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OCCURRENCE AND SENSITIVITY TO ANTIBIOTICS OF BACTERIA FOUND IN GILLS, SKIN, BUCCAL CAVITY OF MORMYRUS RUME, LABEO OGUNENSIS, AND OREOCHROMIS NILOTICUS IN OGUN RIVER

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ABSTRACT

Specimens of *Mormyrus rume*, *Labeo ogunensis and Oreochromis niloticus* collected from Ogun River were examined for bacterial flora from the gills, skin, and buccal cavity of these species. The different bacteria isolated were tested for their sensitivities to different antibiotics. A total of nine (9) bacteria were identified (*Staphylococcus aureaus, Proteus mirabilis, Klebsellia pneumonia, Pseudomonas aerogenosa, Escherichia coli, Proteus vulgaris, Enterobacter aerogenes, Serretia marcescens* and *Salmonella spp*) and incidence of the bacteria count of *Mormyrus rume* was highest in the skin (5.00±0.60, f<0.05) than gills (3.60±0.98, f<0.05) and buccal cavity (1.22±0.76, f<0.05), in *Labeo ogunensis* bacteria count was highest in skin (5.30±0.70, f<0.05) than gills (4.80±0.80, f<0.05) and buccal cavity (3.70±1.07, f<0.05), in *Oreochromis niloticus* bacteria count was also highest in skin (6.20±0.83, f<0.05) than gills (5.95±0.79, f<0.05) and buccal cavity (4.54±0.78, f<0.05). it was noticed that in all nine (9) bacteria, Gentamicin and Ceforoxime were most sensitive but at different levels, while all bacteria isolated are most resistant to Caftazidime followed by Oflaxacin.

Keywords: Antibiotics, Bacteria, Occurrence, Sensitivity, Reservoir and Ogun River

INTRODUCTION

Ogun River is one of the perennial rivers in Nigeria with characteristics synchronizing typical tropical climate. It has coordinates of 3º28"E and 8º41"N from its source in Oyo state to 3º25"E and 6º35"N in Lagos where it enters the Lagos lagoon. (Sowunmi *et al,* 2004). Fish is a consumable and in high demand because of its nutritional components, this is majorly in form of proteins. Fish is affordable and in demand both in the upper and lower living class, this allows the introduction of various forms of fish products; in the bid to meet up with the demand of the economy, the necessary steps in hygiene will be over looked. In such situations, because fish is easily decomposable it will begin degrading there by allowing the quality of the fish reduced and also if consumed could cause food poisoning. The primary attention of the bacteriologists around the turn of the century tended to be focused on human and animal diseases. An extensive investigation of the natural occurrence of enteropathogenic bacteria in fish and other aquatic animals started with the recognition of the involvement of shellfish as carriers of human enteric diseases. Interest in fish by clinical or health-related bacteriologists through continuous attention paid to problems of mussels and oysters (Connell, 1980). Since bacteria are living things they require a source of food, moisture and suitable temperature to grow. When these conditions are adequate, bacteria will grow by a process known as binary fission in which one cell divides into two new cells (Eyo, 2001). The bacteria of fish are mostly psychrophilic, growing between 0^{o}C and about 30^{o}C.. The very large effect in growth rate of temperature changes near 0^{o}C as contrasted to comparable changes at higher

temperature ranges was noted (Connell, 1980). The bacteria in fish intestines are somewhat depending on the food being consumed but normally contain *Vibrio, Achromobacter, Pseudomonas*, and *Peromonas* in addition to smaller numbers of gram-positive bacteria including *Clostridium.* Antimicrobial drugs can either be antibiotics or chemical antimicrobials (chemotherapeutic agents). Anti microbial substances produced by living micro-organisms are antibiotics. They can be culture extract and filtrates of fungi such as Penicillium and bacteria such as *Bacillus spp.* Antimicrobials act on bacteria in various ways by inhibiting cell wall formation leading to cell lysis e.g penicillin, changing the bacterial cell membrane, leading to loss of cell contents and to cell death e.g Polymyxins, inhibiting protein production and therefore arresting bacterial growth e.g Tetracycline, and inhibiting the production of nucleic acids and therefore preventing bacteria from reproducing e.g nalidixic and prevents DNA synthesis (Cheesebrough, 1984).The aim of this study is to provide information on the significance of morphometrics of fish species to the occurrence and prevalence of bacteria flora from the gills, buccal cavity, and skin of *Mormyrus rume, Labeo ogunensis,* and *Oreochromis niloticus* from Arakanga reserviour on river Ogun.

MATERIALS AND METHODOLOGY

All samples were taken either freshly dying or alive from *M. rume, L. ogunensis,* and *O. niloticus* from Ogun River, Arakanga reserviour situated at Akomoje water scheme. The reserviour is used to supply water to the neighbouring areas and also for fishing. it is divided into two sections; the upper stream section dammed at a depth of 30 feet that supplies the water scheme and the lower stream section is used for washing, bathing and more human activities by the locals. This flows through to Lafenwa, along this route there are activities like agriculture, animal slaughter (abattoir) and the likes.

COLLECTION OF SAMPLES

On the field, identification of fish samples and morphometrics; standard length, head length, gill length, and buccal depth in centimetres (cm) were measured after weighing the fish specimen in grams (g). Samples were swabbed from three spots on each fish i.e. skin, gills, and mouth from Arakanga old water scheme dam. When fish species were caught, the swab was done with a swab stick and sealed back into the case carefully taking note of timing because without using a transport media the samples need to get to the laboratory in less than an hour for immediate bacteriological analysis.

ANALYSIS OF SAMPLES

Each swab stick was initially cultured on Nutrient agar for growth and subcultured on MacConkey Agar, Blood Agar and Chocolate Agar and incubated at 37^0C for 24 hours. The organisms were identified using the biochemical tests such as: Catalase, Coagulase, Citrate Utilization, Urease, Nitrate Reduction, Indole Reaction, Oxidase tests etc were carried out according to Akinyemi, 2001 to identify and confirm the presence of the suspected micro-organism by their reaction to the tests.

SENSITIVITY TEST

A total of six different antibiotics were selected based on their effect on the bacteria. Each set (6 discs) was imbedded in a plate of pure isolates with specific bacteria and observed for action after incubating for 24 hours at room temperature. After sometime (30

minutes), the diameter of inhibition zones were measured and interpreted as sensitive or resistant or intermediary sensitive using a common disc diameter value as 2.0mm. Therefore results are inclusive of the antibiotic discs diameter.

RESULTS

The specimen were measured for varying morphometric parameters, and from the result, it was found out that in all parameters except weight and head length, all the three species were significantly different ($P<0.05$). In weight, *M. rume* and *L. ogunensis* had no significant difference but were both found to have significant differences to *O. niloticus* while with the head length measurement*, L. ogunensis* and *O. niloticus* had no significant difference but were both significantly different to *M. rume* (Table 1). Viable count of bacteria growth isolated from the specimen was done on each species and it was revealed that *O. niloticus* had the highest number of growth in general, followed by *L. Ogunensis* and with the lowest number of growth in *M. rume* and the highest indication of no growth (Table 2). Incidence of bacteria flora on buccal cavity, gills and skin of the nine (9) identified bacterial species (*S. aureaus, P. mirabilis, K. pneumonia, P. aerogenosa, E. coli, P. vulgaris, S. marcescens, Salmonella spp* and *Enterobacter aerogenes*) is presented in Table 3 and this further confirms what was earlier mentioned that the most number of growths was found from *O. niloticus* and least number of growths found in *M. rume*. Statistical analysis further proves that the skin of *O. niloticus* (6.20±0.83) recorded higher incidence of isolated bacteria species with the exception of *Proteus* sp, *Salmonella* sp and *Enterobacter aerogenes* (Table 3). In Table 4, antibiotics used were selected based on their different effects on the bacteria. Gentamicin and Ceforoxime were found most active because all the bacteria isolated was either sensitive or intermediately sensitive to them and while Ofloxacin and Caftazidime were found least active in bacteria like *Proteus vulgaris*, Sparfloxacin and Caftazidime were found least active in *Proteus mirabilis* and Caftazidime most active for *staphylococcus aureaus*.

DISCUSSION

This study was carried out with the purpose of knowing if the bacteria assemblage was of public health significance amongst others also to know the morphometric parameters gotten and relating it with the incidence and diversity of bacteria to imply that smaller specimen are more liable to infections and observations indicating that *O. niloticus* as the smallest recorded higher infection than the other specimen. The correlation of morphometrics to occurrence of bacteria in this study has proven the above notion the smaller sized fishes are susceptible to a higher bacterial occurrence and this could be depending on the fact that the smaller fishes are found in shallow or sedimentary part of the water body while the larger sized fishes are majorly found in the water column. In explaining this better, we can refer to the result showing that pertaining to weight there is a significant difference between *O. niloticus* which also further confirms the difference in weight as a factor in the bacterial load in the different species. The incidence of bacteria on the skin compared to other spots of reference is dependent on the interaction of the skin with the water; also increased surface area can encourage the multiplicity of the bacteria. According to the diversity of bacteria found in the skin, gills and buccal cavity, in comparing the result gotten, we can summarise therefore that despite the small size of *O. niloticus* the bacterial load was found higher in the skin. Thus, explaining the morphometric parameters as it affects the bacteria occurrence could be dependent on

certain characteristics common to the species; this can be type of feeder, where the species are mostly found. The interaction of the skin of fish with water is dependent on the water influent and effluents. Generally, environments contaminated with human and animal excreta, excessive use of fertilizer on near by farms, decaying dried grasses, wastes from abattoirs and so on, have a high bacterial load and this also affect the bacterial count on the skin of the fish species dwelling in that water. According to Quenum (2003), there is in 1 gram of faecal material, 10 million viruses, 1 million bacterial, 1 million cysts and a hundred eggs of worms; unfortunately most people are not aware of the link between faecal contamination and occurrence of intestinal worms, diarrhoea, skin troubles, cholera and more. In tracing the source of the bacteria present in water we should endeavour not to disregard other environmental factors like the water quality and seasons: According to Seong Wei *et al*. (2008), many factors could contribute to bacterial infection like poor water quality, crowding, transportation and inadequate nutrition. Horsley (1976) explained that the need to monitor water quality with base-line knowledge might provide an advanced indication of the presence of bacteria's potential to produce diseased conditions. Shewan (1961) proposed the hypothesis that the bacterial flora of fish is a reflection of their environment, Horsley (1973) later in a study confirmed the hypothesis. Temperature, salinity, dissolved oxygen concentration and pH are examples of environmental factors that will influence the bacterial population within the water mass and cause physiological stress to fish. Any influence on the bacterial population present on the skin of fish is the same for that of the gills because the gill comes in contact with particles in water also.

The Southern Nigerian river systems are usually perennial with very large volumes resulting from run off from the systems catchments areas during flood. The increase in the volume of water enables wide dispersal of both the parasites and its host there by reducing the contact between them. In a study by Horsley (1973), a higher bacterial count on the skin of salmon from an upland water sampling pool was attributed to turbulence caused by rainfall, so while during high rainfall there is a wider dispersal between host and parasite, there is an increase in the bacterial content of the water.
From the health point of view, the most important characteristics of good quality water and its resource is an absence of pathogenic organisms or if present, it should be the micro-organism that is easily destructible, not like *Vibro spp* or C*lostridium spp.* The presence of some bacteria can cause food poisoning, cholera, gastroenteritis, respiratory infection, typhoid fever amongst others; when not properly processed or cooked before consumption for man. The presence of these bacteria in the species may be indicative of similar diet, feeding habits and patterns amongst the fresh water fishes. Stratification in the occurrence of these bacteria of public health concern was recorded with more organisms occurring in the sediment than in the pond water column. The result reveals that the pond sediment contains about 5 times the number of bacteria than in the water column (Okpokwasili and Ogbulie, 1993). This can be related that the fish dwelling in the sediments of the water will have a higher bacterial load than those dwelling in the water column. Although, pathogenicity was not the aim of this study, it was found that a lot of the bacterial species encountered are no doubt potentially pathogenic in different fish species under certain condition as reported for *Pseudomonas spp* and *Staphylococcus spp* (Varvarigos, 1997). In past studies, antibiotics have been used to know the sensitivity of bacteria to them so as to be able to use the antibiotics to treat the disease in fish. In this

study, a variety of antibiotics were chosen depending on their characteristics, Ceforoxime and Caftazidime are cephalosporin and act on the cell wall of the bacteria, Ofloxacin, Sparfloxacin and Ciprofloxacin have Quinoline while Gentamicin acts on the protein. In the results Gentamicin and Ceforoxime were found most active possibly because of the presence of the factor that helped disable the bacteria and allowing it to be sensitive to the antibiotics.

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Table 1: Summary of morphometrics for *M. rume, L. ogunensis, O. niloticus*
a, b, c means of the same row with different superscripts are significantly different (P<0.05)
Wt-Weight; SL- Standard Length; HL-Head Length; GL-Gill Length; BD-Buccal Depth

Morphometrics	*M. rume*	*L. ogunensis*	*O. niloticus*	F. value
Wt (g)	77.15± 5.81[a]	68.7±6.40[a]	51.96±4.39[b]	5.24
SL (cm)	20.51±0.39[a]	13.26±0.50[b]	8.17±0.42[c]	199.45
HL (cm)	2.65±0.24[b]	3.93±0.10[a]	3.80±0.06[a]	20.92
GL (cm)	2.37±0.05[c]	3.18±0.07[a]	2.63±0.07[b]	44.39
BD (cm)	1.88±0.08[b]	2.56±0.06[a]	1.62±0.04c	60.24

Table 2: Viable count of bacteria growth isolated from specimen of three spots each from each species (cfu/g).

Species	Spots	Viable count
Oreochromis niloticus	GILLS	$1.5\times10^5 - 8\times10^5$
	MOUTH	1.2×10^5- 9×10^5
	SKIN	2×10^5- 9×10^5
Labeo ogunensis	GILLS	3×10^5- 8×10^5
	MOUTH	2×10^5 - 9×10^5
	SKIN	2×10^5 - 9×10^5
Mormyrus rume	GILLS	3×10^5 - 9×10^5
	MOUTH	1.2×10^5- 7×10^5
	SKIN	2×10^5 - 8×10^5

Table 3: Diversity and incidence of bacterial flora found in the mouth, gills and skin of *Mormyrus rume, L. Ogunensis, O. niloticus*

Species	*Mormyrus rume*			***LABEO OGUNENSIS***			*Oreochromis niloticus*		
	Mouth	Gill	Skin	Mouth	Gill	Skin	Mouth	Gill	Skin
Staphylococcus aureaus	0%	10%	10%	30%	20%	10%	40%	40%	50%
Proteus mirabilis	10%	10%	0%	0%	30%	40%	0%	10%	0%
Klebsiella pneumonia	0%	10%	50%	30%	10%	30%	40%	30%	10%
Pseudomonas aerogenosa	0%	30%	30%	40%	70%	50%	100%	70%	100%
Escherichia coli	20%	30%	40%	20%	40%	50%	10%	10%	0%
Proteus vulgaris	0%	10%	0%	20%	10%	0%	0%	0%	0%
Enterobacter aerogenes	0%	10%	20%	0%	10%	0%	0%	0%	0%
Serretia marcescens	0%	10%	0%	20%	20%	0%	40%	30%	10%
Salmonella spp	0%	20%	20%	10%	30%	50%	0%	0%	0%
Mean bacterial count (cfu)	1.22±0.76	3.60±0.98	5.00±0.60	3.70±1.07	4.80±0.80	5.3±0.70	4.54±0.78	5.95±0.79	6.20±0.83
F statistics	1.86			3.83			7.65		

TABLE 4: SENSITIVITY PATTERN OF ISOLATED ORGANISMS TO SOME ANTIBIOTICS

Organism	Gentamicin	Ofloxacin	Sparfloxacin	Ciprofloxacin	Ceforoxime	Caftazidime
Staphylococcus aureaus	9.0	5.0	10.0	8.0	9.0	10.5
Pseudomonas aerogenosa	8.4	4.0	4.5	8.0	8.0	9.0
Proteus mirabilis	9.5	8.5	5.0	10.5	10.1	2.0
Proteus vulgaris	7.0	4.0	8.0	9.0	8.0	3.0
Escherichia coli	6.0	7.0	9.0	6.0	7.0	6.0
Serretia mercescens	7.0	4.5	9.0	7.0	7.0	6.0
Klebsiella pneumonia	6.5	8.5	8.5	8.0	8.5	4.0
Enterobacter Aerogenes	6.0	10.0	7.0	9.0	9.0	9.0
Salmonella spp	6.0	8.0	7.0	4.0	6.5	3.0

*The above results are inclusive of the diameter of the antibiotic disc, assuming the disc has a diameter of 2.0mm