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Original Research Article

Microbiota Dynamics in *Chrysichthys nigrodigitatus* and *Oreochromis niloticus* from Fresh and Brackish Environment

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Abstract: In recent times, increase in human interference on aquatic environment has tremendously interfere on the biota composition which nonetheless contribute to health status of fish. This study was undertaken to identify the bacterial flora from thirty-six life samples of *C. nigrodigitatus* and *O. niloticus* collected from two environments; fresh water (Osun River) and Brackish water (Lagos Lagoon) South-western Nigeria, Sub Saharan Africa. Specimen were collected from gills, intestines and skins of fish species and homogenized in 10^5 serial dilutions with distilled water. The specimens were cultured on Nutrient agar using pour plate method at 37°C for 24 hours. Two-way ANOVA was used to calculate mean count at $P<0.05$ significant level. Bacterial flora occurrence from the two species are: *Streptococcus spp* (10%), *Vibrio spp* (3%), *Escherichia coli* (15%), *Micrococcus spp* (3%), *Aeromonas spp* (7%), *Bacillius spp* (3%), *Spirillium spp* (6%), *Proteus spp* (4%), *Staphylococcus spp* (18%), *Pseudomonas spp* (14%), *Aerococcus spp* (5%), *Lactobacillius spp* (1%), *Alcogenes spp* (1%), *Citrobacter spp* (1%), *Fusobacterium spp* (2%), *Flexibacter spp* (3%), *Flavobacterium spp* (2%), *Salmonella spp* (1%), *klebisella spp* (1%). Total bacteria count found in each sample from Lagos lagoon was higher than that of samples from Osun River, which may be attributed to dense urbanization around brackish water environment as a result of higher degree of pollution.

Keywords: Bacteria flora; Human influence; fish species; Lagos lagoon, Osun River.

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1. INTRODUCTION

Fish is one of the most important sources of proteins available for humans and other animals in the tropic according to the Food and Agricultural Organization (FAO) of the United Nations fish account for more than 40% of the protein diet of two-third of the global population. Most Nigerians rely on fish as their main source of protein. Fish not only provide food for immediate consumption but people rely directly or indirectly on fishing for their economic survival and a source of job. *Chrysichthys nigrodigitatus* [1], commonly called African forktail catfish. *C. nigrodigitatus* is considered one of the best examples of an omnivore or predator feeding mainly on aquatic insects, crustaceans, bivalves and detritus [2] which exposes it to a variety of parasites which negatively impacts on its health [3].

Oreochromis niloticus is a species of tilapia, a cichlid fish native to Africa from Egypt south to East and Central Africa and numerous introduced populations exist outside its natural range. It is freshwater specie but can tolerate brackish water. It is an omnivore, feeding on planktons as well as higher plants. It is commercially known as mango fish, nilotica or boulti [4].

Disease or infection is common to all living organisms which results in the alteration in their normal physiological function. Fish living in the wild as well as reared in the aquaculture facilities are susceptible to infectious diseases caused by a phylogenetically diverse collection of bacteria pathogens, [5]. Aquatic microorganisms not only influence the water quality but are known to be closely associated with the physiological status of the fish and post-harvest quality of fish [6]. The bacterial flora on newly caught fish depends on the environment in which it is caught rather than on the fish species [7]. It is apparent that many bacterial genera have at various times been described as

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pathogens of fresh water and/or marine fish species. It may be assumed that fish are continually bathed in an aqueous solution/suspension of micro-organisms. Therefore, the external surfaces will be in frequent contact with these organisms. Similarly, any water entering the intestinal tract will contain bacteria because that's the water they consumed from as well as microbial growth on wounds on skins, water borne organisms, food borne organisms and gill microflora [8]. The presence of potential human pathogens suggests the fish improperly handled, undercooked or consumed raw may cause diseases to susceptible individual. Therefore, this study was conducted to investigate the bacterial species present in the gills, intestine and on the skin of *Chrysichthys nigrodigitatus* and *Oreochromis niloticus* gotten from Osun River (fresh water) and Lagos lagoon (brackish environment).

2. MATERIALS AND METHODS

2.1. Collection of Fish samples

Live *Chrysichthys nigrodigitatus* and *Oreochromis niloticus* fish species were obtained from Osun dam in Osun State and Lagos lagoon in Lagos State. 1 g was cut from the required body tissues (skin, gill and intestine) and were put in sample bottles containing 10 ml of distilled water and later transported to the Fisheries and Aquaculture Technology Laboratory of Federal University of Technology, Akure, Ondo State.

2.2. Culture Media Used

Culture media used in the analysis was Nutrient Agar. The medium was weighed according to manufacturer's specialization on a chemical balance and was poured in a conical flask with the appropriate volume of distilled water plugged in with foil paper. The culture medium was autoclaved at 120° C for 15 minutes. The inoculating loops and wire were sterilized by flaming in the spirit lamp until red hot, working bench surfaces were decontaminated by the application of disinfectants (Dettol) and 70% alcohol.

2.3. Microbiological Analysis

2.3.1. Isolation of Bacteria

The organisms were isolated by pour plate technique. Serial dilution was made with distilled water up to 10^5, one ml of the dilution factor 10^5 was then dispensed into the sterilized petri dishes. The sterilized culture medium was then poured into the petri dishes gently and it was rotated clockwise and anticlockwise to form a uniform layer. The mixture was then left to solidify. The plates were turned upside down and set inside incubator for 24 hours at 37ºC. Observation of colonies began after day one.

2.3.2. Stock Culture of Pure Bacteria Isolates

These plates were incubated for 24 hours at 37ºC and pure isolates obtained were stored on slants of Nutrient Agar in the refrigerator at 4ºC. Inoculums from these sources were used for the study as desired.

2.3.3. Bacterial Characterization and Identification

Bacterial colonies were observed after 18-24 hours of incubation for their colonial characteristics such as shape, colour, size, edge elevation, transparency and surface texture. Similarly, the isolates were Gram stained to differentiate the organisms into Gram negative and Gram positive by microscopic examination of stained preparation. Hanging drop preparations of the isolates were made on cavity slides and examined microscopically for motility. A good number of coliform isolates were motile. Other biochemical reactions including coagulase, catalase and sugar fermentation were intensified. One percent of sugars such as glucose, sucrose, lactose, maltose and others were used in a basal fermentative medium to determine the ability of the organisms to utilize the appropriate carbon sources signified by acid production or the change in colour of the medium and production of gas in Durham tube provided for the test.

2.3.4. Total bacterial count

The total bacterial count has been the usual technique for monitoring the living organisms in foods. During the incubation period, growth and multiplication of cells occurred until a viable count was formed. After 24 hours, the total number of growths in the plate was counted through the sight of observation.

2.4. Purification of Isolates

After growth, each distinct colony was inoculated and sterilized in another sterilized medium as pure culture. The plates were then incubated at 37ºC for 24 hours.

2.5. Gram's staining

Gram's staining method was carried out for the identification of each bacterium isolated. A smear of each isolates was made on a clear glass microscope slide with a sterilized inoculating loop and it was air dried and heat fixed by passing the slides gently through the flame. The smear was stained with crystal dye, crystal violet solutions for 1 minute and the solution was washed off with distilled water. It was then stained with Gram iodine solution for 1 minute and was decolourized with 95% alcohol for 30 seconds. The slides were then gently washed with water and counter stained with safranin for 1 minute and was washed off and was allowed to dry. Gram positive bacteria were stained purple colour while Gram negative bacteria were stained pink or red.

2.6. Statistical analysis

The data on the morphometric parameters and the bacteria (cfu) of all samples were recorded as mean ± standard deviation. All data were subjected to two-way analysis of variance (ANOVA) and differences between mean were separated by Duncan Multiple Range Tests using Computer Software SPSS version 16.0.

3. RESULT

Bacteria load on the skin and gills of *Chrysichthys nigrodigitatus* and *Oreochromis niloticus* from Asejire dam and Lagos Lagoon were examined, and Table 1 revealed the bacteria load. The bacteria load isolated from the body of *C. nigrodigitatus* and *O. niloticus* revealed low bacteria load generally in the body of the two fish species samples but high bacteria load on the skin of the two fish species samples. Lagos Lagoon fish samples revealed high bacteria load generally in the body of the two fish species samples but high bacteria flora on the skin of the two fish specie samples.

Table 2 shows the bacteria flora isolated from the fish species. A total of nineteen species of bacteria flora were isolated from the two fish species; they include: *Streptococcus spp, Vibrio spp., Escherichia coli, Micrococcus spp, Aeromonas spp., Bacillus spp., Spirillium spp., Proteus spp., Staphylococcus spp., Pseudomonas spp., Aerococcus spp., Lactobacillius spp., Alcogenes spp., Citrobacter spp., Fusobacterium spp., Flexibacter spp., Flavobacterium spp., Salmonella spp., Klebisella spp.* Figure 1 showed the percentage occurrence of the isolated bacteria flora, and figure 2 showed relative relationship in occurrence of bacteria flora common within and among fish organs.

Table 1: Mean ± standard deviation of bacteria colony forming unit on *Chrysichthys nigrodigitatus* and *Oreochromis niloticus* from Asejire dam and Lagos Lagoon

Sample	Asejire Dam Bacteria load (10^5)CFU/ml	Lagos Lagoon Bacteria load (10^5)CFU/ml
C. nigrodigitatus Skin	22.67 ± 8.08	102.00 ± 12.00
Gill	17.33 ± 8.74	98.33 ± 16.50
Intestine	15.00 ± 9.64	77.33 ± 14.47
O. niloticus Skin	27.67 ± 11.59	121.67 ± 3.51
Gill	19.67 ± 6.43	96.33 ± 17.93
Intestine	13.67 ± 4.73	100.67 ± 17.04

Table 2: Bacteria Isolated from Gills, Skins and Intestine of the fish samples

Fish Part /Tissue	Bacteria pathogen from Asejire Dam		Bacteria pathogen from Lagos lagoon	
	Chrysichthys nigrodigitatus	***Oreochromis niloticus***	***Chrysichthys nigrodigitatus***	***Oreochromis niloticus***
Skin	*Streptococcus feacalis, Vibrio spp, Escherichia coli*	*Staphylococcus aureus, Pseudomonas flourescens, Pseudomonas aeruginosa*	*Pseudomonas syringae, Areococcus aerogenes, Escherichia coli*	*Proteus mirabilis, Alcoligenes feacalis, Flexibacter columnaris*
Gill	*Streptococcus feacalis, Areomonas hydrophyla, Bacillus subtilis*	*Salmonella spp, Streptococcus feacalis, Staphylococcus aureus*	*Escherichia coli, Citrobacter spp, Spirrilium graniferum*	*Micrococcus luteus, Flavobacterium spp, Escherichia coli*
Intenstine	*Staphylococcus epidermis, Klebsiella spp, Lactobacillius planetarium*	*Pseudomonas spp Micrococcus spp Salmonella spp*	*Streptococcus feacalis, Fusobacterium spp, Staphylococcus epidermis*	*Staphylococcus spp, Vibrio cholera, Bacteriodes fragilis*

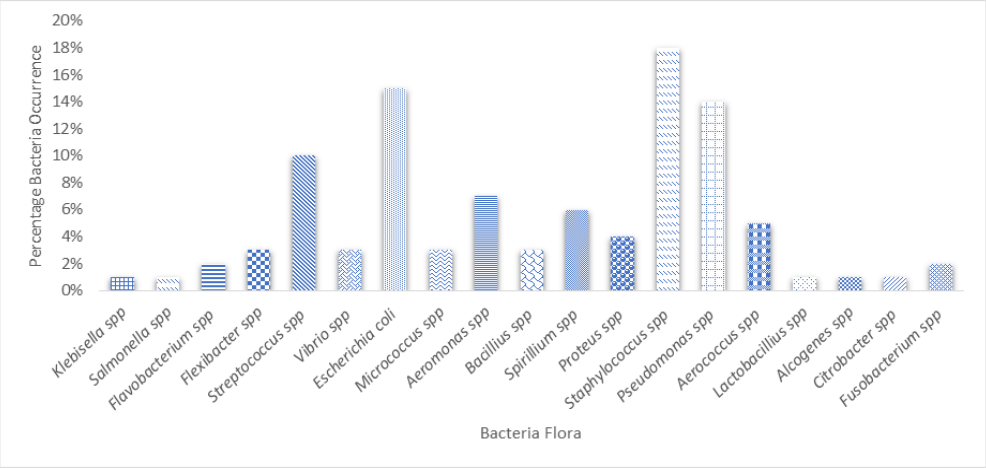

Figure 1: Percentage bacteria flora occurrence in fish organs

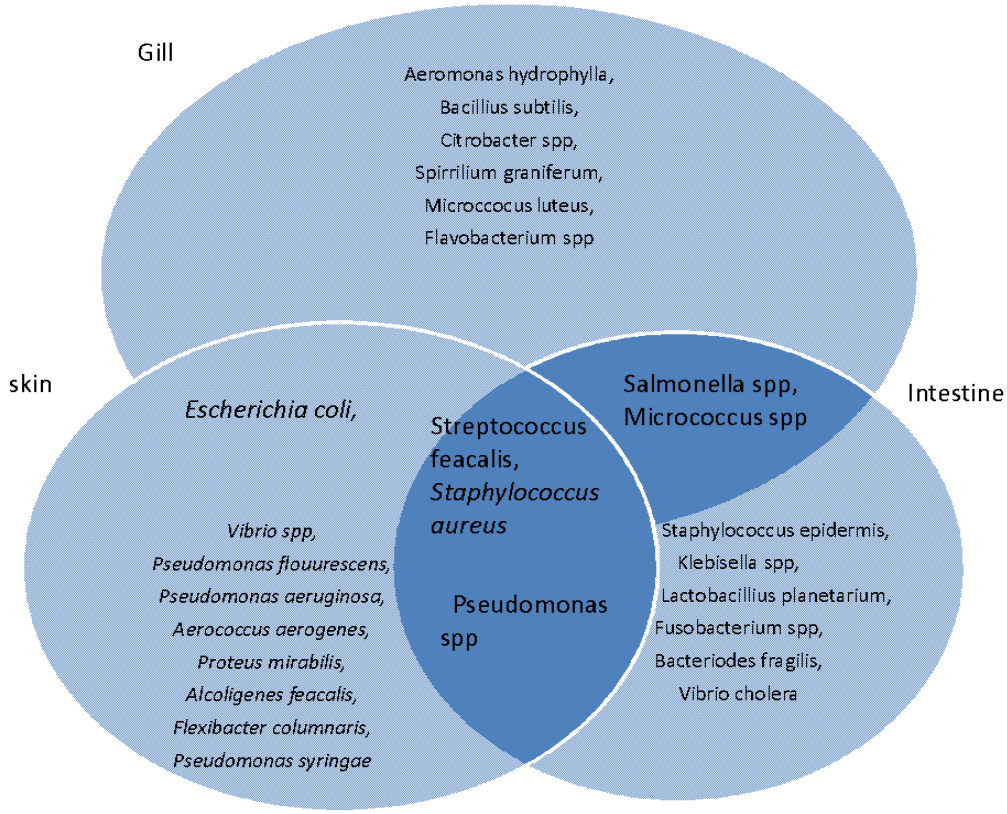

Figure 2: Venn diagram showing bacteria common to the different fish organs.

4. DISCUSSION

The results for the bacteria colony forming unit (cfu) on the bodies of the two fish species from Asejire dam shows significant differences on the skin, gills and intestines. While the results for the bacteria colony forming unit on the bodies of the two species from Lagos lagoon revealed significant differences on the skin of the two fish species and significant differences on the gills and intestine of the two fish species at significant level of $P < 0.05$.

There is high bacterial load from the skin of the two fish species, followed by the intestine and the gills from the two sites but high bacteria load was obtained from the bodies of fish species in Lagos lagoon compared to the fish species in Asejire dam due to high degree of pollution in Lagos lagoon. [9] also observed that high degree of pollution is as a result of discharge of waste material into water bodies upon which the fish species feed or it might result from flooding during rainy season.

The most occurred bacteria spp were *Staphylococcus spp* (20%), *Escherichia coli* (15%), *Pseudomonas spp* (15%), *and Streptococcus spp* (10%). Many investigators [10-12] have isolated different species of bacteria from the skin of the fresh water fish (catfish) including *Bacillus species* from the skin of sea water fish. Isolated *Proteus* species from some fresh water. Sugita 1997 reported that *Staphylococcus spp, Escherichia coli* were isolated frequently from the skin of fresh water fish [13]. He concluded that the skin of fresh water fishes were the natural habitat of these bacteria. Some investigations reported that the skin of the *Clarias species* contained *Klebsiella spp, Pseudomonas spp*, *Micrococcus spp* as the predominant genera.

The presence of *Staphylococcus aureus* in fish samples according to [4] might have been through contamination by handling. The bacteria group of *Staphylococcus aureus* according to [3] reported that it was one of the most common causes of human disease and they constitute the normal flora of the human skin and mucous membrane without resulting in a diseased condition.

5. CONCLUSION

The assessments on occurrence of bacterial flora on *Chrysichthys nigrodigitatus* and *Oreochromis niloticus* indicated that microbial load prevailed on the skin as it is prone to microbial contacts in water, the common environment for micro-organisms and fish. High percentage of losses/spoilage in fish production is as a result of microbial infection. Occurrence of bacteria may be due to pollution through the dumping of waste into the water column (river, lake, sea), therefore the populace especially the local communities should be sensitized on the effect of pollution in water as it is detrimental to the fish. Hence, there is need for water management for fish culture and the feed for the

fish must not be contaminated to reduce the occurrence of pathogens causing diseases in fish.

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