

## BACTERIOLOGICAL DIVERSITY OF THE ORGANS OF *Clarias gariepinus*, *Chrysichthys nigrodigitatus*, AND *Oreochromis niloticus* FROM KATSINA-ALA AND IBI RIVERS

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### ABSTRACT

Knowledge of the bacteriological load of fish is important for public health as fish are easily susceptible to bacterial attack due to their nature. Therefore, this study aimed at assessing the relative microbial loads of gills, intestine, and skin of *Oreochromis niloticus*, *Chrysichthys nigrodigitatus*, and *Clarias gariepinus* from Katsina-Ala and Ibi Rivers. Targeted fishes ( $n = 120$ ) were randomly collected from fishermen at the landing sites, grouped and aseptically transported alive using oxygenated nylon bags to Microbiology Laboratory for bacteriological assay using standard procedures. Aseptically, spread and pour plate methods were carried out for gills, intestinal samples and skin respectively. Bacterial loads from the organs of the fishes in both locations were significantly different ( $P < 0.05$ ). Specifically, the gills of *Oreochromis niloticus* had significantly more bacterial load than *Chrysichthys nigrodigitatus* and *Clarias gariepinus*. Similarly, the intestine and skin of *Clarias gariepinus* had more bacterial loads. In comparison, total bacterial viable counts (CFU/g) of the gills for *Chrysichthys nigrodigitatus* and *Oreochromis niloticus* were significantly higher in Ibi River, same for the intestine and skin of *Oreochromis niloticus*. Generally, bacteriological loads of the targeted fishes were higher in Ibi River; however, in some instance they were below the acceptable limits for food safety.

**Keywords:** Acceptable limits; Bacteriological loads; Fish; public health; Ibi River; Katsina-Ala River

### INTRODUCTION

Fish is one of the most sought-after sources of animal protein food, constituting an integral part of the diet of man worldwide (Eze *et al.*, 2011; Olugbojo and Ayoola, 2015; Svanberg, 2021). They are consumed for their high biological values (HBV) in terms of high protein retention in the body, presence of essential amino acids, and relatively low cholesterol (Mohanty *et al.*, 2014; Tacon *et al.*, 2020). According to Emikpe *et al.* (2011), fish contribute about 60% of global animal protein demand, and 60% of the developing countries derive over 30% of their animal protein from fish. Fish are generally regarded as safe, nutritious, and beneficial for man's consumption and health, however, both wild and aquaculture species are often associated with some food safety issues (WHO, 2007). The increasing human population, activities, and interest in the aquatic ecosystem have led to a huge upset in the survival and propagation of fish due to the high emergence of disease-causing bacteria and their antibiotic effect on man (Meijide *et al.*, 2018; Zhu *et al.*, 2018; Schmeller *et al.*, 2018). Microbes causing disorder in aquatic ecosystems, infections and mortality in fishes are directly linked to food security concerns due to inadequate animal protein sources and post-harvest losses (Ziarati *et al.*, 2022).

The presence of bacteria in fish could play diverse roles, some of which might be beneficial to the fish, but its adverse effect is both dangerous to the fish and the man that consumes it (Wasng, *et al.*, 2019; Vanamala *et al.*, 2022). Bacteria are transmitted by fish that have made contact with other diseased fish. Bacteria fish disease and infections are very common and are one of the most difficult health challenges. Most bacterial diseases display common symptoms, particularly in fish. Bacterial infection can occur in the muscles, internal organs, skin, fins, and exoskeleton (Vanamala *et al.*, 2022).

The African catfish (*Clarias gariepinus*) has been reported to be a very important freshwater fish for aquaculture industries (Federal Department of Fisheries, 2007) due to some of its advantageous qualities like tolerance to wide fluctuations in environmental conditions, fast growth, high fecundity, artificial breeding success, and palatability (Eyo *et al.*, 2014; Fagbuaro *et al.*, 2015). Its consumption is on the increase in both rural and urban areas due to its high nutritive value (Federal Department of Fisheries, 2007; Emikpe *et al.*, 2011). Catfishes generally are important fish species in the

inland water bodies of Africa because of their high commercial value.

The Nile tilapia (*Oreochromis niloticus*) is one of the most commonly farmed and commercialized fish species around the world because of its high rate of growth and consumer preferences (Mortuza and Al-Misned, 2013). It can grow and reproduce in a wide range of environmental conditions and tolerate stress induced by handling (Tsadik and Bart, 2007), and is also considered as a model to reduce the gap in aquaculture nutrition (Kapinga *et al.*, 2014). However, Nile tilapia is commonly associated with many bacterial loads, and studies have shown that different organs of this species carry a wide variety of bacteria (Mandal *et al.*, 2009; Eissa *et al.*, 2010).

The Silver catfish (*Chrysichthys nigrodigitatus*) is a highly valued food fish occurring in several African waters. It is among the dominant fish species of commercial importance, occurring all year round with peak abundance in the rainy season, (Holzloehner *et al.*, 2007; Ama-Abasi *et al.*, 2017). However, Uyoh *et al.*, (2020), reported that *Chrysichthys nigrodigitatus* of some water body has very low genetic diversity and that such low genetic diversity can lead to population eradication in the face of environmental variability and microbial infections.

Fish generally are vulnerable and quick to spoilage primarily by the action of enzymes and bacteria on the fish post-harvest due to its biological nature of high moisture, fat content, and high protein content, and also due to weak muscle tissue and low levels of carbohydrates (Murthy and Jeyakumari,

2019). Therefore, man must be mindful of his anthropogenic activities relating to the aquatic ecosystem. In addition, practice safe use of aquatic resources, healthy fishing activities, and hygienic handling of fish post-harvest to reduce fish susceptibility to microbial attack and spoilage. Microbiological quality study of fish has great importance to public health as it is directly related to spoilage of fish and food poisoning, so it is important to monitor the quality of harvested freshwater fish to ensure that the level of microbes is within the acceptable limits, and also reduce the health risk to end users.

Bacterial infection of fish is generally considered one of the major constraints of output for both wild and aquaculture fish species. This makes it necessary to determine the microorganisms of various fishes of economic importance in the wild and also their safety limits. Therefore, this study aimed at assaying the bacteriological loads of the gills, intestine, and skin of catfish (*Clarias gariepinus*), silver catfish (*Chrysichthys nigrodigitatus*), and tilapia (*Oreochromis niloticus*) from two major fish landing sites in lower River Benue, Ibi Local Government area of Taraba State and major tributary of River Benue in Katsina-Ala Local Government area of Benue State.

## MATERIALS AND METHODS

### Study Area

Katsina-Ala and Ibi Local Government Areas are in Benue State north-central and Taraba State north-east of Nigeria, respectively. River Katsina-Ala is a major tributary of River Benue, and the lower River Benue at Ibi has a confluence with River Katsina-Ala in Agasha town of Guma Local Government Area, Benue State (Figure 1).

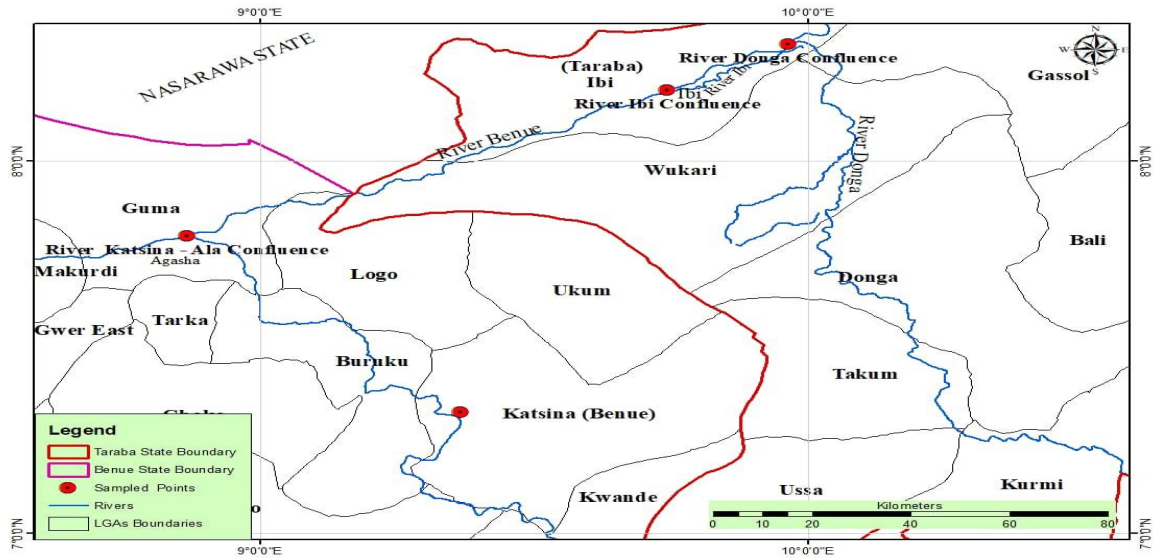


Figure 1: Map showing the sample points in both Katsina-Ala River and lower River Benue at Ibi, and their confluence points

**Collection of fish samples from the landing sites**

A total of one hundred and twenty (n=120) random samples of fishes (*Chrysichthys nigrodigitatus*, *Oreochromis niloticus*, and *Clarias gariepinus*) were immediately collected from the fishermen on arrival at the River Ibi and Katsina-Ala landing sites, and were further separated into three groups of twenty (20) fish species each and transferred aseptically in separate oxygenated nylons to the Microbiology laboratory of the Federal University Wukari for microbial analysis.

**Preparation of culture media**

The culture media used were Plate Count Agar (PCA) and Nutrient Agar (NA). Each of them was prepared according to the manufacturer's instructions (Oxoid Ltd) and autoclaved at a temperature of 121°C and a pressure of 15psi for a period of 15 minutes, then allowed to cool to 45°C before use (Oscroft and Correy, 1991).

**Isolation of bacteria from the gills, intestine, and skin of sampled fishes**

One gram (1g) of both gills and intestine was aseptically collected and pooled from each of the dissected fish samples made up of three groups of 20 fish species each in the separate landing sites. The collected samples were homogenized in 10 mL of normal saline and serially diluted using 10-fold serial dilutions. An aliquot from dilution 10<sup>6</sup> was used. The pour plate method was used for the primary isolation of bacteria from the serially diluted gill and intestinal samples by putting 1mL

from the selected dilutions into Petri dishes with already cooled and solidified molten nutrient agar and swirling properly for inoculation. The spread plate method was used for the primary isolation of bacteria from the fish skin swabbing. The inoculated plates were incubated at 37°C for 24 hours. Bacteria isolates were purified by repeated sub-culturing onto freshly prepared sterile nutrient agar using the streak plate method as described by Katz (2008).

**Microscopic analysis**

The bacteria isolates were morphologically examined for size, color, shape, and pigmentation. The isolates were morphologically identified based on cultural and microscopic characteristics as described by Váradi *et al.* (2017). Pure isolates were picked using a sterile inoculating needle and placed on a sterile grease-free microscope slide, the slide containing the bacteria culture was heat fixed under a hot flame using the Bunsen burner. The heat-fixed slide was stained using the gram staining reagent after which the stained slide was allowed to air dry. The slide was viewed under the microscope using an x100 objective lens (oil immersion lens). This was done to check and determine the colony characteristics and gram reaction of the bacteria isolates.

**Biochemical test**

Briefly, isolates were further identified by gram stain, motility, catalase, oxidase, coagulase, and IMViC reactions as follows:

### Gram staining procedures

A smear of each isolate was made on a grease-free slide and heat-fixed properly. Crystal violet which is the primary stain was added to each slide and allowed to stay for 60 seconds after which it was washed off using clean running water. Grams iodine was added as mordant for 60 seconds and washed off. The slides were decolorized using 95% ethanol to remove excess stains and rinse with water. The slides were counterstained using a secondary stain called Safranin and rinsed with tap water. The slides were air-dried and observed under a microscope using X100 objective (oil immersion) for morphological characteristics as described by Cardoso *et al.* (1998).

### Catalase

2mL of hydrogen peroxide solution was placed inside the test tube. Colonies of the culture were picked using a sterile glass rod after an 18 to 24-hour test and immersed in the hydrogen peroxide to observe the bubbling of gas (Sapkota, 2020).

### Coagulase test

1mL of plasma was added to test tubes and a loopful of the bacteria isolates were inoculated. The test tubes were incubated at 37°C for 1 hour in a water bath to observe for the presence of clouding and clotting.

### Oxidase

The tetramethyl-p-phenylenediamine dihydrochloride substrate was used to soak filter paper. Sterile distilled water was used to moisten the paper and the colony was picked using a sterile platinum loop and observed for any color change to deep blue or purple between 10 to 30 seconds (Shield and Cathcart, 2010).

### Indole test

An 18 to 24-hour culture inoculum was placed aseptically in a sterilized test tube with 4mL of prepared tryptophan broth. It was incubated at 37°C for 24 to 28 hours, and 0.5mL of Kovac's reagent was introduced into the broth culture and observed for the presence of the ring (Kar *et al.*, 2017).

### Methyl red test

The isolated colonies were inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer, and incubated at 37°C for 24 hours. The pH of the medium was tested by the

addition of 5 drops of methyl red reagent, to check for either red color indicating positive results or yellow color indicating negative results.

### Voges-Proskauer test

The isolated colonies were inoculated into glucose phosphate broth and incubated at 37°C for 24 hours. 0.6 ml of alpha-naphthol was added to the test broth and shaken. 0.2 ml of 40% KOH was added to the broth and shaken. The test tube was allowed to stand for 15 minutes, to check for the appearance of a red color indicating a positive result or not.

### Citrate utilization test

Isolated bacterial colonies were picked with a sterile inoculating loop and inoculated into slants of Simmon's citrate agar and incubated at 37°C, to check if the organism can utilize citrate by changing the medium color from green to blue.

### Data Analysis

Anderson-Darling Normality test was used to check the normality of the distribution and the homogeneity of variance obtained. Data on total bacterial viable counts on the gills, intestine, and skin of the fish species were analyzed statistically using ANOVA. The significant difference of the means of bacteria species was carried out using Tukey HSD. Then, a t-test was used to compare the mean difference of bacteria on the organs of the fish species in the two locations. All the statistical analysis was done with the aid of Minitab statistical software program version 19 (Minitab® LLC, Pennsylvania, USA).

## RESULTS

### Morphometric characteristics of the isolated bacteria of the gills, intestine and skin of *Clarias gariepinus*, *Chrysichthys nigrodigitatus*, and *Oreochromis niloticus*

The morphological characteristics of the isolated bacteria (Table 1) indicated their distinct characteristics ranging from small-to large colony sizes, with varying colors. Most of them were non-lactose fermenters, except for *Escherichia coli* and *Klebsiella Sp.* They all indicated negative gram staining reactions and rod-shaped appearance, except *Bacillus sp* which was gram-positive.

**Bacteriological status of the gills, intestine and skin of *Clarias gariepinus*, *Chrysichthys nigrodigitatus* and *Oreochromis niloticus* in Katsina-Ala and Ibi Rivers.**

The microbial loads of the gill, intestine and skin of the three (3) species of fish from Katsina-Ala as shown in Table 2 indicated that there was a significant difference ( $P < 0.05$ ) in the total viable counts in the gill, intestine, and skin of *Clarias gariepinus*, *Chrysichthys nigrodigitatus*, and *Oreochromis niloticus* respectively. In specific, the intestine of *Clarias gariepinus* significantly harbored more of the bacterial species isolated, while the gills of *Oreochromis niloticus* harbored more of the bacterial species isolated except *Flavobacterus sp*, *Proteus sp*, *Salmonella sp*, and *Shigella sp* in *Clarias gariepinus*. The skin of *Clarias gariepinus* was noticed to have the highest levels of the identified bacterial species isolates. The pooled standard deviation was not wide among all the samples ranging from 0.2 – 0.3. Instances where the R-square values of the model were very high indicated a strong relationship between the bacterial isolates (dependent variable) and the various organs (independent variables) of the three (3) species investigated.

Similarly, it was observed that the same fish species (*Clarias gariepinus*, *Chrysichthys nigrodigitatus*, and *Oreochromis niloticus*) in Ibi River were having the same pattern of bacterial loads on the gills as in River Katsina-Ala, with *Oreochromis niloticus* having the highest bacterial loads in all the isolated species of bacteria (Table 3). The intestinal bacterial loads of the isolated bacteria in the Ibi River were higher in *Clarias gariepinus* but not significantly different ( $P > 0.05$ ) between *Chrysichthys nigrodigitatus* and *Oreochromis niloticus*. The skin of *Clarias gariepinus* in the Ibi River like the Katsina-Ala River had a similar trend in bacterial loads among the three (3) fish species investigated except for *Proteus sp*. and *Shigella sp* in *Oreochromis niloticus*. The observed summary of the results in both Rivers shows that *Clarias gariepinus* significantly had more bacteria loads in the intestine and on the skin, while *Oreochromis niloticus* had more in the gills. The pooled standard deviation was not also wide among the samples with a range of 0.18 – 0.22. Instances where the R-square values of the model were very high indicated a strong relationship between the bacterial isolates (dependent variable) and the various organs (independent variables) of the three (3) species investigated.

**Table 1: Morphological characteristics of the isolated bacteria of the gills, intestine, and skin of *Clarias gariepinus*, *Chrysichthys nigrodigitatus*, and *Oreochromis niloticus* in Rivers Katsina-Ala and Ibi**

Bacteria	Morphological characteristics	Gram stain reaction
<i>Pseudomonas aeruginosa</i>	Moderate, moist, blue-green non-lactose fermenter colonies	Gram-negative rod-shaped
<i>Bacillus sp</i>	Small, moist white or pale-yellow non-lactose fermenter colonies	Gram-positive rod-shaped
<i>Escherichia coli</i>	Moderate, moist yellow lactose fermenter colonies	Gram-negative rod-shaped
<i>Flavobacterus sp.</i>	Large, moist yellowish-brown, non-lactose fermenter colonies	Gram-negative rod-shaped
<i>Klebsiella sp.</i>	Large, moist red lactose fermenter colonies	Gram-negative rod-shaped
<i>Aeromonas sp.</i>	Moderate, moist, dark-green non-lactose fermenter colonies	Gram-negative rod-shaped
<i>Proteus sp.</i>	Small, smooth, pale non-lactose fermenter colonies	Gram-negative rod-shaped
<i>Salmonella sp.</i>	Small, moist pale-yellow non-lactose fermenter colonies	Gram-negative rod-shaped
<i>Shigella sp.</i>	Small, moist, blue-green non-lactose fermenter colonies	Gram-negative rod-shaped

**Table 2: Bacteria load (10<sup>6</sup> CFU/g) of the gills, intestine, and Skin of *Clarias gariepinus*, *Chrysichthys nigrodigitatus*, and *Oreochromis niloticus* in River Katsina-Ala**  
 Means with different superscripts along the row are significantly different (P < 0.05). Cg-*Clarias gariepinus*, Cn- *Chrysichthys nigrodigitatus*, On- *Oreochromis niloticus*

Parameters	Gills Cg	Gills Cn	Gills On	Pooled StDev	R <sup>2</sup>	Intestine Cg	Intestine Cn	Intestine On	Pooled StDev.	R <sup>2</sup>	Skin Cg	Skin Cn	Skin On	Pooled StDev.	R <sup>2</sup>
<i>Pseudomonas sp</i>	7.0 <sup>c</sup>	7.8 <sup>b</sup>	9.6 <sup>a</sup>	0.2	97.8	13.6 <sup>a</sup>	11.4 <sup>b</sup>	11.8 <sup>b</sup>	0.2	97.1	9.6 <sup>A</sup>	6.2 <sup>C</sup>	7.2 <sup>B</sup>	0.2	98.7
<i>Bacillus sp</i>	9.5 <sup>b</sup>	11.6 <sup>a</sup>	11.6 <sup>a</sup>	0.2	97.5	18.3 <sup>a</sup>	17.2 <sup>b</sup>	14.3 <sup>c</sup>	0.2	99.2	12.9 <sup>A</sup>	9.2 <sup>B</sup>	8.4 <sup>C</sup>	0.2	99.3
<i>Flavobacterus sp</i>	5.2 <sup>a</sup>	5.4 <sup>a</sup>	4.3 <sup>b</sup>	0.2	89.9	10.1 <sup>a</sup>	8.1 <sup>b</sup>	5.3 <sup>c</sup>	0.2	99.3	7.1 <sup>A</sup>	4.3 <sup>B</sup>	2.8 <sup>C</sup>	0.5	95.6
<i>Klebsiella sp</i>	5.3 <sup>c</sup>	7.1 <sup>b</sup>	7.9 <sup>a</sup>	0.2	98.1	10.3 <sup>a</sup>	10.5 <sup>a</sup>	9.7 <sup>b</sup>	0.2	80.4	7.3 <sup>A</sup>	5.6 <sup>B</sup>	5.9 <sup>B</sup>	0.2	96.6
<i>Aeromonas sp</i>	1.0 <sup>b</sup>	0.6 <sup>b</sup>	1.8 <sup>a</sup>	0.3	81.5	2.0 <sup>a</sup>	0.9 <sup>b</sup>	1.8 <sup>a</sup>	0.2	87.7	1.4 <sup>A</sup>	0.4 <sup>B</sup>	1.0 <sup>A</sup>	0.2	85.0
<i>Proteus sp</i>	3.1 <sup>a</sup>	3.1 <sup>a</sup>	0.8 <sup>b</sup>	0.2	97.9	6.2 <sup>a</sup>	4.7 <sup>b</sup>	1.0 <sup>c</sup>	0.2	99.4	4.3 <sup>A</sup>	2.5 <sup>B</sup>	0.5 <sup>C</sup>	0.2	98.9
<i>Salmonella</i>	2.5 <sup>ab</sup>	2.8 <sup>a</sup>	2.3 <sup>b</sup>	0.2	62.8	5.0 <sup>a</sup>	4.1 <sup>b</sup>	2.8 <sup>c</sup>	0.2	96.9	3.5 <sup>A</sup>	4.9 <sup>A</sup>	1.6 <sup>A</sup>	2.6	28.3
<i>Shigella sp</i>	2.7 <sup>a</sup>	2.4 <sup>a</sup>	2.4 <sup>a</sup>	0.2	46.1	5.4 <sup>a</sup>	3.6 <sup>b</sup>	3.0 <sup>c</sup>	0.2	97.5	3.8 <sup>A</sup>	1.9 <sup>B</sup>	1.8 <sup>B</sup>	0.2	97.3
<i>Escherichia coli</i>	6.3 <sup>c</sup>	8.9 <sup>b</sup>	9.9 <sup>c</sup>	0.2	98.6	12.3 <sup>b</sup>	13.4 <sup>a</sup>	12.2 <sup>b</sup>	0.2	87.6	8.7 <sup>A</sup>	7.1 <sup>B</sup>	7.4 <sup>B</sup>	0.2	94.6

**Table 3: Bacteria load ((10<sup>6</sup> CFU/g) of the gills, intestine, and Skin of *Clarias gariepinus*, *Chrysichthys nigrodigitatus* and *Oreochromis niloticus* in River Ibi**

Parameters	Gills Cg	Gills Cn	Gills On	Pooled StDev	R <sup>2</sup>	Intestine Cg	Intestine Cn	Intestine On	Pooled StDev.	R <sup>2</sup>	Skin Cg	Skin Cn	Skin On	Pooled StDev.	R <sup>2</sup>
<i>Pseudomonas sp</i>	6.2 <sup>c</sup>	8.3 <sup>b</sup>	9.8 <sup>a</sup>	0.2	98.8	13.5 <sup>a</sup>	13.4 <sup>a</sup>	13.4 <sup>a</sup>	0.2	7.7	9.2 <sup>a</sup>	6.2 <sup>b</sup>	3.8 <sup>c</sup>	0.2	99.7
<i>Bacillus sp</i>	9.2 <sup>c</sup>	10.6 <sup>b</sup>	13.6 <sup>a</sup>	0.2	99.2	20.2 <sup>a</sup>	19.2 <sup>b</sup>	19.23 <sup>b</sup>	0.18	90.4	13.7 <sup>a</sup>	9.2 <sup>c</sup>	10.2 <sup>b</sup>	0.2	99.9
<i>Flavobacterus sp</i>	4.3 <sup>c</sup>	6.1 <sup>b</sup>	7.4 <sup>a</sup>	0.2	98.4	11.2 <sup>a</sup>	10.0 <sup>b</sup>	10.1 <sup>b</sup>	0.2	91.0	6.5 <sup>a</sup>	4.3 <sup>c</sup>	5.3 <sup>b</sup>	0.2	96.7
<i>Klebsiella sp</i>	5.6 <sup>b</sup>	9.4 <sup>a</sup>	9.0 <sup>a</sup>	0.3	97.9	13.5 <sup>a</sup>	12.5 <sup>b</sup>	12.5 <sup>b</sup>	0.2	89.7	8.3 <sup>a</sup>	5.6 <sup>b</sup>	3.6 <sup>c</sup>	0.2	99.3
<i>Aeromonas sp</i>	0.4 <sup>c</sup>	4.6 <sup>a</sup>	0.4 <sup>b</sup>	0.2	99.8	2.9 <sup>a</sup>	1.9 <sup>b</sup>	2.9 <sup>b</sup>	0.22	88.2	0.7 <sup>b</sup>	0.4 <sup>b</sup>	2.4 <sup>a</sup>	0.2	96.6
<i>Proteus sp</i>	2.5 <sup>c</sup>	7.1 <sup>a</sup>	5.1 <sup>b</sup>	0.2	99.3	9.7 <sup>a</sup>	6.7 <sup>b</sup>	6.7 <sup>b</sup>	0.2	97.8	3.7 <sup>b</sup>	2.5 <sup>c</sup>	4.5 <sup>a</sup>	0.2	96.4
<i>Salmonella</i>	2.2 <sup>c</sup>	7.8 <sup>a</sup>	4.8 <sup>b</sup>	0.2	99.5	7.2 <sup>a</sup>	6.2 <sup>b</sup>	6.7 <sup>ab</sup>	0.2	86.2	3.3 <sup>a</sup>	2.2 <sup>b</sup>	2.2 <sup>b</sup>	0.2	91.3
<i>Shigella sp</i>	1.9 <sup>c</sup>	7.4 <sup>a</sup>	4.4 <sup>b</sup>	0.2	99.5	7.6 <sup>a</sup>	5.6 <sup>b</sup>	5.6 <sup>b</sup>	0.2	97.1	2.9 <sup>b</sup>	1.9 <sup>c</sup>	3.8 <sup>a</sup>	0.2	95.9
<i>Escherichia coli</i>	7.1 <sup>c</sup>	9.9 <sup>b</sup>	11.0	0.2	99.1	16.4 <sup>a</sup>	15.3 <sup>b</sup>	15.3 <sup>b</sup>	0.2	90.8	10.6	7.1 <sup>b</sup>	7.1 <sup>b</sup>	0.2	99.0

Means with different superscripts along the row are significantly different (P < 0.05). Cg-*Clarias gariepinus*, Cn- *Chrysichthys nigrodigitatus*, On- *Oreochromis niloticus*

**Comparison of the bacteria load of the gills, intestine, and skin of *Clarias gariepinus*, *Chrysichthys nigrodigitatus*, and *Oreochromis niloticus***

The comparison of the total bacteria loads between the fishes of River Katsina-Ala and Ibi (Table 4) indicated that there is no significant difference ( $P < 0.05$ ) in the total viable counts of bacteria on the gills on *Clarias gariepinus* in both Rivers. However, there was a strong significant difference in both *Chrysichthys nigrodigitatus* and

*Oreochromis niloticus* in River Katsina-Ala and Ibi respectively, with the highest loads in Ibi River. The intestinal bacteria loads in the three (3) fish species showed a strongly significant difference ( $P < 0.05$ ) in both the Katsina-Ala River and Ibi River, with the highest loads in the Ibi River. The skin of the fish species in both Rivers showed no significant difference between *Clarias gariepinus* and *Chrysichthys nigrodigitatus*, except *Oreochromis niloticus*, where it was significantly higher in the Ibi River.

**Table 4 Total bacterial viable counts ( $10^6$  CFU/g) of the gills, intestine, and skin of *Clarias gariepinus*, *Chrysichthys nigrodigitatus*, and *Oreochromis niloticus* in the two locations**

Organs	Species	Katsina-Ala	Ibi	T-Value	P-Value	DF	Pooled StDev.
Gills	Cg	4.73±1.80	4.36±1.77	2.20	0.093	4	1.7851
	Cn	5.51±1.80	7.90±1.31	-16.74	0.00	4	1.5767
	On	5.62±1.33	7.52±1.80	-13.27	0.00	4	1.5832
Intestine	Cg	9.25±1.89	11.37±1.85	-12.99	0.00	4	1.7876
	Cn	8.21±1.89	10.10±1.85	-11.15	0.00	4	1.8702
	On	6.88±1.80	10.27±1.75	-21.06	0.00	4	1.7753
Skin	Cg	6.51±1.75	6.55±1.80	-0.25	0.813	4	1.7753
	Cn	4.67±3.20	4.37±1.80	1.29	0.267	4	2.5984
	On	4.07±2.27	4.77±1.80	-3.73	0.02	4	2.0498

T-Value of the means of the two locations along the rows with P-Value less than 0.05 is significantly different ( $P < 0.05$ ). Cg-*Clarias gariepinus*, Cn- *Chrysichthys nigrodigitatus*, On- *Oreochromis niloticus*

**DISCUSSION**

The bacteriological status of fish is of importance to both the aquatic environment and fish consumers. In this instance, the bacterial load of three fish species from two locations was studied, and these obvious indicators show that different water bodies have different levels of bacteria (Cabral 2010; Some *et al.*, 2021). This could probably be from the backdrop of the activities carried out by men in and around the water bodies. It was noticed at the time of this investigation that the lower River Benue in the Ibi local government area of Taraba State had fish with more bacterial load than the major tributary of River Benue in the Katsina-Ala local government area of Benue State. The fish bacterial load can cause severe fish economic losses due to post-harvest spoilage from bacteria activities, and in addition harm the consumers (Safaeian and Khanzadi, 2018; Mishra, Sudhansu, *et al.*, 2019; Maldonado-Miranda *et al.*, 2022; Keerthana *et al.*, 2022; Ogura, 2022). The overview of this study is to compare and quantify the bacteria status of the three common fish species in these locations to raise the necessary awareness

that would reduce anthropogenic activities or unhealthy fishing activities around and within this aquatic ecosystem. Therefore, it was noticed that the fish species in the two locations had similar patterns of bacteria loads on the various organs investigated, where the intestine had more bacterial loads and the skin and gills were trailing in that order. This perhaps could be because of the suitable substrate of the intestine for bacteria growth (Umma *et al.*, 2023), and the more frequent interfacing of the gills and the skin with the flowing water. Furthermore, it was noticed that the bacteria loads of the intestine of *Clarias gariepinus* were significantly higher than that of *Chrysichthys nigrodigitatus* and *Oreochromis niloticus*, this could be due to the feeding habit of the selected fish species, where *Clarias gariepinus* is an omnivore, and also having the propensity for being carnivorous (Khedkar *et al.*, 2002), although *Chrysichthys nigrodigitatus*, and *Oreochromis niloticus* may have an overlapping feeding habit of both omnivorous and herbivorous (Dada and Araoye, 2008), the primary feeding habit of *Oreochromis niloticus* is herbivore (Khallaf and Alne-na-ei 1987). However,

the gills of *Oreochromis niloticus* had more bacterial loads compared to *Clarias gariepinus* and *Chrysichthys nigrodigitatus*. This could be because the gill of *Oreochromis niloticus* tends towards being a good substrate for bacteria, as reported by Bakr *et al.* (2021) where the gills of *Oreochromis niloticus* showed more susceptibility to infection carried out by intraperitoneal injection of 0.1 ml of  $1.5 \times 10^8$  CFU/ml of *A. sobria* than the skin. The skin of *Clarias gariepinus* was seen to have more bacterial loads than the skin of both *Chrysichthys nigrodigitatus* and *Oreochromis niloticus*. This is perhaps due to the nature of the skin where the slime though having a protecting tendency could also support bacteria growth. In general, the bacteria were at the range of 7.90– 4.30 CFU/g for the gills, 11.37– 6.88 CFU/g for the intestine and 6.55– 4.07 CFU/g for the skin of the three species of fish in the two locations while the acceptable maximum microbiological limits for total viable aerobic bacterial counts (TVABC)  $5 \times 10^5$  CFU/g, and the acceptable limits of total coliform (TC) and fecal (FC) for fresh and frozen fish are < 100 most probable number per gram (MPN/g) and < 10 MPN/g respectively (ICMSF- International Commission of Microbiological Specification for Food, 1986).

## CONCLUSION

In conclusion, the two Rivers from different sources had a common confluence point, signifying their link and the need for studying the bacterial diversity of the economic fish species from the two fish landing sites and also comparing their bacterial loads with the acceptable limits for human consumption. The fish collected from River Katsina-Ala landing site had less bacterial load than the fish collected from River Ibi landing site, however, still below the borderline of the acceptable limits. The obvious reason for the differences in fish bacterial loads in the two locations could be due to the nature of activities along the rivers and the handling methods from capture to the landing sites.

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