COMPARATIVE STUDY OF THE BACTERIAL LOAD AND SPECIES DIVERSITY IN THE AFRICAN CATFISH (*CLARIAS GARIEPINUS)* CULTURED IN CONTRASTING AQUACULTURE TANKS IN UYO, NIGERIA

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ABSTRACT

Comparative study on bacterial load and diversity on the intestine, skin and gills of cultured Clarias gariepinus from concrete and tarpaulin tanks were investigated. Determination of bacterial loads, species characterization and composition in fish parts were done using standard microbiological procedures. Results of the assay showed high bacterial count in intestine and gills of fish from both culture tanks. Heterotrophic bacteria count ranged from 1.7 x 10^4 cfu/ml on skin of catfish cultured in tarpaulin tank to 2.6 x 10^4 cfu/ml in gills of catfish from both systems, while the total coliform count ranged from 1.2 x 10^4 cfu/ml in the gills to 3.9 x 10^4 cfu/ml in the intestine. The Salmonella count was higher in the intestine of catfish in both systems, while the highest vibrio counts of 4.2 – 4.6 x 10^4 cfu/ml was recorded in the gills of catfish from tarpaulin tank. In both culture systems, Pseudomonas, Salmonella and Escherichia coli, were not observed on fish skin. The Bacterial organisms isolated included: Escherichia coli, Salmonella enterica, Staphylococcus epidermidis, Vibrio anguillarum, Pseudomonas fluorescens, Serratia liquefaciens, Bacillus licheniformis, Shigella sonnei, Lactobacillus plantarum, Enterobacter aerogenes, Klebsiella pneumoniae and Proteus volgaris. S. epidermidis accounted for the highest frequency of occurrence (75.0 %) in concrete tank, while K. pneumoniae had the least frequency of occurrence (2.0 %) in tarpaulin tank. The bacterial flora recovered composed of potential pathogenic organisms of public health interest.

Keywords: Aquaculture, Bacterial load, Catfish, Concrete and tarpaulin tanks

INTRODUCTION

The African catfish, *Clarias gariepinus* are a diverse group of ray finned fish known by their prominent barbells and are considered to be one of the most important tropical catfish species for aquaculture in West Africa (Amande and Nwaka, 2013). In Nigeria, catfish farming is proving to be a lucrative option for small-scale inland fisheries and the consumption of its products is on the increase (Otubusin, 2011). The gut flora consists of the microorganisms that normally live in the digestive tract of animals (O'Hara and Shanahan, 2006). These microbes are among agents associated with diseases in warm water aquaculture. These microbes not only influence water quality but are also known to be closely associated with the physiological status as well as the postharvest quality of fish (Al-Harbi and Uddin, 2003). Thus fish health and it yield are 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. In an earlier study, Kashiwada *et al*. (1970) showed that vitamin B_{12}

was produced by microorganisms in the intestine of carp. Other studies by Egerton *et al.* (2018) and Butt and Volkoff (2019) opined that microorganisms present in the gut perform a host of functions such as fermenting unused energy substrates, supporting the immune system, preventing growth of harmful species, regulating the development of the gut and producing vitamins for the host. Thus, bacteria can contribute positively to the fish wellbeing.

On the other hand, the presence of some bacterial species could lead to post harvest spoilage and adverse health conditions in farmed fishes. Also, in certain conditions some microbes are capable of causing disease by passing infection to the host (Feldhusen, 2000). Thus, intestinal microflora may be significant in fish spoilage as well as being able to transmit many of the established food borne microbial infections and intoxications. There had been dart of information on the microbial load of farmed catfish in Uyo, Akwa Ibom State, Nigeria. This is however essential for the improvement of fish health. Thus, in the present study, the bacterial load and species diversity in catfish from two fish farms in Uyo metropolis were evaluated.

MATERIALS AND METHODS

Source of Experimental Fish: The experimental fish were procured from two fish farms (Almond Fisheries Limited and Vika Farms Limited) in Uyo, Akwa Ibom State, Nigeria. The Vika farms operated the concrete tank system, while Almond Fisheries raised their fish in tarpaulin tanks. Culture system for both farms was semi-intensive. A total of 60 fish samples having mean weight of 200 ± 5.08 g and of equal sexes (30 fish from each farm) were collected between May to July 2019 (three months) in plastic containers and moved to the wet laboratory of the Department of Animal and Environmental Biology, University of Uyo for the study.

Sample Preparation, Culture and Isolation of Bacteria: The study used one external (skin) and two internal (gills and intestines) organs. Initial visual inspection of the external body surface was performed to identify any gross external lesions. Thereafter, fishes humanly were killed under anesthesia (ICMSF, 1986) and various parts dissected aseptically. 10 grams from each part was separately homogenized in 90 ml of distilled water. The homogenate was allowed to settle for 10 minutes prior to the withdrawal of 5 ml of the supernatant for 10-fold serial dilution. One ml of the diluted sample was inoculated using pour plate technique. 0.5 % Nutrient agar medium was poured at 40°C on the plates. The sample and the medium were properly mixed, allowed to set and incubated at 25°C for 48 hours. The colonies were sub-cultured to get pure cultures which were further screened for the presence of indicator organisms. Microbial assay of the skin, gills and intestine of experimental fish were carried out to determine the microbial load, identification and frequency of occurrence as described by Cheesbrough (2000). Plates with colonies ranging between 50 – 200 were selected for determination of total bacterial count and isolation of individual bacterial groups. Total load of bacteria was estimate thus: Total load of bacteria (cfu/ml) = C x D x 10 x V/W (Cheesbrough, 2000), where: C= Number of colonies found, D= Dilution factor, V= Volume of physiological saline, W= Weight of the material (fish parts).

Characterization of Bacteria: Morphological characteristics (shape, size and color) of the bacterial colonies were observed visually. Shape of the individual isolate was determined by Gram staining method with the young culture. The motility test was performed by hanging drop method. Biochemical tests such as catalase activity, oxidase, indole production, gelatin liquefaction and proteinase test were performed using bacterial isolates from fresh culture according to the methods described by Barrow and Feltham (1993).

Data Analysis: Data collection for bacterial counts were subjected to descriptive statistics and differences in bacterial load and species were analyzed using one-way analysis of variance (ANOVA) at 95 % probability level

using SPSS version 20. The bacterial isolates characterized were presented in tables.

RESULTS

The results of bacterial counts in intestines, skin and gills are presented in Table 1. Bacteria detected in catfish cultured in concrete tank were significantly higher ($p<0.05$) than the numbers detected in catfish cultured in tarpaulin tank. Total heterotrophic bacterial count in the skin ranged between 1.73×10^4 cfu/ml in tarpaulin tank and 2.5×10^4 cfu/ml in concrete tank. The bacterial count in the intestine of catfish showed significant variations ($p<0.05$), with higher indices when compared with other body parts. There was no *S. enterica* counts on the skin of catfish from both systems and in the gills of fish raised in tarpaulin tank. Table 2 showed the primary characterization of different bacterial isolates. Bacterial flora of catfish obtained from the two systems were somewhat similar. The Gram negative rod shaped bacteria dominated the population. The results of biochemical tests for the different bacterial isolates are presented in Table 3. *E. coli* was positive to indole production, while other organisms tested negative. *V. anguillarum* tested positive to gelatin liquefaction and negative to proteinase as against all other organisms. The study further indicated that *S. epidermidis* accounted for the highest frequency of occurrence (75.0%) followed by *E. aerogenes* (69.0%) and *B. licheniformis* (48.0%) and the least (2.0%) in *L. plantarum* from fish cultured in concrete tank (Table 4).

DISCUSSION

The fish samples examined in this study looked physically healthy based on their appearances but they were all infected with bacteria. Strom and Olafsen (1989) reported that bacteria are abundant in the environment in which fish live and it is therefore impossible to avoid them being a component of their diet. Representatives of 25 bacteria genera have been reported as pathogens of freshwater and marine fish (Cameron, 2002). In this study, a total of 12 bacterial species were recovered from intestine, skin and gills of *C. gariepinus*. They included both pathogenic and normal flora (*E. coli, S. enterica, S. epidermidis, V. anguillarum, P. fluorescens, S. liquefaciens, B. licheniformis, S. sonnei, L. plantarum, E. aerogenes, K. pneumoniae and P. volgaris*). The species found were similar to those reported by Jimoh *et al.* (2009) in the gut sections of wild and cultured *C. gariepinus* in Abeokuta, Nigeria and Emikpe *et al*. (2011) on skin and in stomach of *C. gariepinus* and *Oreochromis niloticus* from Ibadan, Nigeria. The occurrence of these species in different organs of fish may be associated with some predisposing factors such as poor handling, feed and feeding, improper pond management, and excessive amounts of decomposing organic matter in the pond (Ampofo and Clerk, 2010). Bacterial counts in intestine were more than those observed in the skin and gills. A similar report was given by Uddin and Al-Harbi (2012) where the highest bacterial load was observed in the gut when compared to the skin and gills of polycultured *Cyprinus carpio* and *C. gariepinus*. Similarly, Al-Harbi and Uddin (2003) reported that the intestine of hybrid *O. niloticus* x *O. aureus* cultured in earthen ponds was heavily colonized by heterotrophic bacteria.

The bacteria entering along with the diet of fish during ingestion may adapt themselves in the gastrointestinal tract and form a symbiotic association within the digestive tract of fish, in which large numbers of microbes are present. This indicates that the digestive tracts of fish provide favourable ecological niches for these organisms. In the present study, the microbial load reported in different body parts of cultured *C. gariepinus* was in agreement with earlier work by Mondal *et al*. (2008). Haniffa and Abdulkader (2011) reported that bacteria especially motile aeromonads were frequently isolated from both fish and other aquatic animals. In a similar study, Musefiu *et al.* (2011) isolated and identified *S. epidermidis, Bacillus* sp.*, Salmonella* sp. and *Streptococcus* sp. from the skin of *C. gariepinus* and *O. niloticus.* Similarly, Al-Harbi and Uddin (2003) in a study carried out on bacterial infestation of *C. gariepinus* isolated *S. aureus, E. coli* and *Pseudomonas* sp. as dominant organisms.

Table 1: Microbial densities in intestine, skin and gills of catfish cultured in different aquaculture tanks in Uyo

Bacterial Counts	Concrete Tank			Tarpaulin Tank		
	Intestine	Skin	Gills	Intestine	Skin	Gills
THBC (cfu/ml)	2.5×10^4	2.17×10^4	2.5×10^4	2.1×10^4	1.73×10^4	2.3×10^4
PC (cfu/ml)	1.23×10^4	-	1.33×10^4	1.07×10^4	-	1.03×10^4
TCC (cfu/ml)	1.5×10^4	1.07×10^4	1.63×10^4	2.8×10^4	2.17×10^4	1.97×10^4
FCC (cfu/ml)	2.21×10^4	0.83×10^4	2.5×10^4	3.3×10^4	2.47×10^4	2.33×10^4
SSC (cfu/ml)	1.10×10^4	-	1.07×10^4	1.4×10^4	-	-
VC (cfu/ml)	2.43×10^4	3.03×10^4	1.93×10^4	3.37×10^4	-	4.27×10^4
SC (cfu/ml)	6.83×10^4	1.07×10^4	2.07×10^4	1.8×10^4	1.43×10^4	1.77×10^4

Where: *THBC: Total heterotrophic bacterial counts; PC: Pseudomonas count; TCC: Total coliform count; FCC: Faecal coliform count: SSC: Salmonella shigella count; VC: Vibro count; SC: Staphylococcus count*

Table 2: Primary characterization of different bacterial isolates from catfish cultured in different aquaculture tanks in Uyo

Characters	A	B	C	D	E	F	G	H	I	J	K	L
Gram stain	+	-	-	-	+	+	-	-	-	+	-	-
Shape	R	R	R	R	R	R	S	R	R	R	R	R
Motility	-	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Coagulase	+	-	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	+	+	-	-	-	+	+	+
Urease	-	-	-	+	-	-	-	-	-	-	-	+
Oxidase	-	-	+	-	-	-	-	-	-	-	-	+
Glucose	A	AG	A	A	AG	A	AG	A	AG	AG	A	A
Maltose	A	AG	-	A	AG	A	AG	A	AG	A	AG	A
Fructose	AG	-	AG	AG	-	A	-	-	-	A	-	A
Sucrose	A	-	AG	AG	-	-	AG	-	AG	A	-	A
Mannitol	AG	AG	AG	-	AG	-	AG	AG	AG	-	AG	-
Galactose	A	-	A	AG	A	A	-	-	A	A	-	A

Where: *A: S. epidermidis; B: E. coli; C: P. fluorescens; D: P. volgaris; E: S. liquefaciens; F: B. licheniformis; G: E. aerogenes; H: S. enterica; I: S. sonnei; J: L. plantarum; K: K. pneumoniae; L: V. anguillarum; R; Rod; -: Negative; +: Positive; A: acid; AG: Acid and Gas*

Table 3: Biochemical characteristics of bacterial isolates from catfish cultured in different aquaculture tanks in Uyo

Characters	Responses by different bacterial isolates											
	A	B	C	D	E	F	G	H	I	J	K	L
Indole production	-	+	-	-	-	-	-	-	-	-	-	-
Proteinase test	+	+	+	+	+	+	+	+	+	+	+	-
Citrate utilization test	+	-	+	-	+	+	+	+	-	-	-	+
H_2S production test	-	-	-	+	+	-	-	+	-	-	-	-
Gelatin liquefaction	-	-	-	-	-	-	-	-	-	-	-	+

Where: *A: S. epidermidis; B: E. coli; C: P. fluorescens; D: P. volgaris; E: S. liquefaciens; F: B. licheniformis; G: E. aerogenes; H: S. enterica; I: S. sonnei; J: L. plantarum; K: K. pneumoniae; L: V. anguillarum; -: Negative; +: Positive*

Table 4: Percentage of occurrence of bacterial isolates from *Clarias gariepinus* cultured in different systems

Bacterial isolates	Percentage of Occurrence	
	Concrete tank	Tarpaulin tank
Staphylococcus epidermidis	75	62
Enterobacter aerogenes	69	65
Pseudomonas fluorescens	56	52
Bacillus licheniformis	48	40
Escherichia coli	27	25
Salmonella enterica	17	20
Vibrio anguillarum	15	13
Shigella sonnei	13	8
Serratia liquefaciens	8	5
Proteus volgaris	4	7
Lactobacillus plantarum	4	5
Klebsiella pneumoniae	3	2

Also, *S. epidermidis*, one of the microorganisms isolated in this study was among the predominant microorganisms isolated from both gills and skin of *C. gariepinus* in a study conducted by El-Sayyad *et al*. (2010). *Bacillus* sp*., E. coli, Salmonella* sp. and *Streptococcus* sp. had been implicated in fish-borne diseases of human (Babu, 2000). Several other studies had recovered these organisms from gills, intestine and other body parts of *C. gariepinus* and other fish species (Al-Harbi and Uddin, 2003; Efuntoye *et al*., 2012; Budiati *et al.,* 2013; Omojowo and Omojasola, 2013). Thus, the presence of these organisms in cultured fish constitutes food safety problem since these fish could be potential agents of transfer of these pathogenic species to unsuspecting consumers. However, results of the present study showed that the bacterial counts of between 0.21 x 10^4 and 5.33 x 10^4 cfu/ml in intestine, 0 to 3.9 x 10^4 cfu/ml in skin and 1.03 x 10^4 to 5.23 x 10^4 cfu/ml in gills where within permissible range of 10^2 to 10^7 as given by Dutta *et al.* (2018).

Conclusion: Bacterial species are normal flora of all living organisms including fish. The presence of these organisms in fish samples from different systems indicated possible contamination of the culture systems. However, the need for proper processing of fish products before consumption to reduce the incidence of fish borne infections is advised.

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