughly mixed using a stomacher at 15,000-20,000 revolution per min. for 2.5 min. The resultant homogenate was used as stock solution. The stock solution was used for analyzing coliforms, faecal coliforms and presence of *Salmonella* and *Shigella*.

Total coliform determination in water and fish
From the 1:10 stock solution, three 1 ml volumes were pipetted into three bottles containing MacConkey broth. The same procedure was repeated for the 10-2 and 10-3 dilutions. The Durham tubes inside the bottles containing MacConkey broth were ensured filled before incubation at 37°C for 48 hours. After 48 hours, the broth was checked for any gas (as indicated by yellow colouration) and acid production (as indicated by gas in Durham tube). The tubes with gas and acid were presumed as positive for coliforms. This process was repeated for each sample. Analysis of water for coliforms was done according to procedures recommended by the World Health Organization (7). The coliform numbers were then determined by use of the Most Probable Number (MPN) tables.

Determination of Escherichia coli in water and fish
From each of the coliform presumptive positive tubes, 0.02 ml of the broth was taken and pipetted into fresh MacConkey broth alongside each of the buffered peptone broth (P3). The broths were then incubated at 44±0.25°C in a water bath for 48 hours. Kovac's reagent was added in each of the P3 broth tubes, mixed and allowed to stand for a few minutes. With the formation of the red or pink ring, it confirmed the presence of indole. The indole production is associated with the presence of *E. coli*, which is the faecal colifom. Analysis of water for faecal coliforms was done according to procedures recommended by the World Health Organization (7). The faecal coliform numbers were then determined by use of the Most Probable Number (MPN) tables.

Isolation of Salmonella and Shigella spp
100 ml of the stock solution were pipetted into a sterile bottle containing a Rappaport Vassiliadis with soya medium and the other containing 100 ml of selenite cystine broth. The inoculated Rappaport Vassiliadis and Selenite cystine were each incubated for 48 hours at 37°C. After 18-24 hours of incubation, the contents of each flask were shaken to mix. Using a platinum wireloop, the Rappaport Vassiliadis culture was shaken and a loopful transferred to the surface of already prepared XLD (Xylose lysine desoxycholate) and Mac Conkey agar plates. The streaked plates were then inverted and incubated for 18-24 hours at 37°C. Similar plates were streaked in the same way with the cultures from the Selenite medium. The cultures were returned to their respective incubators

to complete their 48 hour incubation period. The plating was repeated a second time on a further series of plates at the end of the 48 hour period. The inoculated plates were also incubated for 18-24 hours at 37°C. After incubation, plates on MacConkey were examined and colonies present were sub-cultured onto TSI slants and incubated at 37°C for 48 hours. The slants that were positive for hydrogen sulphide (H2S) were further analyzed using serological tests by use of *Salmonella* and *Shigella antisera*.

RESULTS

Results of this study demonstrate that water sampled at Kolunga had more coliforms than the three landing sites at 1.6×10^3-1.8×10^6 mpn/100cm^2 followed by Tabla at 1.5×10^3-1.8×10^6 mpn/100cm^2 then Nyachebe at 1.3×10^3-1.8×10^6 mpn/100cm^2 (Table1). However, the coliforms range in fish were 1.6×10^3 - 2.4×10^6 mpn/g at Kolunga, 2.4×10^3 - 2.4×10^6 mpn/g at Nyachebe and 2.4×10^3 - 4.0×10^3 mpn/g at Tabla. This implies that Nyachebe had more coliforms range followed by Kolunga and Tabla respectively. Therefore, water at Kolunga landing site was exposed to a lot of contamination compared to Nyachebe and Tabla landing site, whereas fish samples from Nyachebe landing site had been exposed to most contamination

Table 1
Total coliforms in water and fresh *Rastrineobola argentea* at three landing sites in Homa Bay County

Landing site	Coliforms in water (mpn/100cm^2)	Coliforms in fish (mpn/g)
Kolunga	1.6×10^3-1.8×10^6	1.6×10^3 - 2.4×10^6
Nyachebe	1.3×10^3-1.8×10^6	2.4×10^3 - 2.4×10^6
Tabla	1.5×10^3-1.8×10^6	2.4×10^3 - 4.0×10^3

Table 2 shows that *Escherichia coli* ranges in water were 1.8×10^3-3.5×10^1 mpn/100cm^2 at Kolunga, 1.1×10^3-4.6×10^1 mpn/100cm^2 at Nyachebe and 1.2×10^1-1.0×10^2 mpn/100cm^2 at Tabla. It shows that just like coliforms ranges; water sampled at Kolunga had higher *E. coli* among the three landing sites.

For fish samples, *Escherichia coli* ranges were 0.8×10^1-4.8×10^2 mpn/g, 2.4×10^2-2.4×10^6 mpn/g and 1.1×10^6-1.6×10^3 mpn/g for Kolunga, Nyachebe and Tabla respectively. Consequently, Nyachebe had the highest ranges followed by Tabla and Kolunga landing sites.

Table 2
Total *Escherichia coli* in water and Fresh *Rastrineobola argentea* at three landing sites in Homa Bay County

Landing site	*Escherichia coli* in water (mpn/100cm^2)	*Escherichia coli* in fish (mpn/g)
Kolunga	1.8×10^3-3.5×10^1	0.8×10^1-4.8×10^2
Nyachebe	1.1×10^3-4.6×10^1	2.4×10^2-2.4×10^6
Tabla	1.2×10^1-1.0×10^2	1.1×10^6-1.6×10^3

Table 3 shows that Mbita fish market had more coliforms and *E. coli* ranges at 3.0×10^5-1.0×10^6 mpn/g and 8.7×10^3-2.3×10^4 mpn/g respectively on sun dried *R. argentea* than Homa Bay fish market 1.3×10^5-1.1×10^6 mpn/g and 8.8×10^3-1.2×10^4 mpn/g respectively. This indicates that Mbita fish market and its sun dried *R. argentea* are more contaminated than Homa Bay fish market.

Table 3
Coliforms and Escherichia coli on Sundried *Rastrineobola argentea* at two markets in Homa Bay County

Markets	Coliforms **(mpn/g)**	*Escherichia coli* **(mpn/g)**
Mbita	$3.0 \times 10^5 - 1.0 \times 10$	$8.7 \times 10^3 - 2.3 \times 10^4$
Homa-Bay	$1.3 \times 10^5 - 1.1 \times 10^6$	$8.8 \times 10^3 - 1.2 \times 10^4$

Personnel hands from Kolunga and Nyachebe landing sites were found to be contaminated with *Salmonella typhimurium* and *Shigella dysenteriae* respectively. While at Tabla landing site, no incidences of either *Salmonella* or *Shigella* spp were recorded. Again for Mbita and Homa Bay fish markets, only Homa Bay fish market personnel hands of those handling sun dried *R. argentea* were found to be contaminated with *Salmonella spp*. (Table 4).

Table 4
Isolated serotypes of *Salmonella* and *Shigella* as recovered on personnel hands at landing sites and one market in Homa Bay County

Sampling	Incidences	Serotype
Landing site	1	*Salmonella typhymurium*
Kolunga	1	*Shigella dysentariae*
Nyachebe		
Markets		
Homa Bay	1	*Salmonella spp*

DISCUSSION

In this study, water quality was unacceptable as its total coliform and *E. coli* levels were above those recommended for recreational waters which are nil *E. coli*/faecal coliform and 500cfu/100ml for total coliforms (8). Previous monitoring of microbiological quality of water at the Lake Victoria shores has reported relatively unacceptable microbiological quality levels (9). Generally, the microbiological quality of water at the shores could be attributed to human activities such as washing of dishes and clothes within the water body, fetching water, boat launching all stir bottom sediments which are microbial habitats (10), thereby suspending bacteria and leading to increased levels of coliforms at the shoreline.

The ICMSF (11) recommends a limit for fresh fish at 4×10^2 mpn/g while Kenya Bureau of Standards (KEBS) recommends 0 cfu/g in dried *R. argentea* (12). Kolunga landing site had *E. coli* counts in excess of the limit for good quality fish. The high levels of these organisms indicated the direct or indirect contamination of the fish with faecal matter and possible presence of other potential enteric pathogens.

The current study found personnel involved in processing the fish products to be important carriers of *Salmonella typhi* and *Shigella dysenteriae* at Nyachebe and Kolunga landing sites. Personal contamination during fishing expeditions have been reported in other studies (2). Since the fishers were using the lake shores as a source of water for both domestic use and washing of fresh fish, it's possible that such practices of using untreated water on fish could lead to increased bacterial loads on sundried fish products at retail markets. Similarly, poor handling practices could lead to the fish products being exposed to pathogenic bacteria such as *Salmonella* (13).

The study observed high levels of coliforms and faecal coliforms contamination in all the *R. argentea* market samples tested including presence of *E. coli* and *Salmonella*. For example, Mbita market recorded the highest mean levels of 6.78×105 MPN/g whereas Homa Bay recorded 6.68×105 MPN/g. The coliforms and faecal coliforms are measures of possible faecal contamination. This therefore implies that the fish being sold at local markets is of low microbial quality and does not comply with the locally laid down standards of *R. argentea* (12). The Kenya Bureau of Standards, 1998 specifications recommend that sun dried *R. argentea* should have TPC of not more than 105 cfu/g, zero coliform counts and no food-poisoning organisms. Previously, *Escherichia coli* in smoked Nile perch were reported around beaches and markets within Winam Gulf of Lake Victoria

(6). *E. coli* is known to be non-pathogenic in fish but many strains are human pathogens (14). The findings in this study can be attributed to the operations the fish products go through, which include harvesting, processing, distribution and marketing.

Salmonella spp. should be absent from fish samples of 25g (11; 15). This guideline is based on the fact that *Salmonella spp.* are naturally present in the aquatic environment and therefore are likely to be detected in freshly harvested fish. However, locally it is recommended that *Salmonella spp.* should not be present in dried *R. argentea* (12). This study recorded the occurrence of pathogenic microbes such as *Salmonella* that could be attributable to poor handling and preservation techniques employed by the fishermen (13) which eventually could be responsible for the presence of the organism on sundried products found at the retail markets. Sifuna *et al,* (16) has also shown that fish products offered for sale in Kisumu markets were contaminated with *Salmonella spp.* These findings therefore, demonstrate that there are potential health hazards present in Omena retailed in local markets.

In Conclusion, this study shows that handling practices and general processing of *R. argentea* practices were inadequate for maintaining the quality of fish. Such practices were identified as displaying fish over open drains, exposure to ambient temperatures of the same batch of fish and display of fresh fish in dirty containers with drips from previous sales. The repeated spreading of *R. argentea* on mats and dirty sacks for sundrying could inoculate the fish with a lot of organisms.

Based on the results of this work, this study recommends that there is need to carry out further surveys for most landing sites around the lake and retail markets to determine the hygiene status of the surroundings where the product is passed through. It is also recommended that regular monitoring of microbiological quality along the landing sites and markets be enhanced.

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