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OCCURRENCE AND SENSITIVITY TO ANTIBIOTICS OF BACTERIA FOUND IN GILLS, BUCCAL CAVITY AND SKIN OF *Hemichromis fasciatus*, *Brycinus macrolepidotus* AND *Hydrocynus forskalii* FROM OGUN RIVER, ABEOKUTA, OGUN STATE

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ABSTRACT

The morphometrics of *Hemichromis fasciatus*, *Brycinus macrolepidotus* and *Hydrocynus forskalii* from Ogun River were determined. The culture, isolation, and characterization of bacteria species, their sensitivity to antibiotics were carried out. There were significant differences (P>0.05) in the weight, standard length, head length, gill length and buccal depth. The highest mean body weight 113.85 ± 9.38 g was observed in *B. macrolepidotus* while the lowest mean weight of 47.20 ± 6.3 g was observed in H*. forskalii*. Ten (10) bacteria species were isolated from the gill, buccal cavity and skin. These include the Gram positive (*Staphylococcus epidermidis*, *Streptococcus faecium, Micrococcus letus*) and the Gram negative bacteria (*Enterobacter aerogenes, Pseudomonas aerogenes, Klebseilla aerogenes, Aerobacter aerogenes, Proteus vulgaris, Bacillus licheniform and Salmonella sp*). The number of colony forming units per milligram varied from 3.1×10^4 to 3.8×10^5. The highest mean percentage bacteria occurrence of 5.4 ± 4.4 at (P> 0.05) was recorded from the gills of *H. fasciatus* while the lowest value of 4.6 ± 3.8 was recorded in the gill of *H. forskalii S. epidermidis* had the widest inhibition zones of 10.2 mm and 10.5 mm respectively of sensitivity to Sparfloxcin and Caflazidime while *P. vulgaris* had the thinnest inhibition zone 2.1 mm of resistance to Caflazidime. From the foregoing results, this study confirmed that the bacterial organisms isolated from the fish were pathogenic and of public health importance. Caflazidime and Oflaxacin were the best antibiotic substances with the highest inhibition zones of 10.5 mm and 10.0 mm for the Gram positive bacteria (*S. epidermidis*) and the Gram negative bacteria (*E. aerogenes*) respectively.

Keywords: Bacteria, Ogun River, sensitivity, antibiotics and morphometrics

INTRODUCTION

Fresh water is a complex ecosystem consisting of microbial organisms as well as species of great diversity and different importance. Ita and Sado (1985) established total Nigeria Inland water mass to be about 13 million hectares but this has increased over the years as a result of new dams. Associated with this enormous water mass is a vast and varied assemblage of fish stocks and other aquatic organisms which support huge artisanal and culture fishery. FDF (2007) estimated Nigeria fish resources as well as potential yield estimate per annum from rivers, flood plains, pools and reservoirs as 226550 metric tonnes. Among the commercially im-

portant fresh water fish genera in Nigeria are the *Tilapia, Lates, Chrysichthys, Mormyrus, Lutjanus, Hemichromis, Hydrocynus* and *Brycinus*. Mostly Cichlids and Characids are abundantly common in rivers and reservoirs such as Arankanga Reservoir, along Ogun River in Abeokuta, Ogun State. Ayansanwo (1999) also reported that it occupies an approximate size of 10.0 hectares managed for the purpose of water supply and fishing. These species of fish depending on the size, Epiya *(Hemichromis fasciatus*), Aga (*Hydrocynus forskalii*) and Kranpo (*Brycinus macrolepidotus*) in Yoruba vernacular language are of high economic value in Nigeria.

Microorganisms known are viruses and bacteria, however, certain algae and fungi can also be considered as microorganisms because they are microscopic in size (Jay, 1986 and Akinyemi, 2001). Microorganisms occurrence in captured fisheries of tropical waters and infection compared with fishes from the temperate waters have been well recognized (Sarig, 1976; Ballarin and Hatton, 1979). Essentially, bacteria may occur in fresh water bodies as pathogenic, natural and spoilage organisms.

Fish is reservoir for large number of microorganisms (Sowunmi, *et al.,* 2008). Some are inherent, coming from where the fish is caught, and others are traced to contamination at various stages of handling, from the time of catch until it reaches the consumer. Majority of these organisms are non pathogenic, causing only spoilage of fish, but there are some which are pathogenic causing food poisoning. Quality standards have been prescribed for the fish and fishery products meant for export and they needed to be monitored strictly (Ashokkumar, 2008). Bacteria are unicellular microscopic organisms smaller in size than mould or yeasts and are ubiquitous in their occurrence. They occur in air, on the land, in lakes, in oceans, in the intestinal tracts of man and animals. They eat dead organic matter while few even cause diseases in human, animals and plants (Bisen and Verma, 1994). Bacterial organisms may cause extensive losses of captured fish populations and mortalities caused by bacteria are often chronic rather than acute, but may also cause high percentage of death. Nevertheless, bacteria are among the most important pathogens of fish. Bacterially caused mortalities are frequently associated with environmental stress as the causative organisms are usually saprophytic, facultative or opportunistic and ubiquitous (Lewis and Plumb, 1979). Under natural water environment, microbes such as bacteria occur in both man made and natural fresh water bodies as pathogenic, natural and spoilage micro organisms. It is important to study their occurrence on these commercially important fish species with the intention to investigate the tendency to cause food poisoning or death from the consumption of fish (Wei *et al*, 2008).

The general objective of the study is to provide information on bacteria flora from gills, buccal cavity and skin of *Hemichromis fasciatus, Hydrocynus forskalii* and *Brycinus macrolepidotus*. The specific objectives are to determine the morphometrics of fish samples, culture and isolate bacteria stock from the fish species and to determine the sensitivity / resistance of bacteria isolates to specific antibiotics.

MATERIALS AND METHODS

The study was carried out at Arakanga Reservoir. It has its source from Arakanga Stream along Ogun River. It is located at Iberekodo, Abeokuta North Local Government Area of Ogun State. It lies between Long; 3^0 21' S and Lat; 7^0 12' E, North of

Abeokuta (Ogun State Bureau of Land and Survey).

Collection of samples

Live fish samples were procured from Arakanga Reservoir. Bacteria isolates from each fish sample were obtained from the gills surfaces, buccal cavity and skin by swabbing method with the use of sterile cotton swabs aseptically. All swabs were then inoculated into universal bottles containing 10 ml of saline water as a transport media. The universal bottles were then arranged and preserved in an ice box with block-ice. Cultures and sub-cultures of bacterial isolates were carried out within 7 to 12 hours of sample collection. The standard length, head length, gill length and buccal depth in centimeters (cm) were measured and recorded after weighing the fish samples in grams (g).

Microbiological analysis

All the universal bottles containing the stock culture from swabs were microbiologically analyzed for the gill, buccal cavity and skin. The media used in the experiment were prepared according to the manufacture's directions; these include Nutrient agar, MacConkey and Blood agar. The agar media were allowed to cool to 45^0C before dispensing aseptically into sterile plastic Petri dishes.

Preparation of sample for serial dilution

The original stock culture was serially diluted. 1 ml of the original stock solution was aseptically poured into 9 ml sterile distilled water and mixed thoroughly to give 10^{-1} dilution of original stock culture. 1 ml of dilution $10^{-1}$of the original stock culture was poured into another tube of 9 ml sterile distilled water to give 10 ml of 10^{-2} dilution of the original stock culture. Repeating the above procedure, dilutions of 10^{-2}, 10^{-6}, 10^{-8} and 10^{-10} were obtained.

Microbial isolation of different bacteria

Nutrient agar was poured into sterile Petri dishes and allowed to solidify. Using the pour plate method, a volume of 0.1 ml of dilution 10^{-2} and 10^{-6} was poured on the already solidified nutrient agar using a sterile 1 ml pipette. Ten plates were inoculated for each dilution. The plates were incubated at 37^0C for 24 hours. After incubation, different developed colony was transferred to fresh corresponding agar plate for purification of isolates. Pure cultures were stocked in agar slant and kept in the refrigerator for identification; the same procedure was repeated for MacConkey and Blood agar.

Viable bacterial count on nutrient agar

The pour plate method was employed. 0.5 ml of dilution 10^{-1} of the stock culture was introduced into each of ninety sterilized nutrient agar plates. Sterilized molten nutrient agar at 45^0C was added and then mixed thoroughly and allowed to set undisturbed. The set agar plates were incubated at 37^0C for 24 hours. This procedure was repeated using dilutions 10^{-2}, 10^{-6}, 10^{-8}, and 10^{-10}. Finally, the number of colonies per plates were counted and recorded.

Identification

All the isolates were transferred from the slants into appropriate agar plates, incubated appropriately and used for identification such as the cultural and morphological characteristics, biochemical and antibiotics sensitivity tests.

Antibiotics sensitivity test

Nutrient agar was prepared according to the manufacturer's specification and it was sterilized at 121^0C for 15 minutes at 1.06 mmHg. The bacteria organisms kept on nutrient agar

slant were retrieved and streaked on a fresh nutrient agar plates, an antibiotic disc was introduced into each nutrient agar plates and these were incubated at 37^{0}C for 24 hours. After sometime a clear zone (inhibition zone) was observed and the diameter measured.

RESULTS

Table 1 indicate that *B. macrolepidotus* had the highest mean value in weight and standard length and buccal depth 113.85 ± 9.38 g, 17.82 ± 0.55 cm and 4.10 ± 0.14 cm. *H. forskalii* had the highest head length and gill length 4.46 ± 0.53 cm and 3.33 ± 0.45 cm. Table 2 indicate that *H. forskalii* gill had the highest range of viable bacteria count 1.2×10^5 to 3.8×10^5. *H. fasciatus* buccal cavity had the lowest range of viable bacteria count 1.7×10^4 to 3.1×10^4. Table 3 indicate that *Proteus vulgaris* had the highest percentage of occurrence 10 %, 8 %, 8 %, 9 %, 9 %, 10 %, 10 %, 9 % and 10 % respectively in the gill, buccal cavity and skin of *H. fasciatus, B. macrolepidotus and H. forskalii. Salmonella sp.* recorded the least percentage occurrence of 1 % in the *H. fasciatus* while nothing was detected in the gills, buccal cavity and skin for other fish species. Table 4 indicate that *S. epidermidis* had the widest inhibition zone of sensitivity to Gentamicin and Sparfloxacin 9.5 and 10.2 mm respectively. *E. aerogenes* had the widest inhibition zone of sensitivity to Oflaxacin and Ciprofloxime 10.0 mm and 9.1 mm respectively.

However, *P. vulgaris* had the thinnest inhibition zone of resistance to Caflazidime 2.1 mm and *Salmonella sp.* had less inhibition zone of resistance 4.6 mm to Sparfloxacin.

Table 1: Summary of the morphometrics characteristics

Morphometrics	H. *fasciatus*	B. *macrolepidotus*	H. *forskalii*	F *statistic*
Weight (g)	47.20 ± 6.3^a	113.85 ± 9.38^b	83.79 ± 2.70^c	30.44±7.58
Standard length (cm)	9.63 ± 0.48^a	17.82 ± 0.55^b	17.29 ± 2.38^b	57.47±0.98
Head length (cm)	2.89 ± 0.25^a	4.02 ± 0.48^b	4.46 ± 0.53^b	17.40±0.50
Gill length (cm)	2.67 ± 0.15^a	2.94 ± 0.14^{ab}	3.33 ± 0.45^b	5.50±0.35
Buccal depth (cm)	3.97 ± 0.29^b	4.10 ± 0.14^b	3.11 ± 0.45^a	6.29±0.38

$^{a, b, c}$ Means along the same row with different superscripts are significantly different at ($P < 0.05$)

Table 2: Summary of viable bacteria count (cfu/ml in log_{10})

Fish Species	Body Parts	Range
H. fasciatus	Gill	$1.4\times10^4 - 3.1\times10^5$
	Buccal cavity	$1.7\times10^4 - 3.1\times10^4$
	Skin	$1.7\times10^4 - 3.3\times10^5$
B. macrolepidotus	Gill	$2.0\times10^4 - 2.9\times10^5$
	Buccal cavity	$3.1\times10^4 - 9.2\times10^4$
	Skin	$1.1\times10^5 - 2.8\times10^5$
H. forskalii	Gill	$1.2\times10^5 - 3.8\times10^5$
	Buccal cavity	$3.3\times10^4 - 2.5\times10^5$
	Skin	$2.2\times10^4 - 3.1\times10^5$

Table 3: Percentage occurrence of bacteria flora from gill, buccal cavity and skin

Bacteria species	H. *faciatus*			B. *macrolepidotus*			H. *forskalii*		
	G	B	S	G	B	S	G	B	S
S. epidermidis	2	ND	1	1	1	1	1	3	ND
Enterobacter aerogenes	5	5	4	6	6	5	1	ND	4
Pseudomonas aerogenes	1	2	3	ND	ND	ND	ND	7	1
Klebsiella aerogenes	8	5	4	ND	ND	4	7	4	5
Micrococcus letus	5	7	4	8	7	8	3	3	5
Streptococcus faecium	4	3	3	5	8	ND	2	1	7
Bacillus licheniform	ND	4	2	3	1	9	ND	3	2
Aerobacter aerogenes	ND	1	1	1	3	4	2	3	3
Proteus vulgaris	10	8	8	9	9	10	10	9	10
Salmonella sp	1	ND	ND	ND	ND	ND	ND	ND	ND
Mean bacteria counts (cfu/ml) in log10	5.4 ± 0.4^b	5.2 ± 0.5^b	5.2 ± 0.6^a	5.3 ± 0.5^{ab}	4.9 ± 0.3^a	5.3 ± 0.5^a	5.2 ± 0.3^a	4.6 ± 0.8^a	5.2 ± 0.1^a
F statistics	2.50±0.65			9.41±0.56			0.41±0.06		

Key: G = gill, B = buccal cavity, S = skin ND=Not Detected
[a,b,c,] Means along the same row with different superscripts are significantly different at $P<0.05$

Table 4: Zone of inhibition of bacteria isolates to anti-biotics (mm)

Organisms	Gentamicin	Oflaxacin	Sparfloxacin	Ciprofloxime	Caforaxime	Caflazidime
S. epidermidis	9.5	5.4	10.2	8.0	9.0	10.5
P. aerogenes	8.8	5.0	4.5	9.0	8.2	9.0
P. vulgaris	7.0	4.6	8.2	9.0	8.2	2.1
K. aerogenes	6.5	8.5	8.5	8.0	8.6	4.0
E. aerogenes	6.2	10.0	7.1	9.1	9.0	6.0
A. aerogenes	8.0	5.0	8.2	8.0	7.8	4.0
S. faecium	6.2	8.5	7.1	6.0	7.0	9.2
Salmonella sp.	6.0	5.2	4.6	8.0	9.0	8.0
B. licheniform	7.0	5.0	8.0	6.0	8.0	6.4
M. letus	6.1	8.0	6.2	8.4	7.1	6.4

The morphometric features evaluated were significantly different (P>0.05) except for gill length of *B. macrolepidotus* with double superscripts 2.94 ± 0.14^{ab}. The highest mean weight (g), standard length (cm), head length (cm), gill length (cm), and body depth (cm) at (P<0.05) were $11.3.85 \pm 9.86^{b}$, 17.82 ± 0.55^{b}, 4.02 ± 0.48^{b}, 2.94 ± 0.14^{ab} and 4.10 ± 0.14^{b} respectively. Sowunmi *et al.,* (2008) also compared these morphometric features of *Clarias gariepinus* and *Tilapia zilli* from Lekki lagoon, South West Nigeria and recorded these values, weight 7.84 ± 20.06 and 60.67 ± 13.84 g; standard length 19.99 ± 2.51 and 11.50 ± 18.55 cm; head length 4.02 ± 0.43 and 4.16 ± 0.40 cm; gill length 3.43 ± 0.31 and 3.18 ± 0.40 cm; body depth 2.17 ± 0.68 and 1.76 ± 0.21 cm respectively.

In this study a total of Ten (10) species of bacteria were recovered from the gills, buccal cavity and skin of *H. fasciatus, B. macrolepidotus and H. forskalii*. Seven (7) out of the isolated bacteria species were Gram negative *Enterobacter aerogenes*, *Pseudomonas aerogenes, Klebseilla aerogenes, Aerobacter aerogenes, Proteus vulgaris, Bacillus licheniform and Salmonella sp.* 68 %. This also confirmed the findings of Ahmed and Naim (2002) who reported that the bacteria identified from the brackish pond water, sediment, gills and intestine of healthy Tilapia cultured in Saudi Arabia were predominantly Gram negative rods 87 %. Sowunmi, *et al., (*2008) isolated highest percentage of *Pseudomonas flourescens* 50 % and *Proteus sp* 24 % from the gills and buccal cavity of *Clarias gariepinus* and *Tilapia zilli.* This was not in support of this study that recorded the highest 83 % for *Proteus vulgaris*.

The highest viable bacteria count of 3.8×10^{5} Cfu/ml was observed for the gills of *H. forskalii* and 3.1×10^{5} Cfu/ml for *H. fasciatus* .The lowest viable bacteria count of 3.1×10^{4} Cfu/ml was recorded for the buccal cavity of *H .fasciatus* and 9.2×10^{4} Cfu/ml for *B. macrolepidotus*. This is in support of the argument of Jara and Chodyniecki (1999) that fish gills come into direct contact with the environment and any of the pathogen. It might harbor and provide a unique niche for many bacteria and parasite present in water. Although the gill protected by a single layered epithelium and a layer of mucus which neutralizes the impact of bacteria and fungi present, the gills are particularly susceptible to pathogen. Ashokkamar, (2000) observed *Esheriachia coli* total plate counts

(TPC) from dried fishes and recorded the highest value 3.50×10^5 Cfu/g for *Sardinella fimbriata* and lowest value 2.5×10^3 Cfu/g for *Upeneus spp.* in South East Coast of India.

In terms of mean percentage bacteria occurrence; there was no significant difference ($P>0.05$) in the gills of *H. fasciatus and H .forskalii* 5.4 ± 4.4^b and 5.3 ± 4.5^b respectively. *B. macrolepidotus* indicated both super scripts 5.3 ± 4.5^{ab}. Sowunmi *et al.,* (2008) recorded the mean percentage of the diversity and incidence of bacteria from gills and the buccal cavity of *C. gariepinus* and *Tilapia zilli* 7.94 ± 0.29 and 7.85 ± 0.24; 6.65 ± 0.38 and 6.62 ± 0.35 respectively. *Proteus vulgaris* recorded the highest mean percentage bacteria occurrence 83 %. This could be as a result of the presence of sewage, waste from abattoir or manure in the reservoir. *Salmonella sp.* recorded the lowest value 1%. This is in accordance with the fact that the principal habitat of *Salmonella sp* is intestinal tract of man and animals (including fish).

From the result, the morphological characteristics showed that three bacterial isolates *Staphylococcus epidermidis, Micrococus letus* and *Streptococcus faecium* were Gram positive while *Enterobacter aerogenes, Pseudomanas aerogenes*, *Proteus vulgaris* and *Salmonella sp.* were Gram negative. The fermentative characteristics indicated that *S. epidermidis, P. aerogenes, K. aerogenes*, *Streptococcus faecium* and *B. licheniform* fully produced acid while *Salmonella sp*. partially produced acid from utilizing all the sugars. On the other hand *E. aerogenes, Aerobacter aerogenes* and *Proteus vulgaris* produced acid as well as gas. Catalase test and Starch hydrolysis were highly tested positive by *S. epidermidis, E. aerogenes, P. aerogenes*, *M. letus, S. faecium, B lichenform, A. aerogenes,* and *P. vulgaris* while none of the bacteria isolate tested positive to Coagulase test.

The result of the present study revealed the prevalence of the highest antibiotic sensitivity (i.e. the widest inhibition zone) in Gram positive bacteria isolated from all the spots of the fish, *S. epidermidis* at 10.5 mm diameter to Caflazidime. *E. aerogenes* recorded 10.0 mm diameter to Oflaxacin and *Streptococous faecium* at 9.2 mm diameter to Caflazidime. The highest antibiotic resistance (i.e. the thinnest inhibition zone) was observed in Gram negative bacteria *Proteus vulgaris* at 2.1 mm diameter to Caflazidime. Ashokkumar (2008) recorded the highest sensitivity of 18 mm diameter clear zone to Coramphenicol and the lowest sensitivity of 8 mm to Bacitracin for *E. coli.*

CONCLUSION

This work provided information on the bacterial flora from the gill, buccal cavity and skin of commercially important fresh water fish species such as *H. fasciatus*, *B. macrolepidotus* and *H. forskalii* which support huge artisanal and culture fisheries in Nigeria. Hence, this study confirms the existence of pathogenic bacterial organisms which are of public health importance. The findings have confirmed that fish can be infected with variety of microbial species especially those of bacteria in the freshwater environment. The isolates from the gill, buccal cavity and skin can be accounted for mainly by the filter effect of the gill, the feeding habit or the slime layer of the skin, also partly as a result of the active bacteria multiplication and adaptation. The isolates have the potentials to cause serious infections to fish, to the animals that feed on them and finally to man. Caflazidime and Oflaxacin were the best antibiotic substances with the highest inhibition zones of 10.5 mm and 10.0 mm for the Gram positive bacteria (*S. epidermidis*) and the Gram negative bacteria (*E. aerogenes*) respectively.

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