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Bacteria load on the skin and stomach of *Clarias Gariepinus* and *Oreochromis Niloticus* from Ibadan, South West Nigeria: Public health implications

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ABSTRACT

The studies about the human pathogenic bacterial load, particularly enterobacteria in fresh water is scanty in literature. The total aerobic bacteria and enterobacteria load in African Catfish (Clarias gariepinus) and Nile Tilapia (Oreochromis niloticus) randomly sampled from different aquatic environments in Ibadan, Southwest Nigeria were examined and compared. Densities of total aerobic bacteria and enterobacteriaceae count were measured from the skin and stomach. All fish samples examined were heavily contaminated with high indices of 10^{12}-10^{13} (log_{10}cfu/cm^2 =13.07 – 13.20; log_{10}cfu/g = 13.02-13.16). Significantly, higher microbial load was obtained in captured tilapia (log_{10} cfu/cm^2 13.20, t = 3.369; p< 0.001) than captured catfish while there was no significant difference between that of the skin and stomach samples (p>0.05) in cat fish. The microbial load of skin of O. niloticus was significantly higher than that of the stomach of O. niloticus. Significantly higher count of enterobacterial count was obtained in the stomach of captured catfish (log_{10} cfu/g 13.04; t = 3.235, p <0.001) and captured tilapia (log10cfu/g 12.86; t = 3.629, p <0.001) when ccompared with their respective skin samples. The high microbial load of the wild catfish and wild tilapia in this study may be due to mass pollution of the environments where the fish were caught. The public health implications are discussed.

Key Words: Bacterial load , catfish, tilapia, public health

INTRODUCTION

FAO (1) asserted that fish contributes about 60% of the world supply of protein and that 60% of the developing world derives more than 30% of their animal protein from fish. Fish allows for protein improved nutrition in that it has a high biological value in term of high protein retention in the body, low cholesterol level and presence of essential amino acids (2,3). Fish are generally regarded as safe, nutritious and beneficial but aquaculture products have sometimes been associated with certain food safety issues (4). Several studies have demonstrated many bacteria species encountered in different fish which are potentially pathogenic under certain conditions as reported for *Pseudomonas angulluseptica* (5) *Streptococcus sp*. (6,7).

Disease cause economic losses not only from mortality but also treatment expenses, postponement or loss of the opportunity to sell the fish and contraction of zoonotic diseases by the handler and final consumer of the affected fish. Contamination of hands and surfaces during cleaning and evisceration of fish is a common route of pathogen infection through contamination of other food (8). Fish and Shellfish not only transmit disease to man but are themselves subject to many diseases and capable of transmitting many of the established food borne microbial infections and intoxications (9).

The microbiology of fish skin and gastro intestinal tract has been subjected to many researches. Fish can spoil from both outer surface and inner surfaces as fish stomach contain digested and partially digested food which can pass into the intestine. After fish is being caught and dying the immune system collapses and bacteria are allowed to proliferate freely on the skin surface and the stomach. The walls of intestines do break down sufficiently for bacteria to move into the flesh through the muscle fibre. It has been suggested that intestinal microflora is the causative agent for food spoilage (10). Contamination of fish from enteric bacteria of human and animal origin may also be responsible for various food spoilages (11).

Fish take a large number of bacteria into their gut from water sediment and food (12). It has been well known that both fresh and brackish water fishes can harbor human pathogenic bacteria particularly the coliform group (13). Faecal coliform in fish demonstrates the level of pollution in their environment because coliform are not named flora of bacteria in fish (14).

The consumption of fresh African Catfish (*Clarias gariepinus*) and wild Tilapia fish (*Oreochromis niloticus*) is on the increase in both rural and urban centres (15) in Nigeria. However, there is dearth of information on the bacterial load in African catfish and Nile Tilapia sampled from ponds and natural water. Thus the present study was designed to investigate the total bacterial and enterobacteria count on skin and stomach of both wild and cultured Tilapia source from different aquatic environments in Ibadan southwest Nigeria.

MATERIALS AND METHODS

Study Location

Three study sites in Ibadan, Southwest Nigeria were used for this study. A commercial fish pond, (A), A fishery institute fish ponds (B), and a River (C). The area and depth of the ponds were determined.

Table 1: The Descriptive analysis of the study sites

Sites	*Latitude	*Longitude	Area (m^2)	Water depth (m)
A	7.3878 N	3.8964 E	120,270	1.1
B	7.3878 N	3.8964 E	20,045	1.0
C	7.3878 N	3.8964 E	NA	NA

*A, commercial fish pond; B, pond of fishery institute; C, A river, NA- Not available *Source: http// www.Wikipedia.com/june 2010.*

Collection and processing of Fish Samples

Live African catfish (*Clarias gariepinus)* and tilapia species (*Oreochromis niloticus)* were randomly collected from the study sites. Wild African catfish and wild tilapia fish were collected from Eleyele River, while cultured African catfish and cultured tilapia fish were collected from ponds of the commercial farm and the fisheries institute respectively. A total of 210 tissue samples (skin and stomach) harvested from 48 fishes (24 *Clarias gariepinus*- 12 wild and 12 cultured), with average weight (grams) of 814 ± 82.95 and 1146.67 ± 36.98 for wild and cultured respectively (24 *Oreochromis niloticus* – 12 wild, and 12 cultured) with average weight (grams) of 99.83 ± 21.76 and 65.63 ± 9.6 for wild and cultured respectively were analyzed in this study. Eighteen (18) samples of feral (natural) and cultured pond water were randomly collected from different locations and analyzed. Fish were caught by a local fishing gear and by cast net. Sampling was drawn between 8.00 and 10.00 am in each occasion at periodic intervals of seven days for three consecutive times. Fish samples were transported directly to the Food and Meat Hygiene Laboratory of Department of Veterinary Public Health and Preventive Medicine, University of Ibadan within 2hrs of sampling.

Sample preparation

Bacterial isolates from each specimen were obtained from skin and stomach tissue samples by macerating aseptically skin ($1cm^2$) and stomach (1g portion) separate and shaking in 10ml distilled water. The stock solution was serially diluted ten folds. 0.1ml of (10^{-10}) dilution was spread on to nutrient agar and MacConkey Agar (MCA) in duplicate and incubated for 18-24 hrs at 37^0C. The bacteriological media namely nutrient agar (NA) and MacConkey Agar (MCA) (Micrometer, Theme, India) were prepared according to manufacturer's instructions. The media was sterilized at 121^0C for 15 minutes in an autoclave (Fishers scientific, USA) and was poured into sterile disposable petri dishes (Fishers scientific).

The colony forming counts per $1cm^2$ for skin and per 1gm of stomach was determined using standard methods (16,17). The results obtained were converted to logarithms in base ten. Each distinct colony was further subcultured on freshly prepared NA and MCA for evaluation of purity and colonial morphology. The isolates were then identified using gram staining, physiological, biochemical reaction and fermentation of sugars according to standard taxonomic schemes (18).

Statistics
The bacterial load of the skin and stomach of fish samples were compared using the student T test.

RESULTS

The density of total aerobic bacteria found in the skin and stomach of both wild and cultured African catfish and Nile tilapia were compared. The result of means shown in table 2 reveal that there was no significant difference between all skin and stomach samples of their total bacterial count ($p>0.05$). Though, the table revealed that highest bacteria count 159.50±13.721 was recorded in the skin of the wild tilapia while lowest count of 104.17±9.025 was recorded from the stomach of the same species.

The mean microbial loads of skin and stomach of the fish samples compared within are presented in table 3. All the fish samples were contaminated with the microbial load (log_{10}cfu/cm^2 or g) in the range of 13.02-13.20. The highest microbial load was obtained from the skin of *O. niloticus*, while at least occurrence of bacteria was found in the stomach of *O. niloticus*. The microbial load of skin of *O. niloticus* was significantly higher than that of the stomach of *O. niloticus*.

Enterobacteriaceae count
The result of the means shown in table 4 reveals that there was no significant difference between skin of fish samples of their enterobacteriaceae count ($p>.05$) when compared.

The results of enterobacteriaceae count in the skin and stomach of the fish samples are presented in table 5. The log_{10}cfu/cm^2/g varied from 12.69-13.04. The highest enterobacteria load was found in the stomach of wild *C. gariepinus* while the least load was found on the skin of *O. niloticus*. The enterobacteria loads obtained from the stomach of the wild fish samples were significantly higher than those obtained from the skin of the wild fish samples. However, there was no significant difference in the enterobacteria loads of the stomach and skin of the cultured fish samples.

The concentration of enterobacteriaceae count detected in the skin and stomach of different fish samples were indifference ($p > 0.05$) however, there was significantly higher count in the stomach of wild catfish compared to other fish samples as shown in table 3. Table 4 compared the densities of enterobacteriaceae count (mean ±5cm) of skin and stomach of wild and cultured *Clarias gariepinus* and *O niloticus* individually. The result revealed that there is no significance in enterobacteriaceae count of cultured catfish and cultured tilapia. However, the t-test shows that there is significant effect of samples on enterobacteriaceae count of wild catfish and wild tilapia with value obtained from their stomach significantly higher value in their stomach than the skin.

Table 2: Means of Total bacteria count obtained from skin and stomach of fish samples

Samples	Skin	Stomach
	Mean±SEM*	**Mean±SEM***
Cultured cat fish	117.33±11.554[a] (13.07)*	144.50±22.39[a] (13.16)**
Wild cat fish	121.08±8.837[a] (13.08)	137.33±17.215 (13.14)[a]
Cultured tilapia	143.67±19.245[a] (13.16)*	104.72±17.185[a] (13.62)**
Wild tilapia	159.50±13.721[a] (13.20)*	104.17±9.025[a](13.02)**

**Means with same letter are not significantly different from the other vertically according to Duncan Multiple Range Test at p<0.05. *$Log_{10}cfu/cm^2$ **$Log_{10}cfu/g$*

Table 3: Total bacteria load (10^{11}) of skin and stomach of *Clarias gariepinus* and *Oreochromis niloticus*

Body parts	*Clarias gariepinus*		*Oreochromis niloticus*	
	Wild	Cultured	Wild	Cultured
Skin	121.08± 8.84 (13.08)*	117.33± 11.55 (13.07)*	159.50± 13.72 (13.20)*	143.67± 19.25 (13.16)*
Stomach	137.33± 17.22 (13.14)**	144.50± 22.39 (13.16)**	104.17± 9.03 (13.02)**	104.72± 17.19 (13.02)**
t value	-1.078	-0.840	3.369***	1.509

$Log_{10}CFU/cm^2$ skin; **$Log_{10}CFU/g$; * significant difference at (P<0.001); each value is a mean of duplicate readings of 12 fish samples*

Table 4: Means of Enterobacteriaceae count obtained from skin and stomach of fish samples

Samples	Skin	Stomach
	Mean±SEM*	**Mean±SEM***
Cultured cat fish	66.08±5.51[a] (12.82)*	75.33±10.64[b] (13.16)**
Wild cat fish	66.75±7.113[a] (12.81)	110.42±11.471[a] (13.04)*
Cultured tilapia	63.83±7.33[a] (12.80)*	67.17±10.74[a] (12.82)**
Wild tilapia	49.00±4.125[a] (12.69)*	74.08±5.545[b] (12.86)**

**Means with same letter are not significantly different from the other vertically according to Duncan Multiple Range Test at p<0.05. *$Log_{10}cfu/cm^2$ **$Log_{10}cfu/g$*

Table 5: Enterobacteria count (x10^{11}) of skin and stomach of *C. gariepinus* and *O. niloticus*

Body parts	*Clarias gariepinus*		*Oreochromis niloticus*	
	Wild	Cultured	Wild	Cultured
Skin	66.75± 7.11 (12.81)*	66.08± 5.51 (12.82)*	49.00± 4.13 (12.69)*	63.83± 7.33 (12.80)*
Stomach	110.42± 11.47 (13.04)**	75.33± 10.64 (12.86)**	74.08± 5.55 (12.86)**	67.17± 10.74 (12.82)**
t value	3.235***	-0.77	3.629***	-0.256

$Log_{10}CFU/cm^2$ skin; **$Log_{10}CFU/g$ stomach; * significant difference at (P<0.001); each value is a mean of duplicate readings of 12 fish samples*

DISCUSSION

The mean bacterial load of all the skin and stomach of all the fish sampled from different aquatic environments were generally high with cfu of 10^{12}-10^{13}. The permissible count of heterotrophic bacteria in the $1cm^2$ of skin ranges from 10^2 – 10^7 (19) or bacteria of $\log_{10}$ cfu/cm^2 ≤ 5.70 according to Internal Commission on the Microbiology Specification of Foods (20). The skin bacteria load found in this study for African catfish and tilapia species ranged from 10^{12}-10^{13} (log cfu/cm^2 13.02 – 13.20). This also similar to that obtained for the stomach in this investigation, (the bacterial load ranged from 104.17-144.50 x 10^{11}/cfu/g) which was beyond other workers' report of 10^3-10^9 per gm (12,21)

The load of different bacteria observed in the external fish mucus and on the fish skin samples were also higher than the recommended value for fish culture. The higher density in fish may be due to their consumption of bacteria for long time through food and water (22). The survival of these bacteria is dependent on the conditions prevailing in the aquatic environment and fish are often simply their hosts (19,23,24,25).

The total bacteria load in the stomach of wild and cultured catfish and tilapia were not different. However, there was a significantly higher count on the skin of wild tilapia than obtained from the stomach, this could be due to mass pollution of one environment where the fish were caught and scales on the skin of the tilapia that can harbor feed matter suitable for the survival of the bacteria than the stomach. The lower pH environment (pH below 2) of tilapia stomach which ruptures the cell wall of bacteria could be responsible lower bacterial count as well.

The ccontaminations observed may result from rupturing fish intestine during poor processing or inadequate washing as intestinal microflora of human or animal origin are the causative agent for food spoilage (10,12). The higher density of total aerobic bacteria found in the skin and stomach might be due to quick proliferation after catching and during transportation and storage. Preservation in low quality ice, handling with contaminated hands could also be responsible for higher density of aerobic bacteria. Fish are very much susceptible to contamination with different bacteria because of their perishable protein content (22).

The ponds and rivers that harbour the fish may be the source of contaminates due to indiscriminate deposition of human, animal excreta and other environmental wastes into natural water, land and during the rainy season especially, as the faecal matter from various sources are washed from contaminated land into different water bodies. Free roaming animals and pets especially dogs also contribute to faecal contamination of surface water. Run-off from roads, parking lots and yards can carry animal wastes into natural water course and ponds. Birds can also be a significant source of bacteria. Swans; Geese and other water fowl can all elevate bacteria counts in water bodies and ponds (26).

In this present study, fish samples of different sources were contaminated with total aerobic bacteria as well as enterobacteria. Fish of good quality should have bacterial count less than 10^5 per gram (27) and what obtained from fish samples examined in this study exceeded the

acceptable limit recommended by Food and Agricultural Organisation (27). This indicates human health risks due to consumption of fish collected from pond and river from this area.

Depending on the sources and other environmental factors, a wide range of variation in distribution of microflora in fish has been reported (28). The present study clearly showed variation in bacterial load in fish of different sources. Therefore, precaution should be taken to prevent water contamination during harvesting as well as post harvest handling of fish.

This study established the poor microbial quality of fish, both wild and cultures *C. gariepinus* and *O. niloticus* in some areas of Ibadan, southwest Nigeria. The safety of the public then depends on the improvement of sanitation within the metropolis by provision of public toilets, and enactment of effective policy for the collection and disposal (management) of municipal solid waste as these would drastically reduce the pollution of running water and rivers with human and domestic waste.

The sanitary conditions under which fishes are reared or cultured in ponds should be improved by following standard or good practices; such as use of good quality water, use of feeds with high microbial quality, regular draining of pond water after specific period of time, closure of ponds to the public among other things.

The farmers should embrace standard operating practices as applicable to fish farming. The workforce should be educated on the maintenance of good hygienic practices, and should be provided with necessary working and safety equipment.

The microbial load of fish can also be improved through regular disinfection of catching gears or working equipment, and brief immersion of caught fishes in disinfecting solution such as brine water to reduce the microbial load on the fish before storing at cold temperature or sold to the public.

The public should be enlightened on the inherent danger that may accompany handling fresh fish or consumption of improperly cooked fish.

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