

Prevalence of Bacterial Microbiota in *Tilapia Guineensis* Harvested from Buguma Brackish Water Environment River State, Nigeria

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Abstract: Brackish water fishes usually harbour human pathogenic bacteria particularly the enterics and coliform groups in their mucosal surfaces. Thus, this study was undertaken to investigate the prevalence of skin and gills microbiota of *Tilapia guineensis* as to understand their microbial assemblages and as well provide useful insights into the broad dynamics of the fish host-microbial relationship and health status. Thirty samples of *Tilapia guineensis* were collected from Buguma brackish water environment, River State, Nigeria. The skin and the gills of the tilapia were analyzed microbiologically. Specimen were collected by swabbing aseptically over the skin and gills segment of the fish and then processed for total bacterial count, followed by isolation and identification of isolated bacteria. Microbial load analysis revealed that the total viable count of bacteria ranged from 2.4 to 7.6×10^5 cfu/ml for skin and 2.1 to 5.7×10^6 cfu/ml for gills, and it was observed to fall within the recommended microbiological standard safety limit. A total of thirty-seven (37) bacteria, belonging to fifteen different genera in the family of Vibrionaceae, enterobacteriaceae and Morganellaceae were isolated from the fish. The prevalent rate of the bacterial shows that *Proteus vulgaris* recorded the highest frequency of occurrence of 16.22%, followed by *Vibrio parahaemolyticus* with 10.81% occurrence and *Providencia rettgeri*, *Serratia mercenscens* and *Salmonella sp.* trailed them with 8.11%. *Kluyvera ascorbate*, *Citrobacter amalonaticus*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Plesiomonas shigelloides* and *Pseudomonas sp.* have the least frequency of occurrence with 2.7% respectively for each. Though the bacterial count of *Tilapia guineensis* harvested from the water and fish sample did not exceed the standard recommended safety limit. There were several potential pathogenic and spoilage bacteria isolated as part of the fish flora. The presence of these bacteria could poses or constitute a public health risk.

Keywords: Bacterial Flora, *Tilapia guineensis*, Prevalence, Brackish Water, Pathogen

1. Introduction

Tilapia Guineensis is a widespread fish species with great aquaculture potentials in all the tropical and sub-tropical regions of the world [1]. *Tilapia Guineensis* have been classified prominent in the ecology of tropical waters as well as in the resources of their aquatic systems [2]. *Tilapia Guineensis* is very common in the brackish water zone of Nigeria and most especially in the Niger Delta region [3]. There is an increasing interest in the *Tilapia guineensis*

production due to their several nutritional advantage of being firm in texture, possession of low cholesterol and relatively easy to cook [4]. Regardless of these numerous advantages, *Tilapia guineensis* and other fish species in general are susceptible to contamination with pathogenic bacterial organisms because of their flesh texture, their living habits as well as their habitat which is loaded with pathogenic bacteria. Among these pathogenic bacteria are the *Vibrio sp.*, *Aeromonas sp.*, *Edwardsiella sp.*, and *Salmonella sp.*, which belong to water and food borne group of pathogens. These

bacteria are widely distributed in brackish water environment and they have been implicated as opportunistic pathogens causing gastroenteritis and other severe health conditions to man. Their occurrence has been reported in some African, Asian, European countries and virtually across the globe. Several researchers have reported that the release of pathogenic bacteria in faeces dispersed into aquatic environments can contaminate fish and shellfish harvested from these waters [5]. Once these bacteria are in the aquatic environment, plasmid exchange between the bacteria will be readily facilitated and can result in a higher frequency of multiple antibiotic resistant strains which perhaps could exert selective pressures to influence the antibiotic resistance [6].

Bacteria, a large group of single-celled, prokaryotic and ubiquitous microscopic organisms whose single cells have neither a membrane bounded nucleus nor other membrane bounded organelles like mitochondria and chloroplasts [7]; and are noted for most fish diseases, fish spoilage and threats to public health [8].

Evaluation of the prevalence of bacterial microbiota associated with the skin and gills of *Tilapia guineensis* harvested from brackish water environment usually give information on the relationship between the fish and the environment. Moreover, Water with high organic load have been reported to predisposes fish to disease condition [9]. Therefore, this study was embarked on to investigate the prevalence of bacterial microbiota associated with the skin and gills of *Tilapia guineensis* caught from Buguma brackish water environment.

2. Materials and Methods

Thirty (30) fish samples of *Tilapia guineensis* were collected from Buguma brackish water environment, River State, Nigeria, and transported in an oxygenated bag to Nigerian Institute for Oceanography and Marine Research, Lagos, where the skin and the gills of the fish sample were aseptically swabbed with a sterile swab stick for bacteriological studies.

Table 1. Total bacteria count from the Gills, Skin and Brackish Water Sample from Buguma, River State.

Sample	Total bacteria count (cfu/ml)	Range	Mean
Skin	34.2×10^5	$2.4 - 7.6 \times 10^5$	5×10^5
Gills	26.3×10^6	$2.1 - 5.7 \times 10^6$	4×10^6
Water	29.4×10^6	$3.3 - 8.5 \times 10^6$	6×10^6

Table 2. Bacterial isolates obtained from Water, Skin and Gills of *Tilapia guineensis* from Buguma, River State.

Sample	Bacterial Isolates
Skin	<i>Proteus vulgaris</i> , <i>Pseudomonas</i> sp., <i>Flavimonas oryzihabitans</i> , <i>Salmonella</i> sp., <i>Aerobacter aerogenes</i> , <i>Serratia mercerscens</i> , <i>Enterobacter aerogenes</i> , <i>Morganella morganii</i> , <i>Vibrio parahaemolyticus</i> , <i>Salmonella</i> sp., <i>Vibrio metschnikovii</i> .
Gills	<i>Shigella</i> sp., <i>Shigella sonnei</i> , <i>Proteus vulgaris</i> , <i>Plesiomonas shigelloides</i> , <i>E. coli</i> , <i>Morganella morganii</i> , <i>Proteus mirabilis</i> , <i>Vibrio metschnikovii</i> , <i>Vibrio parahaemolyticus</i> , <i>Serratia mercerscens</i> , <i>Providencia rettgeri</i> .
Water	<i>Providencia rettgeri</i> , <i>Serratia mercerscens</i> , <i>E. coli</i> , <i>Kluyvera ascorbate</i> , <i>Citrobacter amalonaticus</i> , <i>Vibrio parahaemolyticus</i> , <i>Salmonella</i> sp., <i>Citrobacter freundii</i> .

The cultural and morphological characteristics used in the identification processes of the bacterial isolates from Buguma are represented in Table 3. Table 4 shows the Gram stain reaction and biochemical tests for presumptive

2.1. Total Bacteria Count Analysis

Swabs were aseptically taking from the gills and skin of the fish with a clean sterile swab stick. The swabs were immersed into a 100 ml conical flask containing buffered peptone water (0.1%) (Merck, Germany). After overnight incubation for 18 hours at 37°C, 1 ml was transferred for further analysis from the peptone water to Tryptone soy agar. Ten- fold serial dilutions (10^{-5}) were carried out, and viable bacterial counts of the samples were obtained.

2.2. Bacterial Enumeration and Identification

Enumeration of bacteria was carried out using spread plate method. The plates containing Tryptone Soy Agar (Himedia, India) were inoculated and incubated at 37°C for 24 hours after which the discrete colonies were observed and sub-cultured to get pure colonies of the isolates. Pure colonies of the isolates were further sub-cultured on Eosin methylene blue (EMB) agar, Salmonella-Shigella agar, Thiosulfate Citrate bile salts sucrose (TCBS) agar and Mannitol salt agar. The bacteria isolates were identified using some parameters such as Gram staining reaction, cultural and morphological characteristics, and series of biochemical tests to confirm the presence of the suspected microorganism by their reactions to the tests according to Bergey's manual of determinative bacteriology, 8th Edition [10].

3. Results

Total bacteria count result of water sample, skin and gills of *Tilapia guineensis* from Buguma brackish water, River State are shown in Table 1. The bacterial load from the skin are significantly lower ($P < 0.05$) than the bacteria count from the gills and the water sample. The highest microbial load was observed in the water sample, followed by the gills. All the bacterial isolated from the water sample, skin and gills of *Tilapia guineensis* from Buguma are shown in Table 2.

confirmation of the bacterial isolates obtained from the gills and skin of the fish samples. Table 5, on the other hand represent the percentage frequency of occurrence of the bacterial isolates from *Tilapia guineensis*.

Table 3. Cultural and Morphological Characteristics of bacterial isolates of *Tilapia guineensis* from Buguma, River State.

Bacterial isolates	Colonial characteristics	Cell morphology under microscope
<i>Proteus vulgaris</i>	Circular and convex with Swarming motility	Appeared as negative short rod
<i>Pseudomonas</i> sp.	Circular shape with raised undulate margin	Cell appears as asporogenous negative rod
<i>Flavimonas oryzihabitans</i>	Rough shape with yellow pigment	Appeared as negative rod.
<i>Salmonella</i> sp.	Translucent colony with black center	Appears negative straight rod.
<i>Aerobacter aerogens</i>	Shiny, convex colony with entire margins.	Appears negative short rod
<i>Serratia marcescens</i>	Pinpoint circular and mucoid colony with entire margins and umbonate elevation	Cells appear as negative short rod-shape
<i>Morganella morganii</i>	Circular shape with opaque colour	Appears negative straight rod
<i>Vibrio parahaemolyticus</i>	Circular blue-green colony	Appears negative curved-rod
<i>Vibrio metschnikovii</i>	Greyish, opaque colony	Appears slightly curved-rod
<i>Shigella</i> sp.	Small, circular convex, smooth and transparent colony	Appears negative short rod
<i>plesiomonas shigelloides</i>	Round ended, greyish, shiny colony with smooth surface	Appears negative straight rod.
<i>Escherichia coli</i>	Large, thick, greyish white, moist and smooth opaque colony	Appears negative short rod
<i>Providencia rettgeri</i>	Large, dull grey colony	Appears negative straight rod
<i>Kluyyera ascorbate</i>	Small circular colony with entire edge	Appears negative straight rod
<i>Citrobacter amalonaticus</i>	Low, smooth, convex and moist translucent colony	Appears negative rod

Table 4. Biochemical tests and Gram stain reaction of bacterial isolates of *Tilapia guineensis* from Buguma, River state.

Isolates Code	Organ	Gram reaction	morphology	Motility	Oxidase	Glucose	Lactose	Maltose
RM1	skin	-ve	rod	+	-	+	+	+
RM2	Skin	-ve	rod	-	-	+	+	+
RM3	Skin	-ve	rod	+	-	+	-	-
RF2	Skin	-ve	rod	+	-	+	-	-
RF1	Skin	-ve	rod	+	+	+	+	+
RF2	Gill	-ve	rod	-	-	+	-	-
RF3	Gill	-ve	rod	-	-	+	-	-
RM4	Gill	-ve	rod	+	+	+	-	-
RF4	Gill	-ve	rod	+	-	+	-	-
RF5	Gill	-ve	rod	+	-	+	-	-
RM5	Skin	-ve	rod	+	-	+	+	+
RM6	Gill	-ve	rod	+	-	+	+	+
RM7	Gill	-ve	Curve-rod	+	-	+	-	-
RF6	Skin	-ve	rod	+	-	+	-	-
RF7	Skin	-ve	rod	+	-	+	-	-
RM8	Skin	-ve	Curve-rod	+	-	+	-	-
RM9	skin	-ve	rod	+	-	+	+	+
RM10	skin	-ve	rod	+	-	+	+	+
RM11	Gill	-ve	Curve-rod	+	-	+	-	-
RM8	Gill	-ve	rod	+	-	+	+	+
RF9	Gill	-ve	rod	+	-	+	-	-
RF10	Gill	-ve	rod	+	+	+	+	+
RF11	Gill	-ve	rod	-	-	+	-	-

H ₂ S	Gas production	Citrate	Methyl red	Voges-proskauer	Indole	Nitrate reduction	Catalase	Organism isolated
+	-	+	+	-	+	+	+	<i>Proteus vulgaris</i>
+	-	+	-	-	-	-	-	<i>Pseudomonas</i> sp.
-	+	+	-	-	+	-	+	<i>Flavimonas oryzihabitans</i>
-	+	-	+	-	-	-	+	<i>Salmonella</i> sp.
+	-	+	-	+	-	+	+	<i>Aerobacter aerogenes</i>
-	+	-	-	-	-	-	-	<i>Shigella</i> sp.
-	-	+	-	-	-	+	-	<i>Providencia rettgeri</i>
-	+	-	-	-	+	-	+	<i>Plesiomonas shigelloides</i>
-	-	-	+	-	+	-	-	<i>E. coli</i>
-	+	-	+	-	+	-	+	<i>Morgenella morganii</i>
-	+	+	+	-	+	-	-	<i>Kluyyera ascorbata</i>
+	+	+	-	-	-	+	+	<i>Proteus mirabilis</i>
-	+	-	+	+	+	-	-	<i>Vibrio metschnikovii</i>
-	+	+	-	+	+	-	-	<i>Serratia marcescens</i>
-	+	-	+	-	+	-	+	<i>Morgenella morganii</i>
+	+	-	-	-	+	-	-	<i>Vibrio parahaemolyticus</i>
+	-	+	+	-	+	+	+	<i>Proteus vulgaris</i>
+	+	-	-	-	+	-	-	<i>Proteus vulgaris</i>
+	+	-	-	-	+	-	-	<i>Vibrio</i> sp.
+	+	+	-	-	-	+	+	<i>Proteus mirabilis</i>

H ₂ S	Gas production	Citrate	Methyl red	Voges-proskauer	Indole	Nitrate reduction	Catalase	Organism isolated
-	+	+	-	+	+	-	-	<i>Serratia marcescens</i>
+	+	+	+	-	-	-	-	<i>Citrobacter freundii</i>
-	-	+	-	-	-	+	-	<i>Providencia rettgeri</i>

Table 5. Frequency of occurrence of bacterial isolates in *Tilapia guineensis* from Buguma, River State.

Bacterial Isolates	Number of Occurrence	Percentage of Occurrence
<i>Proteus vulgaris</i>	6	16.22
<i>Pseudomonas sp.</i>	1	2.70
<i>Flavimonas oryzihabitans</i>	2	5.41
<i>Salmonella sp.</i>	3	8.11
<i>Aerobacter aerogenes</i>	1	2.70
<i>Serratia marcescens</i>	3	8.11
<i>Enterobacter aerogenes</i>	1	2.70
<i>Morganella morganii</i>	2	5.41
<i>Vibrio parahaemolyticus</i>	4	10.81
<i>Vibrio metschnikovii</i>	2	5.41
<i>Shigella sp.</i>	2	5.41
<i>Plesiomonas shigelloides</i>	1	2.70
<i>E. coli</i>	2	5.41
<i>Proteus mirabilis</i>	1	2.70
<i>Providencia rettgeri</i>	3	8.11
<i>Kluyvera ascorbate</i>	1	2.70
<i>Citrobacter amalonaticus</i>	1	2.70
<i>Citrobacter freundii</i>	1	2.70
Total	37	100

4. Discussion

Total bacteria count in brackish water sample, skin and gills of the *Tilapia guineensis* varies significantly. The bacterial load in each of the sample analyzed was high but does not exceed the standard microbiological recommended limit. The high bacterial load in each of the sample segment could be as a result of high temperature where the fish samples were collected which is close to optimum temperature for several mesophilic bacterial [10]. Total bacteria load in the gills ranged from 2.1 to 5.7×10^6 , 2.4 to 7.6×10^5 for the skin and 3.3 to 8.5×10^6 for the water sample. High bacteria load observed from this study is in agreement with the study of [11] who reported the presence of high bacterial load in tilapia gills, but in contrast with the work of Chessbrought [12], who reported that bacteria associated with the gills are actively maintained at low level to enable the fish keep the bacterial number low, and therefore afford the fish some degree of protection against bacterial invasion by the gills micro flora.

The bacterial flora from the water sample, skin and gills of *Tilapia guineensis* were majorly gram negative bacteria belonging to the family of enterobacteriaceae, Vibrionaceae and Morganellaceae, and it is a reflection of the bacteria composition from their environment. This finding is in agreement with an earlier report by Al-Harbi and Uddin [13] 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 *Tilapia guineensis* harvested from Buguma brackish water environment include: *Proteus sp.*, *Pseudomonas sp.*, *Flavimonas oryzihabitans*, *Salmonella sp.*, *Aerobacter*

aerogenes, *Serratia marcescens*, *Enterobacter aerogenes*, *Morganella morganii*, *Vibrio parahaemolyticus*, *Salmonella sp.*, *Vibrio metschnikovii*, *Shigella sp.*, *Providencia rettgeri*, *E. coli*, *Kluyvera ascorbate*, and *Citrobacter species*. Nganou Donkeng *et al.* [14] reported different bacterial species in their study of Characterization of bacterial flora of tilapia harvested from four lakes in the north of Cameroon. *Proteus sp.*, *Serratia marcescens*, *Vibrio species*, *Salmonella sp.*, *Morganella morganii*, *Providencia rettgeri* dominated other bacterial groups in the water sample, skin and gills of the *Tilapia guineensis* as shown in Table 2. However, the commensal bacterial flora from this fish is a facultative opportunistic pathogen which under stress could give rise to fish disease and could as well be zoonotic in nature. *Salmonella sp.*, *E. coli*, *Shigella sp.*, and *Vibrio sp.* have been implicated in so many fish borne infection of human.

A total of thirty-seven [37] bacteria, belonging to fifteen different genera in the family of Vibrionaceae, enterobacteriaceae and Morganellaceae were isolated from the fish sample [15].

The prevalence rate shows that *Proteus vulgaris* recorded the highest frequency of occurrence of 16.22%, followed by *Vibrio parahaemolyticus* with 10.81% occurrence and *Providencia rettgeri*, *Serratia marcescens* and *Salmonella sp.* trailed them with 8.11%. *Kluyvera ascorbate*, *Citrobacter amalonaticus*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Plesiomonas shigelloides* and *Pseudomonas sp.* have the least frequency of occurrence with 2.7% respectively for each. In conclusion, Though the bacterial count of *Tilapia guineensis* harvested from the water and fish sample did not exceed the standard recommended limit. There were several potential pathogenic and spoilage bacteria isolated as part of the fish

flora. The presence of these bacteria could poses or constitute a public health risk. This study showed that the *Tilapia guineensis* and the water sample from Buguma brackish water environment host a ridiculous and diverse bacterial flora. However, Microbiota of fishes and brackish water from Buguma, River State, Nigeria is mainly composed of Gram-negative bacilli.

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