BACTERIOLOGICAL QUALITY OF FRESHWATER FISH CAUGHT FROM TWO NATURAL LAKES IN THE RAINFOREST REGION OF SOUTH-EASTERN NIGERIA

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ABSTRACT

The study evaluated the bacteria quality of freshwater fish caught from Oguta and Agulu Lakes. A total of 24 fish samples consisting of 12 samples from each Lake were sampled for four months. The fish samples were transported to the laboratory, processed and compared. Using a sterile scapel and a pair of sterile scissors, section of fish scales, gills, gut, skin, liver, intestine and gonads were removed and subjected for bacteriological analysis. A total of eight (8) bacteria isolates were identified from the fish samples. The bacterial isolates were Escherichia coli, Aerobic mesophlic bacteria, Salmonella typhi, Listera monocytogen, Vibrio cholerae, Coliforms and Staphylococcus aureus. The gill yielded the maximum isolation rate of 4310(22.3%) of the total bacteria isolates from fish of Oguta Lake, while scale had the maximum isolation rate of 2530(27.1%) of the total isolates from fish of Agulu Lake. The common isolates namely Escherichia coli, Salmonella typhi and Coliforms of the family Enterobacteriaceae formed 79.9% of the total bacteria isolates from Oguta Lake fish and 74.1% from Agulu Lake fish. Staphylococcus aureus was insignificant in Oguta Lake fish (19, 0.1%) but was prominent in Agulu Lake fish where it contributed 150 (1.6%) of the total isolates. Of all the bacteria species identified, only Escherichia coli was isolated from the body parts of fish of both Lakes. Aerobic mesophilic bacteria and Staphylococcus aureus were encountered only in the scales and gut of fish of both Lakes. Salmonella typhi occurred only in the scale of Oguta fish. Vibrio parahaemolyticus was recorded only in the gonads of fish of Oguta Lake and in the intestinal parts of fish of Agulu Lake. The study revealed that the fish from both Lakes is of poor quality and this has a serious health implication. Therefore, sewage should be properly treated before disposal into aquatic ecosystems. Fish should be harvested with unpolluted water as well as handled with caution at post harvest.

Keywords: Bacteria, Freshwater, Fish, Lakes, Public health, Oguta, Agulu, Rainforest, Nigeria

INTRODUCTION

Fishes are vertebrates, poikilotherms and live predominantly in water (Ugwu and Mgbenka, 2006). Their bodies may be elongate, dorsoventrally, laterally compressed or rounded in cross section but recognizable into head, trunk and post anal tail (Egborge, 1993).

Fish are highly important in the development of Nigeria both economically and health wise. Fish have been one of the main foods for humans for many centuries (Leisner *et al.*, 1995; Shinkafi and Ukwaja, 2010).

Fish are known for their high nutritional quality, relatively low in fat, saturated fat, cholesterol and high in poly-saturated fatty acids, protein and minerals such as calcium, phosphorus, sodium, potassium and magnesium (Hany El-Said, 2004; Salihu *et al*., 2012). Millions of people in the world today depend on fish for protein. Fish contributes about 60% of the world supply of protein and 60% of the developing world derives more than 30% of their animal protein from fish (FAO, 1994).

In sub-Saharan Africa, fish accounts for 10% of the animal protein consumed and 98% of this is fin-fish (Delgado and McKenna, 1997; Imam *et al*., 2010). In Africa, fish is widely consumed as a remarkable source of animal protein. Thus, the average per capita world of fish in Africa in 1992 was about 8 kg having increased from an average of 7 kg per annum, from 1969 – 1974 (Ahmed, 1997; Imam *et al*., 2010). In Nigeria, the short supplies of animal protein together with the increasing human population have raised the cost of animal protein to a level almost beyond the reach of the low income group (Ezeri, 2001). The resultant effect is a considerable increase in the demand for fish as an alternative, source of animal protein in the face of the ever increasing population. Fish is more fancied and widely consumed than meat in the Niger Delta area of Nigeria. In Mali, Breuil and Quensiere (1995) stated that the fish consumption in the area was about 10.5kg/person/year compared to 10kg/person/year elsewhere in Africa.

Aquatic environments are easily polluted by both wastes from homes, farmlands and industries. This endangers the life of aquatic biota such as fish since fish take in a large quantity of bacteria into their alimentary tract from water and food. However, Infection due to microbial contamination does not according to Olayemi *et al*. (1990) and Salihu *et al*. (2012) result in disease; environmental stresses may upset the balance between the potential pathogens and their hosts.

Fish are usually infected with a wide range of microbes in aquatic ecosystems. The types of micro-organisms found associated with fish depend on the aquatic habitats of fish and are known to be affected by certain factors like the saltiness level and bacterial load of the habitat (Diler *et al*., 2000; Salihu *et al*., 2012).

Bacteria often occur in parts of fish such as scales, gills, gut and alimentary tract. The bacteria present on the body or internal organs of fish indicate the extent of pollution of aquatic ecosystems.

In faecal polluted water, *Aeromonas* sp., Coliforms, *Shigella flexineri*, *Salmonella* sp. etc are common bacteria which can penetrate the body of fish through different routes including wounds, natural openings, ingestion and engulfment with food water. Kvenberg (1991) and Rodricks (1991) classified bacterial pathogens into non-indigenous and indigenous pathogens.

The indigenous pathogens include *Closteridium botulinum, Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* species, *Escherichia coli* and others (Salihu *et al*., 2012). However, non-indigenous pathogens which are not harmful to fish may on consumption be harmful to man.

Oreochromis niloticus, Clarias gariepinus and *Synodontis budgetti* are highly cherished for their tasty and social significance, particularly among the Igbo people of Nigeria. They are also used in educational institutions because of their readily availability.

Bacteriological quality is of importance to public health since it directly relate to fish spoilage and may cause food poisoning. It is therefore important to monitor the quality of harvested freshwater fish from Oguta Lake and Agulu Lake to ensure that the fishery products does not pose health risks to end users and that the level of potential organisms is within the limits of acceptable product shelf-life. By monitoring the bacteria content of fish organs, the quality of fish can be measured since these will affect the spoilage life and quality of fishery products.

This study was aimed at investigating the bacteria load on the scales, gills, guts liver, intestines and gonads of fishes caught from Oguta and Agulu Lakes within the rainforest region, south-eastern Nigeria.

MATERIALS AND METHODS

Study Area

Fishes from two Lakes namely Oguta Lake (Figure 1) and Agulu Lake (Figure 2) within the rainforest region, south-eastern Nigerian were used for study. The climate is tropical with rainy season (April – September) and dry season (October / November – March). Both Lakes are natural lacusterine Lakes. Human activities in and around the Lake include fetching of water for domestic purposes, fishing, swimming, defaecation, dumping of sewage, laundry, dredging of soil, farming and deforestation.

Aquatic macrophytes in and around the Lake include *Vossia cuspidata, Ipomoea bartata, Pistia stratiotus, Nymphaea lotus and Ceratophyllum* sp.

Figure 1: Map of Oguta Lake, Nigeria

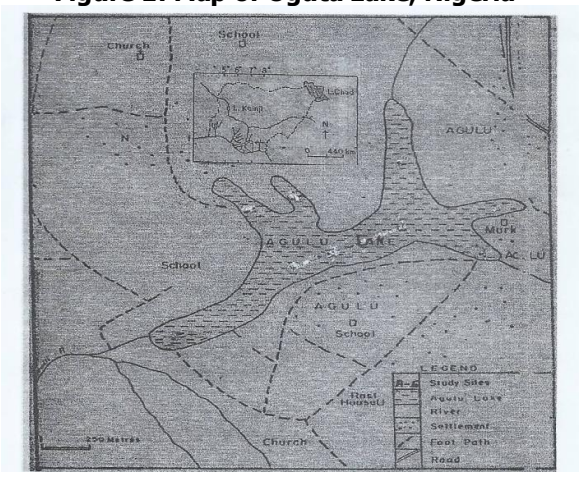

Figure 2: Map of Agulu lake, Nigeria

Agulu Lake is a six armed medium-sized Lake located between latitude 6^0, 01^1 and 6^010^1N and longitude 6^0 35^1 and 7^0 03^1E (Inyang and Ezenwaji, 2004). The Lake empties into River Niger at Atani via the Idemili River. Among the riparian vegetation macrophytes around the Lake include *Elaeis guneensis* and grasses.

Sample Collection and Processing

Four different samples each of *Oreochromis niloticus*, *Clarias gariepinus* and *Synodontis budgetti* were bought from fishermen at fishing landing sites around the Lakes. The sampling was conducted fortnightly between 7.00 am and 9.00am in each sampling day for a period of four months. Each fish sample was put in a sterile polythene bag and transported to National Agency for Food and Drug, Administration and Control (NAFDAC) Zonal Laboratory, Agulu for analysis.

Preparation of Stock Cultures

Section of the scales, gills, guts, skin, liver, intestines and gonads of 24 randomly selected fish samples were removed by means of a sterile scapel and a pair of sterile scissors and kept in sterile Petri dishes. 4g each of these sections were separately pounded with mortar and pestle, and homogenized to ensure that the cells were evenly distributed.

Enumeration, Isolation and Identification

Six serial dilutions of the stock culture from the scales, gills, guts, skin, liver, intestines and gonads, were prepared with 9 ml of peptone water and 1ml of stock culture from different fish parts (scales, gills, guts, skin, liver intestines and gonads). Each stock dilution was placed on freshly prepared media and incubated at 37^{0C} for 24 hours after which the colonies that developed were identified and counted and the number recorded as cfu/ ml.

The identification of isolates was done using gram staining, physiological biochemical reaction and fermentation of sugars according to standard taxonomic scheme (Buchanan and Gibbons, 1974).

Statistical Analysis

One way analysis of variance (ANOVA) was used to analyze the bacterial load of the scales, gills, guts, skin, liver, intestines and gonads of fish samples.

RESULTS

Three species of fish consisting of 24 individuals collected from Oguta and Agulu Lakes were studied for bacterial loads (Table 1).

Table 1: Fish species and number examined from each Lake

Species of fish	Oguta Lake	Agulu Lake
	Number examined	
Oreochromis niloticus	4	4
Clarias gariepinus	4	4
Synodontis budgetti	4	4
Total	12	12
Grand Total	24	

The mean total count of bacteria identified from the scales, gills, guts, liver, intestines and gonads of fish samples from Oguta and Agulu Lakes is presented in Tables 2 and 3. There was significant difference ($p<0.05$) between the total viable count of bacteria isolates from the scales, gills, guts, liver, intestines and gonads of *Oreochromis niloticus, Clarias gariepinus* and *Synodontis budgetti* from Oguta and Agulu Lakes. For Oguta Lake, 1300 cfu/ml and 1000 cfu/ml bacteria count were respectively recorded in the gills of *Clarias gariepinus* and guts of *Oreochromis niloticus.* In the case of Agulu Lake, *Oreochromis niloticus*, had the maximum (1000 cfu/ml) and minimum (7 cfu/ml) bacteria count in its gills and guts, respectively.

Fish of Oguta Lake had maximum bacterial load (9042.33 cfu/ml) while fish of Agulu Lake had minimum bacterial load (7392.5 cfu/ml) *Oreochromis niloticus* of Oguta Lake had the highest bacterial count in the scale (1300 cfu/ml) and while its counter part of Agulu Lake had the lowest bacterial count of (7 cfu/ml) in its gut. The 24 fish samples studied were positive for bacterial isolations.

The total bacteria isolate from the different fish parts was higher (19326 cfu/ml) in fish samples of Oguta Lake and lower (9350 cfu/ml) in fish samples of Agulu Lake. The gill yielded the maximum isolation rate of 4310 (22.3%) of the total bacteria isolates from fish of Oguta Lake while scale yielded the maximum isolation rate of (2530) 27.1% of the total bacteria isolates from fish of Agulu Lake. There was variation in the frequency isolation from body parts of the sampled fish of both Lakes (Table 4).

In all 8 species of bacteria were identified from the studied fish species. The common isolates namely *Escherichia coli* (3250) in the gills of fish of Oguta Lake and 2130 of Agulu Lake. *Escherichia coli*, *Salmonella typhi* and Coliforms, of the family Enterobacteriaeae, formed 79.9% of the total bacteria isolates for fish of Oguta Lake and 74.1% for fish of Agulu Lake (Table 5). *Staphylococcus*, a gram positive genus which was insignificant in fish of Oguta Lake (10, 0.1%) was prominent in fish of Agulu Lake where it contributed (150) 1.6% of the total isolates. Of all the bacteria species identified, only *Escherichia coli* were isolated from the different body parts of fish of both Lakes (Table 5).

Aerobic mesophilic bacteria and *Staphylococcus aureus* were encountered only in the scale and gut of fish of both Lakes. *Salmonella typhi* was obtained only in the scale of Oguta fish. *Vibrio parahaemolyticus* was recorded only in the gonad intestinal parts of fish of Oguta and of fish of Agulu Lakes.

The study revealed that four bacteria isolates namely *Escherichia coli*, *Salmonella typhi,* Coliforms and *Vibrio cholera* were prominent in the two Lakes.

DISCUSSION

A number of microbial tests of fish and fish products are used by authorities to check whether the microbial status is satisfactory. The purpose of these tests is to detect pathogenic bacteria found in fish. The results of the study showed that freshly caught fish are contaminated with different species of bacteria.

Table 2: Mean bacterial viable count of from parts of fish sampled from Oguta Lake, Nigeria

Fish part	*Oreochromis niloticus*	*Clarias gariepinus*	*Synodontis budgetti*	Total
Scale	1300	90	372.67	1762.67
Gill	1000	1050	70	2120
Gut	10	75	503.5	588.5
Liver	503.5	550	552.5	1606
Gonad	550	436.33	49.5	1035.83
Total	4032.83	2956.33	2053.17	9042.33

Table 3: Mean bacterial viable count of from parts of fish sampled from Agulu Lake, Nigeria

Fish part	*Oreochromis niloticus*	*Clarias gariepinus*	*Synodontis budgetti*	Total
Scale	600	436.67	505	1541.67
Gill	1000	370	340	1710
Gut	7	575	10.5	592.5
Liver	505	557	505	1567
Intestine	763.33	82	19	864.33
Gonad	550	550	17	1117
Total	3425.33	2570.67	1396.5	7392.5

Table 4: Percentage bacterial isolation from fish parts

Fish parts	Oguta Lake		Agulu Lake	
	Number of isolates	Percentage isolates	Number of isolates	Percentage isolates
Scale	3898	20.2	2530	27.1
Gill	4310	22.3	2125	22.7
Gut	1167	6.0	1328	14.2
Liver	3905	20.2	1030	11.0
Intestine	3638	18.8	1202	12.9
Gonad	2408	12.5	1135	12.1
Total	19326	100.0	9350	100.0

In both Lakes indigenous and non-indigenous bacterial species were isolated from the freshwater fish samples. This implies that fish are passive carrier of non-indigenous bacteria pathogens (Salihu *et al*., 2012). Many of the Bacteria species such as coliforms, *Salmonella typhi* encountered in fish in this study from Oguta and Agulu Lakes belong to the family Enterobacteriaceae. The Coliforms which are Gram-negative, oxidase – negative, non – sporing rods are the most frequently used indicators of faecal pollution (Mason, 1996). The *Escherichia coli* isolated in the study appeared in large numbers. Although *Escherichia coli* obtained from fish samples of Oguta Lake (11139, 57.6%) was higher than that obtained from fish samples of Agulu Lake (4427, 47.3%), the values were relatively high and may be attributed to faecal contamination of water in which fish live and mishandling which occurs after fish harvest. The isolation of the organism *Escherichia coli* is particularly useful as an indicator of contamination of fish when appeared in small numbers or as an indicator of mishandling when appeared in large numbers (Salihu, 2012). Fish harvested from contaminated waters (Pelzar *et al*., 1993) can cause health hazards to fish consumers including man. The isolation ration of *Salmonella typhi* which are gram-negative facultative rods with petritrichous flagella had close isolation rate in fish samples from the Lakes. The shells are passive carriers of Salmonella (Salihu *et al*., 2012) thus isolation in this study may be from soil. Direct relationships have been found between *Escherichia coli* count and the numbers of pathogenic organisms such

Table 5: Isolation frequency of bacteria from various fish parts

Bacteria	Scale	Gill	Gut	Liver	Intestine	Gonad	Total	Percentage
					Oguta Lake			
Escherichia coli	2130	3250	1140	1100	2510	1009	11139	57.6
Aerobic mesophilic bacteria	1760	-	-	-	-	-	1760	9.1
Salmonella typhimirium	8	50	10	-	-	1200	1268	6.6
Listera monocytrgen	-	-	-	1005	-	7	1012	5.2
Vibrio cholarae	-	-	7	800	108	92	1007	5.7
Vibrio parahaemolyticus	-	-	-	-	-	100	100	0.5
Coliforms	-	1010	-	1000	1020	-	3030	15.7
Staphylococcus aureus	-	-	10	-	-	-	10	0.1
Total	3898	4310	1167	3905	3638	2408	19326	100%
					Agulu Lake			
Escherichia coli	2130	2000	163	20	2	110	44,27	47.3
Aerobic mesophilic bacteria	90	-	-	-	-	-	90	1.0
Salmonella typhimirium	200	-	-	-	-	1008	1208	12.9
Listera monocytrgen	-	10	15	-	-	17	42	0.4
Vibrio cholarae	110	-	1000	-	1019	-	2129	22.8
Vibrio parahaemolyticus	-	-	-	-	9	-	9	0.1
Coliforms	-	115	-	1010	170	-	1295	13.9
Staphylococcus aureus	-	-	150	-	-	-	150	1.6
Total	2530	2125	1328	1030	1202	1135	9350	100%

as *Samonella* (Mason, 1996). Fish harvested from contaminated waters can carry *Salmonella* sp. (Pelczar *et al.*, 1993) which are pathogenic to man and other animals. *Staphylococcus aureus* was obtained only in the gut of fish from both Lakes. However it was isolated more (150) in fish of Agulu Lake compared to fish of Oguta Lake (10) with equal percentage isolation of 0.1% each. Its presence in the fish samples may be due to post harvest handling. During handling of the commodity, the natural flora may be contaminated with organisms associated with man such as *Staphylococcus aureus* which can grow well between 30 - 37^{0}C (Shinkafi and Ukwaja, 2010). Enterotoxins produced by *Staphylococcus aureus* is of public health importance, because the toxins are serious cause of gasteroentritis after consumption of fish and related products (Salihu *et al.,* 2012).

The study revealed that the fish of Oguta and Agulu Lakes are contaminated with bacteria. The poorer fish quality of Oguta Lake may be due to sewage that empties into it.

Conclusion: The fish from both Lakes are of poor quality and this has a serious health implication. Therefore, sewage should be properly treated before disposal into aquatic ecosystems. Also fish should be carefully harvested with unpolluted water and the fish should be handled with caution at post harvest.

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