BACTERIOLOGICAL ANALYSIS OF CATFISH (*Clarias gariepinus)* IN OWO AREA, ONDO STATE, NIGERIA

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ABSTRACT

A total of eight catfish (*Clarias gariepinus*) were collected from different fishponds in Iyere and Otapete areas of Owo local government in Ondo State. The skin, gills, and intestines of the fish were investigated for presence of bacteria. The species of bacteria isolated from the fish samples and their percentage occurrence include *Serratia spp. (2%), Salmonella spp. (32%), Escherichia coli (24%), Proteus spp. (3%), Enterococcus spp. (7%), Staphylococcus spp. (9%), Streptococcus spp. (9%), Klebsiella spp. (3%), Bacillus spp. (6%) and Pseudomonas spp. (5%).* The highest microbial count (total viable count) from *Clarias gariepinus* was 6.5 x 10^2 CFU/ml from skin samples, 3.5 x 10^7 CFU/ml from gill samples, and 4.5 x 10^2 CFU/ml from intestine samples. Correspondingly, the highest microbial count (total coliform count) recorded was 7.0 x 10^2 CFU/ml from skin samples, 2.5 x 10^3 CFU/ml from gill samples, and 3.0 x 10^1 CFU/ml from intestine samples. Finally, the highest microbial count (total anaerobic count) from *Clarias gariepinus* was 2.0 x 10^2 CFU/ml from skin samples, 2.5 x 10^4 CFU/ml from gill samples, and 2.5 x 10^2 CFU/ml from intestine samples. The occurrence of such isolated bacteria if not properly checked could endanger both the fish and the ultimate consumers particularly if the fish harvested from these farms are undercooked.

KEYWORDS: Catfish, *Clarias gariepinus*, skin, gills, intestine

1.0 INTRODUCTION

The Catfish belong to the family Claridae that are referred to as 'omnivorous scavengers'. This category of fish lack scale and they possess feelers. They display an anguilliform body; dorsal body and extremely long fins. They also have pectoral fins, head, mouth and respiratory origins. These enable fish to stay for some time outside water, making use of atmospheric oxygen (Lundberg, 2007). Catfish species live in inland or coastal waters except Antarctica. Catfish have inhabited all continents at one time or the other. The sharp tooth catfish (*Clarias gariepinus*) is one of the most important fresh water fish in Nigeria (Randall et al., 2002).

The production and consumption of fish in Nigeria has been a major source of animal protein, which has competed favorably with meat. Catfish (*Clarias gariepinus)* has been reported to be a very important freshwater fish in Nigeria. It has enjoyed wide acceptability in most parts of the country because of its unique taste, flavor and good texture. It is widely distributed, extensively cultivated in ponds. Fish is one of the best sources of proteins, vitamins and minerals and are essential nutrients required for supplementing both infant and adult diets (Abdullahi *et al.*, 2001). In Nigeria, it has also been noticed that fish is eaten fresh, preserved or processed (smoked) and form a much-cherished delicacy that cuts across socio-economic, age, religious and educational barriers (Adebayo-Tayo *et al*., 2008). The fishery sector accounts for about 2 percent of national GDP, 40 percent of animal protein intake and a substantial proportion of employment, especially in rural areas. The sector is a principal source of livelihood for over 3 million people. Nigeria is the largest African aquaculture producer, at 15,489 tons per year, Egypt (5,645 tons) follows Nigeria and then there are only five other countries (Zambia, Madagascar, Togo, Kenya and Sudan) that each produce more than 1000 tons (Hussein and Zolondi, 2002).

Fish take a large number of bacteria into their gut from water sediment and food (Adedeji *et al*., 2011). It has been well known that both freshwater and brackish water fishes can harbor human pathogenic bacteria particularly the coliform group (Adedeji *et al*., 2011). Fecal coliform in fish demonstrates the level of pollution in their environment because coliform are not named flora of bacteria in fish (Adedeji *et al*., 2011). According to Pillay

(Pillay, 1990) fish living in natural environment are known to harbor pathogenic Enterobacteriaceae. More than 140 invasive bacteria species have been identified in great lakes and other water bodies (Udeze *et al.*, 2012). Invasion of fish muscles due to the breakage of immunological barrier of fish by pathogens is likely to occur, when the fish is raised in ponds with fecal coliform, E. coli and Salmonella of greater than 10^3 per ml in pond water respectively (Guzman *et al.*, 2004). This study was aimed at investigating bacteria associated with catfish and to observe the organ-wise distribution of the associated bacterial organisms.

2.0 MATERIALS AND METHODS

2.1 Collection of Samples

Eight samples of *Clarias gariepinus* were collected from four different unrelated fishponds in Iyere and Otapete, Owo local government area, Ondo state, South West Nigeria. The fishes were caught using fishnets with each fish weighing an average of 400gm. Samples were collected twice, with 7 days interval between each collection. The samples collected were always visually examined and confirmed to be all reasonably healthy.

2.2 Processing and Enrichment of Samples

The skin, gills, and intestines of the fish samples were aseptically obtained, minced, and grinded separately. 0.5 ml of each sample was then transferred to 4.5ml of 1% peptone and 10 fold serial dilutions were prepared for each of the samples.

2.3 Determination of Total Viable Count

An aliquot of 0.5ml of each ten-fold diluted sample was inoculated unto prepared nutrient agar plates using spread-plate method. The plates were then incubated for 24-48 hours at 37^{o}C. The number of colonies in each dilution was multiplied by the dilution factor to determine the total viable count.

2.4 Isolation of pure bacterial colonies

An aliquot of each diluted sample was inoculated in nutrient broth at 37^{o}C for 24 hours and isolation of bacteria was performed according to the method described by Carter, 1986.

2.5 Identification of bacterial isolates

Biochemical testing and bacteria identification were performed on bacterial isolates in accordance with guides from Bergey's manual of systemic bacteriology.

2.6 Determination of total Coliform Count

For each sample, two Eosin-Methylene Blue (EMB) agar plates were prepared. One was cultured with 100 L of the water sample while the second was cultured with 500 L of the water sample. All plates were incubated at 37 C for 48 hours. After the incubation period, the colonies were counted to assess the presence of fecal bacteria.

2.7 Determination of Total Anaerobic Bacterial Count

DeMan Rogosa Sharpe (MRS) medium was used to determine the total anaerobic count of bacteria from the catfish samples. The medium was prepared by suspending 33.35g per 500ml of distilled water, and heated to dissolve completely. The agar was then sterilized, cooled, and poured into petri dishes.

0.1ml diluents of the fish samples were inoculated unto the prepared medium surface and spread. The Plates were then incubated in a microaerophilic atmosphere (5% CO2) at 30 ± 1°C and examined after 3 days (72 hours).

3.0 RESULTS

This study was aimed at investigating the bacterial load on the skin, gill and intestine of catfish (*Clarias gariepinus*). A total of eight catfish were collected from different fishponds in Iyere and Otapete areas of Owo local government in Ondo State. The skin, gills, and intestines of the fish where then investigated for presence of bacteria.

The species of bacteria isolated from the fish samples and their percentage occurrence include *Serratia spp. (2%), Salmonella spp. (32%), Escherichia coli (24%), Proteus spp. (3%), Enterococcus spp. (7%), Staphylococcus spp. (9%), Streptococcus spp. (9%), Klebsiella spp. (3%), Bacillus spp. (6%) and Pseudomonas spp. (5%).*

The highest microbial count (total viable count) from *Clarias gariepinus* was 6.5×10^2 CFU/ml from skin samples, 3.5×10^7 CFU/ml from gill samples, and 4.5×10^2 CFU/ml from intestine samples. Correspondingly, the highest microbial count (total coliform count) observed was 7.0×10^2 CFU/ml from skin samples, 2.5×10^3 CFU/ml from gill samples, and 3.0×10^1 CFU/ml from intestine samples. Finally, the highest microbial

count (total anaerobic count) from *Clarias gariepinus* was 2.0 x 10^2 CFU/ml from skin samples, 2.5 x 10^4 CFU/ml from gill samples, and 2.5 x 10^2 CFU/ml from intestine samples.

Table 1: Total bacteria count from skin of catfish samples

	Sample	Total Bacteria Count (CFU/ml)	Total Coliform Count (CFU/ml)	Total Anaerobic Count (CFU/ml)
Pond A	1	6.5 x 10^2	3.0 x 10^1	1.5 x 10^1
	2	4.0 x 10^2	4.5 x 10^2	2.0 x 10^1
Pond B	3	3.5 x 10^4	3.5 x 10^2	1.5 x 10^1
	4	2.5 x 10^4	5.0 x 10^1	2.0 x 10^2
Pond C	5	5.0 x 10^2	2.5 x 10^1	1.5 x 10^1
	6	2.0 x 10^4	7.0 x 10^2	1.0 x 10^2
Pond D	7	5.5 x 10^2	1.0 x 10^1	2.0 x 10^1
	8	3.0 x 10^4	6.0 x 10^2	1.1 x 10^2

Table 2: Total bacteria count from gills of catfish samples

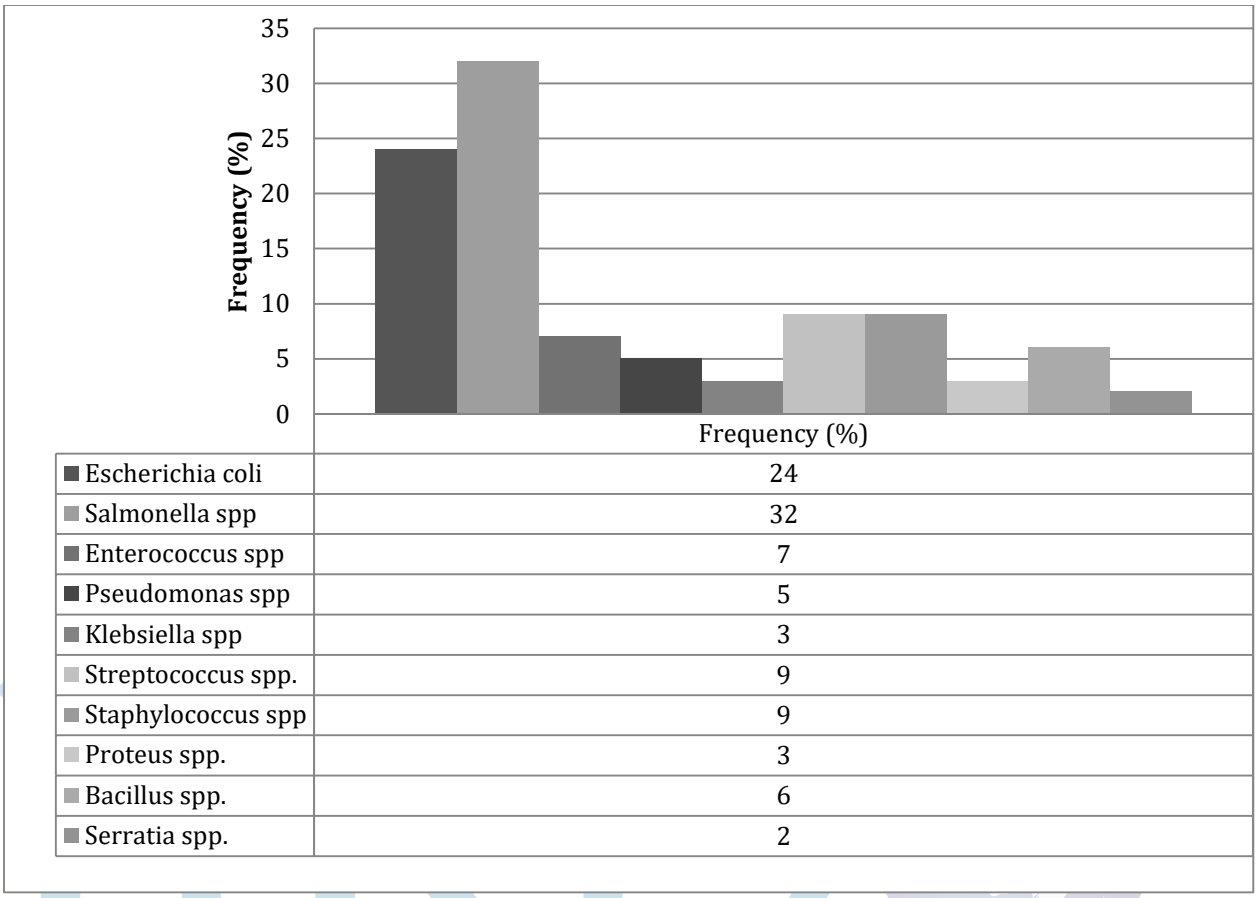
	Sample	Total Bacteria Count (CFU/ml)	Total Coliform Count (CFU/ml)	Total Anaerobic Count (CFU/ml)
Pond A	1	2.25 x 10^7	1.0 x 10^3	1.5 x 10^4
	2	3.5 x 10^7	2.0 x 10^4	1.0 x 10^4
Pond B	3	2.0 x 10^7	2.5 x 10^3	2.0 x 10^4
	4	1.0 x 10^7	1.5 x 10^4	1.0 x 10^4
Pond C	5	3.0 x 10^7	2.0 x 10^3	1.2 x 10^4
	6	2.14 x 10^7	1.51 x 10^4	1.1 x 10^4
Pond D	7	1.41 x 10^7	1.72 x 10^4	1.4 x 10^4
	8	2.21 x 10^7	1.0 x 10^3	2.5 x 10^4

Table 3: Total bacteria count from intestine of catfish samples

	Sample	Total Bacteria Count (CFU/ml)	Total Coliform Count (CFU/ml)	Total Anaerobic Count (CFU/ml)
Pond A	1	4.5×10^2	3.0×10^1	2.5×10^2
	2	3.0×10^4	2.5×10^1	1.0×10^1
Pond B	3	4.0×10^2	2.0×10^2	1.5×10^1
	4	2.0×10^2	3.0×10^1	1.0×10^2
Pond C	5	3.5×10^2	2.5×10^2	2.0×10^1
	6	2.2×10^4	2.0×10^2	2.0×10^2
Pond D	7	4.2×10^2	1.1×10^1	1.5×10^2
	8	4.0×10^4	2.0×10^2	2.0×10^2

Table 4: Bacterial Isolates Obtained from different parts of Catfish

Gills	*Salmonella spp., Escherichia coli, Klebsiella spp., Pseudomonas spp., Enterococcus spp., Proteus spp.,*
Skin	*Staphylococcus spp., Pseudomonas spp., Escherichia coli,*
Skin	*Staphylococcus spp., Proteus spp., Pseudomonas spp., Serratia spp., Enterococcus spp.*



Escherichia coli	24
Salmonella spp	32
Enterococcus spp	7
Pseudomonas spp	5
Klebsiella spp	3
Streptococcus spp.	9
Staphylococcus spp	9
Proteus spp.	3
Bacillus spp.	6
Serratia spp.	2

Figure 1: Percentage frequency of occurrence of bacterial Isolates from *Clarias gariepinus*

4.0 DISCUSSIONS

Bacteria were isolated from eight samples of *Clarias gariepinus* that were collected from four different unrelated fishponds in Iyere and Otapete, Owo local government area, Ondo state, South West Nigeria. The skin, gills, and intestines were investigated separately. The different species of bacteria isolated from all samples include *Serratia spp., Salmonella spp., Escherichia spp., Proteus spp., Enterococcus spp., Staphylococcus spp., Streptococcus spp., Klebsiella spp., Bacillus spp. and Pseudomonas spp.*

The ponds that harbor the fish may be the sources of contamination due to indiscriminate deposition of human stool, animal excreta and other environmental wastes. During the rainy season, fecal matter (as well as other forms of wastes) from various sources is washed from contaminated land into different water bodies. Free roaming animals and pets such as dogs also contribute to fecal contamination of surface water. Besides that,

stream and hold water used in earthen ponds and examining pools might be contaminated by coliform bacteria.

Investigations on the bacterial flora of fish and its influence on fish spoilage (and infections from fish) have been confined mostly to marine species than to fresh water species of fish. This study investigated the bacterial flora of *Clarias gariepinus*. There were variations in the bacterial load of skin, gills, and intestines.

The skin had the highest microbial load with a mean maximum total of 10^7CFU in comparison with other parts of the fish studied. This could be due to its constant exposure and contact with the environment and its many pollutants, as observed by Adebayo-Tayo *et al*., 2012. This result is however low in comparison with an earlier report by Adedeji *et al*., 2011, who reported counts in the range of 10^{12}- 10^{13}CFU. The highest Salmonellae count was recorded in the intestine. Salmonellae tend to be associated with the skin, gills and intestines o catfish, but the most potential reservoir of Salmonella is the intestine (Adedeji *et al*., 2011).

The bacteria isolated included facultative pathogens which under stress, could give rise to disease of fish, and subsequently, to humans. *E. coli*, *Salmonella spp*., *Streptococcus spp.* and *Staphylococcus spp.* have been implicated in fish-borne diseases (Babu, 2000). Staphylococcus frequently causes septicemia, osteomyelitis, bacteremia and otitis (Udeze *et al.*, 2012). *Pseudomonas aeruginosa* could cause general inflammation and sepsis in critical body organs such as lungs, kidneys, urinary tract, which can be fatal because it thrives in most surfaces (Udeze *et al.*, 2012). *E. coli* and *Shigella spp*. have been implicated for a number of gastroenteric diseases such as diarrhea (traveller's disease), dysentery, vomiting, fever, colitis, hemolytic uremic syndrome with renal failure. *Salmonella spp.* causes salmonellosis, which in humans could result in severe typhoid fever (enteric fever) or salmonella fever and bacteremia (Egbere *et al.*, 2010). *Enterococcus spp.* is a causative agent of dental plagues and scarlet fever and has been implicated in human infections like pharyngitis, scarlet fever and pneumonia (Adebayo-Tayo *et al.*, 2009).

5.0 CONCLUSION AND RECOMMENDATION

It is evident from this study that the skin of fish is more exposed to bacterial contamination than other parts, due to its direct contact with surrounding waters. Control

of contamination of fishponds is required to check the rate of fish diseases and infections to humans. This could be through proper water management and good fishery culture. Indiscriminate dumping of refuse and situation of sewages close to natural fishponds Should also be avoided. Ultimately, proper washing of catfish (especially the skin) and adequate cooking should be ensured before consumption to avoid possible bacterial contamination.

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