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Bacterial Flora of African Catfish (Clarias gariepinus) Harvested From Ponds In Uyo South-South Nigeria

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Abstract: *The bacteria flora of Clarias gariepinus was investigated using standard microbiological procedures. The total heterotrophic bacteria count of pond water, skin, gills and intestine of Clarias gariepinus ranged from 1.2 x 10^4 cfu/g in the gills to 2.16 x 10^6 cfu/cm^2 on the skin while the total coliform count ranged from nil in pond water to 3.5 x 10^4 cfu/g of the gills. The Salmonella – Shigella count was higher in the intestine of the catfish obtained from all the ponds under investigation while the total vibrio count was higher in pond water and intestine of catfish from all the ponds studied but was low in gills and skin. The bacteria flora included Streptococcus sp., Escherichia coli, Salmonella sp., Staphylococcus sp., Vibrio sp., Pseudomonas sp., Serratia sp., Klebsiella sp., Shigella sp. , Enterococcus sp. and Proteus sp. Enterococcus sp had the highest frequency of occurrence of 77% followed by Salmonella sp (75%) while Klebsiella sp had the least frequency of occurrence of 8%. The bacterial flora was composed of potential spoilage and pathogenic organisms that could constitute a public heath risk and economic loss. Catfish should be properly processed before consumption or preservation.*
Keywords*: Aquaculture, Bacterial load, Consumers, Pathogens, Spoilage*

I. Introduction

Clarias gariepinus belong to the family Claridae, the air breathing catfish. They belong to a superorder called the Ostariophysi, which also includes the Cypriniformes, Characiformes, Gonorynchiformis and Gymnotiformes, a super order characterized by the weberian apparatus [1]. They are a diverse group of ray finned fish named for their prominent barbells which resembles a cats whiskers. Catfish range in size and behavior from the heaviest and longest Mekong giant catfish of south east Asia to the smallest parasitic fish commonly known as Candiru. All catfishes have either smooth or armored naked bodies with bony plate. The dorsal and pectoral fins are often edged with sharp spines that are used for defense.

Clarias gariepinus BURCHELL, 1822, the African catfish is generally considered to be one of the most important tropical catfish species for aquaculture in West Africa [2]. It is widely distributed throughout Africa, inhabiting tropical swamps, lakes, and rivers, some of which are subjected to seasonal drying [3]. In Nigeria, the rearing of African catfish (Clarias gariepinus) is proving to be a lucrative option for small-scale inland fisheries [4] and its consumption is on the increase [5]. It is also known as the African sharp tooth catfish which is a large eel-like fish, usually of dark grey or black coloration on the back fading to a white belly. It is a highly nutritious fish that contains high amount of vitamins, proteins, minerals and a little or no saturated fat and is low in carbohydrates [6].

Bacterial agents are among the highly encountered causes of diseases in stressed warm water aquaculture . Aquatic microorganisms not only influence the water quality but are known to be closely associated with the physiological status of the fish and the postharvest quality of fish [7]. The health of the fish and it yield is therefore dependent on the quality of the water from which it was harvested.

The presence of bacteria in fish could play diverse roles some of which might be beneficial to the fish itself. However, the presence of some bacterial species could lead to post harvest spoilage and adverse health conditions. The intestinal microflora may be significant in fish spoilage [8] and may be involved in spread of fecal contaminants [9]. Intestinal bacterial flora are also important in nutrition. Kwashiwada et al. [10] showed that vitamin B_{12} was produced by microorganisms in the intestine of carp. It is generally believed that bacteria can contribute to the diet of fish [11]. The presence of potential human pathogens suggests that the fish improperly handled, undercooked , or consumed raw may cause diseases to susceptible individuals.

This study was designed to investigate the bacterial species present in the pond water, gills , intestine and on the skin of Clarias gariepinus harvested from some fish ponds in uyo metropolis.

II. Materials and Methods

2.1 Study Area

The study area for this research was Uyo metropolis. Uyo is the capital of Akwa Ibom state , south – south Nigeria, located between latitude 4°30′N and **5**°30′N and longitude 7°30′E and 8°30′E. The area is within the Niger Delta Basin. Four commercial fish ponds were investigated.

2.2 Bacteriological Sampling and Analysis

2.2.1 Pond water

Pond water were collected in sterile glass bottles (250ml) 15 – 20cm below the water surface from three different locations in each pond. The three samples were combined to make a composite sample for bacteriological analysis in the laboratory. Appropriate sample dilution were made (10^{-1} - 10^{-3}) with sterile peptone water. Aliquots of 1ml of serial dilutions were inoculated using pour plate technique on Nutrient Agar (NA), MacConkey Agar, Salmonella Shigella agar, & Thiosulphate citrate bile salt sucrose agar (TCBS). The plates were incubated at 37°C for 24 – 48 hrs.

2.2.2 Fish (Gills, Intestine and Skin).

Scoop net was used to collect fish samples from the various ponds. Four live catfish were randomly selected for studies. Non of the fish sampled had gross lesions, thus were assumed to be clinically normal. The fish were killed by physical destruction of the brain. The fish were aseptically dissected with the aid of dissecting kits and samples were collected from the skin, gills and intestine of Clarias gariepinus. The skin samples were obtained by macerating aseptically 1cm^2 of skin in 10ml distilled water [12]. The gills and intestines were obtained as described by Uddin and Al-Harbi[13]. 1g sample each were blended with 10ml of sterile 0.1% peptone water. Appropriate sample dilutions were made (10^{-1} to 10^{-6}) with sterile peptone water. Aliquots of 1ml of serial dilutions were inoculated using pour plate technique on Nutrient Agar (NA), MacConkey Agar, Salmonella Shigella agar, & Thiosulphate citrate bile salt sucrose agar (TCBS). The plates were incubated at 37°C for 24 – 48 hrs.

2.3 Identification of Bacteria

Bacteria cultures were characterized and identified using various morphological and biochemical tests such as Grams stain, motility, morphology, oxidase test, catalase, glucose oxidation – fermentation, amylases, gelatinase, lipase, indole, H_2S production and nitrate reduction following the criteria described in the Bergey's Manual of Systematic Bacteriology [14].

III. Results

The total heterotrophic bacterial count of pond water, skin, gills and intestine of Clarias gariepinus ranged from 1.2 x 10^4 cfu/g in the gills to 2.16 x 10^6 cfu/cm^2 on the skin (Table 1) . In general, the bacterial load of the skin was higher than all the other parts of the fish from all the fish ponds sampled. The total coliform count ranged from nil in pond water to 3.5 x 10^4 cfu/g of the gills **(**Table 2). Figure 1 and 2 show the Log values of the Salmonella Shigella counts (SSC) and Total Vibro Count (TVC) in pond water (cfu/ml), skin (cfu/cm^2), Gill (cfu/g) and intestine (cfu/g) respectively. SSC was higher in the intestine of the catfish obtained from all the ponds under investigation (Fig. 1) However, in pond 3 the SSC was also high in pond water and skin. The TVC was higher in pond water and intestine of catfish from all the ponds studied but was low in gills and skin (Fig. 2). There was no vibrio count on the skin of catfish from pond 1 and 4 and gills of the fish from pond 3.

The bacterial flora of the catfish obtained from the three ponds were somewhat similar. The gram – negative rod shaped bacteria dominated all populations. In total, 11 bacteria genera were identified from pond water, skin, gill and intestine . The bacteria isolates were Streptococcus sp., Escherichia coli, Salmonella sp., Staphylococcus sp., Vibrio sp., Pseudomonas sp., Serratia sp., Klebsiella sp., Shigella sp. , Enterococcus sp., Proteus sp. The bacteria isolates obtained from pond water and different parts of the fish is shown in Table 3.

Enterococcus sp had the highest frequency of occurrence of 77% followed by Salmonella sp (75%) while Klebsiella sp had the least frequency of occurrence of 8% (Table 4).

Table 1. Total Heterotrophic Bacteria Count (Bacterial Load) in Pond Water, Skin, Gills and Intestine of African Catfish (Clarias gariepinus) Harvested from Some Ponds in Uyo

Pond	Water (cfu/ml)	Skin(cfu/cm^2)	Gill (cfu/g)	Intestine (cfu/g)
1	1.05 x 10^5	8.0 x 10^4	1.2 x 10^4	4.5 x 10^4
2	1.5 x 10^4	1.72 x 10^6	2.01 x 10^6	4.3 x 10^4
3	6.5 x 10^4	2.16 x 10^6	1.8 x 10^4	5.0 x 10^4
4	2.4 x 10^4	2.9 x 10^4	1.7 x 10^4	7.1 x 10^4

Table 2. Total coliform count in pond water, skin, gills and intestine of African catfish (Clarias gariepinus) harvested from some ponds in Uyo

Pond	Water (cfu/ml)	Skin (cfu/cm^2)	Gill (cfu/g)	Intestine (cfu/g)
1	-	2.0 x 10^3	9.0 x 10^3	5.0 x 10^3
2	1.0 x 10^3	1.5 x 10^4	3.5 x 10^4	1.2 x 10^4
3	3.0 x 10^3	2.2 x 10^4	1.7 x 10^4	1.6 x 10^4
4	4.0 x 10^3	9.0 x 10^3	8.0 x 10^3	1.2 x 10^4

Table 3. Bacterial Isolates Obtained from Pond Water and Different Parts of African Catfish Fish

Sample	Bacterial isolates
Pond water	Streptococcus sp., Escherichia coli, Salmonella sp., Staphylococcus sp., Vibrio sp., Pseudomonas sp., Serratia sp., Klebsiella sp., Shigella sp. , Enterococcus sp., Proteus sp.
Gills	Salmonella sp., Escherichia coli, Klebsiella sp., Pseudomonas sp., Enterococcus sp., Proteus sp. Vibrio sp.
Intestine	Salmonella sp., Proteus sp., Staphylococcus sp., Pseudomonas sp., Escherichia coli, Shigella sp., Vibrio sp.
Skin	Escherichia coli, Salmonella sp., Staphylococcus sp., Proteus sp., Vibrio sp., Pseudomonas sp., Serratia sp., Shigella sp., Enterococcus sp.

Table 4. Percentage Frequency of Occurrence of Bacterial Isolates from Clarias gariepinus

Bacterial Isolates	Percentage frequency of occurrence (%)
Escherichia coli	42
Salmonella sp.	75
Enterococcus sp.	77
Pseudomonas sp.	50
Serratia sp.	17
Klebsiella sp.	8
Streptococcus sp.	25
Staphylococcus sp.	58
Vibrio sp.	33
Shigella sp.	25
Proteus sp.	58

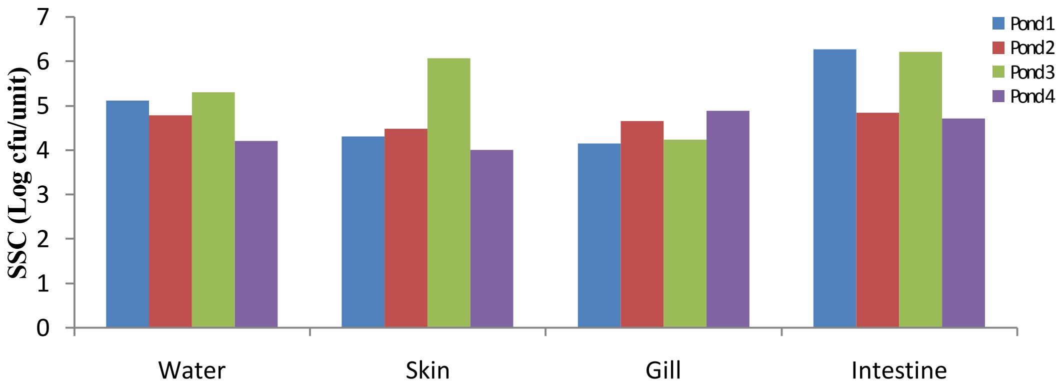

Figure 1. Log values of the Salmonella Shigella counts (SSC) in pond water (cfu/ml), skin (cfu/cm^2), Gill (cfu/g) and intestine (cfu/g) of Clarias gariepinus

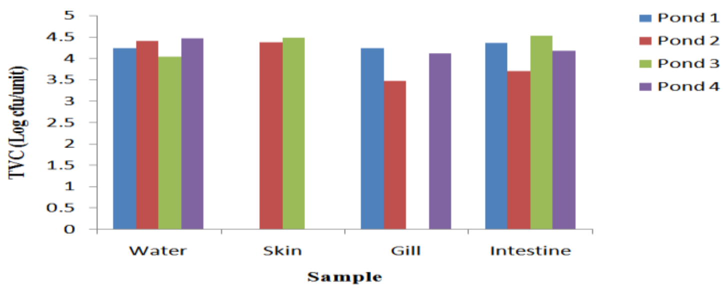

Figure 2. Log values of the Total Vibrio Count (TVC) in pond water (cfu/ml), skin (cfu/cm^2), Gill (cfu/g) and intestine (cfu/g) of Clarias gariepinus

IV. Discussion

Investigation of the bacterial flora of fish and its influence on the fish spoilage has been confined largely to marine species with little attention paid to fresh water fish . In this study the bacterial flora of Clarias gariepinus was investigated. There were variations in the bacterial load of pond water, skin, gills and intestine.

The maximum mean total heterotrophic bacterial count in cfu was 10^6 obtained on the skin of the catfish samples. This result is low as compared to an earlier report by Adedeji et al. [12] who reported counts in the range of $10^{12} – 10^{13}$ cfu. Egbere et al. [1] reported counts in the range of 10^6 to 10^8 in their study of water samples from catfish ponds in Jos metropolis. Wantanabe [15] reported lower counts in the range of $10^4 – 10^5$ on the skin of Clarias sp. The result of our study fall within the acceptable rang of $10^2 – 10^7$ cfu ([16],[17],[18]). The skin had the highest microbial load as compared to all other parts of the fish studied. This could be due to its constant exposure and contact with the environment such as pond water, air, etc. Adebayo – Tayo et al. [19] also made a similar observation.

The microflora was dominated by gram negative bacteria belonging to the family enterobacteriaceae. The bacteria flora of pond water was a reflection of the bacteria composition of the skin, gills and stomach of the Clarias gariepinus. This finding is in agreement with an earlier report by Uddin and Al- Harbi [13]. Pelzar et al. [20] stated that the microflora of caught fish and other aquatic specimens is largely a reflection of the microbial quality of the water where they were harvested.

The microflora of the Clarias gariepinus harvested from ponds in Uyo metropolis included Salmonella sp, Esherichia coli, Enterococcus sp, Pseudomonas sp., Serratia sp. Klebsiella sp., Vibrio sp. Staphylococcus sp, Streptococcus sp., Shigella sp., and Proteus sp. Musefiu et al. [21] reported similar bacterial species in their study of the bacterial flora of Clarias gariepinus and Oreochromis niloticus from Ibadan South west Nigeria.

The commensal bacterial flora included facultative pathogens which under stress could give rise to fish disease. These isolates are potential pathogens to humans as well. E. coli, Salmonella sp. , Streptococcus sp. and S. aureus are implicated to be fish borne [22] . Serratia sp. cause bacteruria while Pseudomonas had been isolated in wounds, burns, eyes and ear infections [23] . Raj and Liston [24] found that some pathogenic and potentially pathogenic microorganisms including E. coli and Staphylococcus survived when undercooked and precooked fish foods were stored at freezing temperatures. Roberts [25] showed that Pseudomonas and Vibrio sp. cause infectious diseases in fish.

Some of the isolates were potential spoilage organisms. Live healthy fish can be covered in bacteria while the flesh remain sterile. After death, incorrect or inadequate handling can introduce bacteria to the flesh resulting in spoilage. The natural flora of fish that play a predominant role in spoilage include the genera Pseudomonas, Vibrio, Micrococcus, Achromonas, Corynebacterium and Flavobacterium [26]. Proteus and Pseudomonas sp. are among the major spoilage bacteria at near freezing temperatures [27]. In order to prolong the shelf life of fish, it is essential to control these spoilage bacteria [13].

The highest salmonella –shigella count was recorded in the intestine of the Clarias gariepinus. Salmonella tend to be associated with the intestinal tracts of warm blooded animals. They have been detected in the gut of tilapia and carp grown in waste –fed and non- waste fed aquaculture ponds ([28],[29]). Hatha [30] reported that this bacteria existed on the skin, gills and intestine of catfish but the most potential reservoir of Salmonella was the intestine. Salmonella sp. is a potential pathogen for humans and fish [31]. It is among the most important cause of human gastrointestinal disease worldwide and many seafood importing countries will not accept products containing these pathogens [21].

The pond water could be a source of contamination of the catfish cultured in them. This is in agreement with Adedeji et al. [12] and Egbere et al. [1]. Egbere et al. [1] reported that the most contaminated water source for catfish ponds was well while tapwater was the least contaminated water source. Contamination of ponds could result form indiscriminate deposition of human and animal excreta . During rainy seasons, environmental wastes including fecal matter are washed from polluted lands into water bodies such as fish ponds. This could be the reason why enteric organism were isolated from the catfish pond water.

V. Conclusion

Although the microbial load of the Clarias gariepinus harvested from some fish ponds in Uyo did not exceed the recommended standards, several potential spoilage and pathogenic organism were components of the bacterial flora. The presence of these organism could constitute a public heath risk and economic loss if the fish is improperly handled, undercooked , or consumed raw. Catfish should be properly processed before consumption. The sanitary conditions under which fishes are reared or cultured should be improved by following standards or good practices such as good quality water, use of feeds with high microbial quality, regular draining of pond water after specific period of time and closure of ponds to the public. The farmers should embrace standard operating practices as applicable in fish farming and the workers should be educated on good hygienic practices. They should be provided with necessary working and safety equipments. The need for proper processing and adequate cooking of Clarias gariepinus, the African catfish is advocated since it is in high demand at fast food joints popularly known as “point and kill” in most parts of Nigeria.

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