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Occurrence of Bacteria Found in Gills, Skin, Buccal Cavity of *Lutjanus agennes*, *Pseudotolithus elongatus* and *Sphyraena barracuda* from Lagos Lagoon, Nigeria

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ABSTRACT

The diversity of bacteria flora in skin, buccal cavity and gills of *Lutjanus agennes, Pseudotolithus elongatus, Sphyraena barracuda* from of Lagos lagoon were examined and compared. Different identification procedure and techniques (colonial morphology, isolation and culturing) were used to determine if pathogenic bacteria groups were present. Gram staining, motility, sugar fermentation as well as biochemical tests were also carried out. The bacteria isolated were *Klebsiella* spp. *Proteus* spp. *Staphylococcus aureus, Citrobacter* spp. *Streptococcus pyogenes, Salmonella* spp. *Micrococcus spp. Bacillus licheniformis, Alcaligenes spp. Pseudomonas aerugenosa, Shigella* spp. *Enterobacter aerogenes, Escherichia coli and Serratia* spp. from the skin, buccal cavity and the gill of randomly selected species. The bacterial count of *L. agennes* was highest in the buccal cavity (cfu = 10.9±0.2, f<0.05) than gills (cfu = 6.2±0.1, f<0.05) and skin (cfu = 4.2±0.3, f<0.05), *P. elongates* highest also in buccal cavity (cfu = 11.02±0.53, f<0.05) than gills (cfu = 6.2±0.2, f<0.05) and skin (cfu = 4.6±0.2, f<0.05), *S. barracuda* highest also in buccal cavity (cfu = 8.6±0.5, f<0.05) than gills (cfu = 5.6±0.1, f<0.05) and skin (cfu = 5.3±0.1, f<0.05). The bacteria flora isolated from the water sample were not totally different from the ones isolated from the fish except for *Clostridium perfringenes, Staphylococcus epidermidis* and *Vibrio parahaemolyticus*. The bacteria assemblage wereassemblages were of public health significance.

Key words: Bacteria, lagoon, *Lutjanus agennes, Pseudotolithus elongatus* and *Sphyraena barracuda* microorganisms, marine, fish species, morphometrics

INTRODUCTION

Lagos Lagoon is a lagoon sharing its name with the city of Lagos, An investigation was carried out on the fish species availability in the fish landing centers of Khulna district. Almost all major fish landing centers in this area were surveyed. A total of 139 inland and marine water fish and crustacean species were observed. Out of 139, 126 species belonged to fin fish and the rest 13 species to crustacean. Amongst the fin fish species, 53 were fresh water, 23 were brackish water, 11 exotic and 39 marine water species. Twenty two fish species were found both as inland and marine water species. Nineteen species were detected endangered (*Ompok pabda, Hilsa toil, Puntius sarana, Notopterus chitala, Mustus aor, Rita rita, Nandus nandus, Eutroeiichthys vacha, Notopterus notopterus, Wallago attu, Channa marulius, Labeo bata, Pangus pangus, Bagarius*

bagarius, *Rasbora rasbora*, *Puntius ticto*, *Rohtee cotio*, *Labeo calbasu* and *Chanda nama*). In summer, 30 species and in winter, 43 species were more available and the rest of the species were found all the year round. On the basis of abundance, Carp species, *Lates calcarifer*, *Pelamys chiliensis*, *Trichiurus haumela*, *Katengus typus*, *Penaeus monodon* and *Scylla serrata* were recorded most abundant, respectively (Ali *et al*., 2004). Nigeria which lies on its southwestern side. The name is Portuguese and means lakes in the Portuguese language so Lagos Lagoon is an example of a tautological place name. The lagoon is more than 50 km long and 3 to 13 km wide, separated from the Atlantic Ocean by a long sand spit 2 to 5 km wide which has swampy margins on the lagoon side. Its surface area is approximately 6, 54.7 sq km (Obafemi, 2008). Lagos lagoon is a coastal body of shallow water formed where low lying rock, sand or coral presents a partial barrier to the open sea. Lagoon as an example of brackish water has a varying salinity. It has salinity close to that of fresh water during the peak of raining season because of increase in precipitation and salinity close to that of marine environment during the dry season because of high rate of evaporation. It lies on latitude 6°30.40'N and 3°24.52 'E longitude. It lies on marshland of vast mangrove and freshwater swamps, surrounding a small and much dissected table land consisting of freshwater swamp forest, mangrove swamp forest, sandy plain vegetation and rainforest vegetation (Ayolabi, 2004).

The fight against bacterial infection represents one of the high points of modern medicine. Bacterial infections can be caused by a wide range of bacteria, resulting in mild to life-threatening illnesses (such as bacterial meningitis) that require immediate intervention. Adult fish and fish larvae ingest bacteria by drinking and are, thus, primed with antigens before active feeding commences. This may result in the formation of an indigenous larval microflora. The microflora of marine invertebrates may harbour bacteria that are pathogenic to other organisms and thus, invertebrate co-inhabitants or food organisms in aquaculture may serve as vectors for transfixion of fish pathogens. In intensive egg production and larviculture, the numbers of bacteria are kept low by various forms of water treatment and disinfection. Total aerobic bacteria and mold counts were higher in corn than in other raw material. The bacterial and fungal counts and moisture levels in mixed feeds were significantly higher ($p<0.05$) in samples taken from stores. Results obtained in the study indicated that temperature, moisture levels and duration of storage are critical factors which affect the microbiological quality of fish feeds (Nuri Cakmak and Sahin, 2002). In a study conducted by Mihdhdir (2009) to evaluate the bacteriological and sanitary quality of drinking water produced in Makkah Al-Mokaramah during the high season in the month of Ramadan. Water samples were collected both from the drinking water stations and the water tankers (in Arabic language called whitats) used to transport and distribute water in different places in the Holy city. Water samples were analyzed to determine the densities of HPC at 22 and 37°C, total coliforms, *E. coli* and *S. aureus*. The bacteriological analysis of drinking water samples at 37°C proved that 6.7-33.3, 20-46.7, 0-20 and 0-6.7% of total water samples contained HPC, total coliforms, *E. coli* and *S. aureus*, respectively which were higher than the safe limits for drinking water. The bacterial analysis of drinking water varied from one water station to another. On the other hand, drinking water transported by tankers appeared to be in the lowest category of water quality. Because out of total water samples 40-59, 60-68.8, 31.2-37.5, 10-25% contained HPC, total coliforms, *E. coli* and *S. aureus*, respectively which were higher than the established safe limits of drinking water. One possible reason for poor quality of drinking water could be attributed to the application of inadequate water disinfection treatments and also the absence of sanitary aspects as supported by the bacteriological analysis which holds true especially for water supplied by

tankers. These approaches, however, may disturb the balance between microbial communities, or favour proliferation of opportunistic bacteria or unpredictable development of bacterial communities. Thus, there is a need for better microbial control during intensive larval production. The use of probiotics has proven advantageous in domestic animal production and microbial management may also have a potential in aquaculture. Better control of host-microbe interactions is a prerequisite for stable production of marine larvae in intensive systems (Olafsen, 2001). The type and number of microorganisms that live in fish vary according to the season, the species and the natural habitat. Additional contamination may occur during the harvesting, handling, or processing of the fish. It was reported that common spoilage microorganisms of fish include species of *Pseudomonas*, *Moraxella* and *Acinetobacter*, found mainly in marine fish and *Bacillus* and *Micrococcus*, found in freshwater fish. Fish may also contain pathogenic (disease-causing) *microorganisms* such as *Salmonella* and *Escherichia coli* (Michelle, 2009). Pathogenic contamination is of special concern with mollusks because they are often eaten raw and as whole animals.

This study will provide the objective of this study was to provide information on the morphometrics, occurrence and diversity of bacteria flora found in skin, gills and buccal cavity of some brackish water species such as *Lutjanus agennes, Pseudotolithus elongatus* and *Sphyraena barracuda*. Different identification procedure and techniques like colonial morphology, isolation and culturing to determine if pathogenic bacteria groups are present. Gram staining, motility, sugar fermentation as well as biochemical tests were also carried out.

MATERIALS AND METHODS

Study area: All samples were collected from live or dying *Lutjanus agennes, Pseudotolithus elongatus, Sphyraena barracuda* from Lagos lagoon at Falomo landing site in Victoria Island Lagos between April 2009 and September 2010. The lagoon is used for fishing and transportation. The water flows to Lekki area of Lagos State to form Lekki lagoon and also to Epe.

Collection of samples: On the field, identification of fish and morphometrics; standard length, head length, gill length and buccal cavity in Centimeters (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 buccal cavity. The swabbing was done with the aid of swab stick and after swabbing it was cocked back in the case that already contained peptone water that serves as transport media. The swab sticks were preserved with ice parks before getting to the laboratory for necessary bacteriological analysis.

Serial dillution: Each sample was sepeatelyseparately analysed by ensuring homogeneity of the samples using a sterile pipette. The 1 mL of each sample was suspended into 9 mL sterile water asceptically aseptically in a macCartney bottle which was then shaken together. Further dilution of 10, 10, 10^{-3} were carried out which 10^{-2} dilution was later used.

Pour plate method: Unto all the disposable Petri dishes, aliquots of 1 mL of different dilution of the sample type were pipette and the plates were labeled. Thereafter, sterilized media were added respectively onto the samples and swirled gently. This method allows for the growth of anaerobic and falcultativefacultative anaerobic organisms.

Microbiological analysis: Each swab stick was initially cultured on Nutrient agar for growth and subcultured on Pseudomonas Agar (PSA), Salmonella-Shigella Agar (SSA) and Eosine Methylene Blue Agar (EMBA) and incubated at 37°C.

Total bacteria count (cfu mL^{-1}): The total bacteria count for each sample was determined with the pour plate technique using the necessary agar. The plates were incubated between 24 h at 37°C. All colonies appearing at the end of the incubation period were counted using digital illuminated colony counter and the counts were expressed in colony forming unit per mL (cfu mL^{-1}) of the sample.

Identification of microorganism: The organisms were identified using the biochemical tests and some other test to confirm the presence of the suspected microorganism by their reaction to the tests (Akinyemi, 2009).

Statistical analysis: The morphometrics results were subjected to a one-way analysis of variance (ANOVA), the bacteria isolates were subjected to descriptive statistics and f-tests. The significant level was $p<0.05$.

RESULTS

Summary of the morphometrics parameters of the randomly selected species of *Lutjanus agennes*, *Pseudotolithus elongatus* and *Sphyraena barracuda* are presented in Table 1. The morphometrics parameters of *Lutjanus agennes, Pseudotolithus elongatus, Sphyraena barracuda* from of Lagos lagoon were measured accordingly and also the diversity of bacteria flora in skin, buccal cavity and gills were examined. There was significant difference ($p<0.05$) in all the parameters measured except for the standard length and gill length of *P. elongatus* and *S. barracuda* that there was no significant difference ($p>0.05$). *P. elongatus* recorded the highest mean body weight (356±77.67) g while *L. agennes* recorded the lowest mean body weight (35±10.43) g. *S. barracuda* recorded the highest mean standard length (27.38±4.57) cm while *L. agennes* recorded the lowest mean standard length (10.28±11.1) cm. *S. barracuda* recorded the highest mean head length (8.50±1.38) cm while *L. agennes* recorded the lowest mean head length (3.78±0.32) cm. *S. barracuda* recorded the highest mean gill length (5.03±0.99) cm while *L. agennes* recorded the lowest mean gill length (2.18±0.38) cm. *S. barracuda* recorded the highest mean buccal depth (4.08±0.62) cm while *L. agennes* recorded the lowest mean buccal depth (1.60±0.17) cm. It was discovered that all parameters measured were significantly different from one another except for the Standard Length (SL) *P. elongatus* and *S. barracuda* had no significance difference but both were significantly different to *L. agennes*. In Gill Length (GL)

Table 1: Summary of the morphometrics for *L. agennes, P. elongatus, S. barrracuda*

Morphometrics	*L. agennes*	*P. elongates*	*S. barracuda*	F value
Wt (g)	35.00±10.43^{c}	356.13±77.67^{a}	230.70±104.35^{b}	4.594
SL (cm)	10.28±1.11^{b}	24.45±4.57^{a}	27.38±4.57^{a}	5.817
HL (cm)	3.78±0.32^{c}	7.00±1.34^{a}	8.50±1.38^{b}	4.593
GL (cm)	2.18±0.38^{b}	4.55±0.43^{a}	5.03±0.99^{a}	5.297
BD (cm)	1.60±0.17^{c}	3.48±0.21^{b}	4.08±0.62^{a}	10.881

Wt: Weight, SL: Standard length, HL: Head length, GL: Gill length, BD: Buccal depth. $^{a, b, c}$ means of the same row with different superscripts are significantly different ($p<0.05$)

P. elongatus and *S. barracuda* had no significant difference but both were significantly different to *L. agennes*. Viable bacteria count of bacteria growth isolated from three (3) spots/sections of the three different species was done for each. The gill of *L. agennes* recorded the highest viable count while the skin of *P. elongatus* recorded the least viable count. In Table 2, it revealed that *P. elongatus* had the highest number of growth (2.7×10^{3}-2.98×10^{4}) in general followed by *L. agennes* (3.3×10^{3}-2.48×10^{4}) and the least seen in *S. barracuda* (2.5×10^{3}-2.18×10^{4}).

Also, a total of fourteen identified bacteria flora from the buccal cavity, gills and skin of all the three species were presented in Table 3, it shows that the bacteria load in the buccal cavity was highest in *P. elongatus* (11.0±0.5) with no significant difference with *L. agennes* (10.9±0.2) both were significantly different to *S. barracuda* (8.6±0.5). The bacterial load in the gill was highest in *L. agennes* (6.6±0.1) with no significant difference with *P. elongatus* (6.2±0.2 but both were significantly different to *S. barracuda* (5.6±0.1). In contrast, the bacterial load on the skin was highest in *S. barracuda* (5.3±0.1) with no significant difference with *P. elongatus* (4.6±0.2) but significantly different to *L. agennes* (4.2±0.3). The isolated bacteria from the water were not totally different from those found in the sample of fish except for *Vibrio parahaemolyticus, Staphylococcus epidermidis* and *Clostridium perfringenes*.

Table 2: Summary of viable bacteria count (cfu mL^{-1})

Species	Spots/Section	Viable count
L. agennes	Skin	0.26×10^{4}-2.21×10^{4}
	Buccal cavity	0.32×10^{4}-2.81×10^{4}
	Gills	0.27×10^{4}-2.98×10^{4}
P. elongates	Skin	0.25×10^{4}-2.18×10^{4}
	Buccal cavity	0.31×10^{4}-2.92×10^{4}
	Gills	0.31×10^{4}-2.56×10^{4}
S. barracuda	Skin	0.45×10^{4}-2.34×10^{4}
	Buccal cavity	0.36×10^{4}-2.72×10^{4}
	Gills	0.33×10^{4}-2.48×10^{4}

Table 3: Percentage total occurrence, diversity and incidence of bacteria flora found in Skin, Buccal cavity and Gills

	L. agennes			*P. elongates*			*S. barracuda*		
Bacterial species	S	B	G	S	B	G	S	B	G
Klebsiella spp.	3	ND	8	ND	ND	8	3	6	6
Proteus spp.	11	8	8	11	11	3	ND	ND	8
Staphylococcus aureus	6	ND	8	8	3	8	8	ND	8
Citrobacter spp.	6	ND	ND	ND	ND	ND	ND	6	ND
Streptococcus pyogenes	3	6	ND	ND	ND	ND	ND	ND	ND
Salmonella spp.	ND	8	ND	ND	11	ND	ND	3	ND
Micrococcus spp.	8	6	6	6	3	3	3	6	6
Bacillus licheniform	6	ND	8	ND	6	11	3	6	8
Alcaligenes spp.	ND	ND	6	6	3	ND	11	ND	ND
Pseudomonas aeruginosa	ND	ND	ND	ND	ND	6	ND	ND	ND
Shigella spp.	ND	6	ND	ND	8	ND	ND	8	ND
Enterobacter spp.	6	6	ND	8	8	8	8	8	8
Escherichia coli	11	8	8	8	8	6	11	8	8
Serretia spp.	ND	6	ND	ND	ND	ND	ND	3	6
Mean bacteria counts (cfu mL^{-1})	4.2 ± 0.3^{a}	10.9 ± 0.2^{b}	6.6 ± 0.1^{b}	4.6 ± 0.2^{ab}	11.0 ± 0.5^{b}	6.2 ± 0.2^{b}	5.3 ± 0.1^{b}	8.6 ± 0.5^{a}	5.6 ± 0.1^{a}
F statistics		6.44			4.35			10.44	

ND: Not detected. S: Skin, B: buccal cavity, G: Gills. Means along the same row with different superscript are significantly different at ($p<0.05$)

DISCUSSION

In this study, a total of fourteen species of bacteria were isolated from the gills, buccal cavity and skin of *L. agennes, P. elongatus* and *S. barracuda*. Ten out of the isolated bacteria species were gram negative (-ve) while the remaining four were gram positive (+ve). This result was in support of an earlier research findings by Al-Harbi and Uddin (2005) who reported that the bacteria identified from the brackish pond water to be predominantly gram negative (-ve). In a different study, Balarin and Hatton (1979); attributed that infected liver and intestines observed in *Tilapia* is caused by *Proteus* spp. and *Streptococcus pyogenes*. In this study, *Streptococcus pyogenes* was found to be high in the buccal cavity of *L. agennes* which could be attributed to the detection of Streptococcus in the analysis of the water sample which would get in through taking in of water by the fish. Jeyasekaran *et al.* (2006) reported that *Moraxella* constituted 50% of the total bacterial flora in raw pomfret and became 2%, when they were pre-chilled with ice. *Moraxella* was also found to be the dominant (29%) flora in pre-chilled stored pomfret, whereas *Flavobacterium* was dominant in control pack Proteus recorded highestrecasthighest in the skin of *L. agennes*, also in the skin and buccal cavity of *P. elongatus* which is similar to the findings of Sowunmi *et al.* (2008); a study carried out on Lekki lagoon that *Proteus vulgaris* recorded highest mean percentage bacteria occurrence which could be as a result of sewage, waste from abattoir or manure in the reservoir. The finding was carried out on Lekki lagoon which is an extension of Lagos lagoon but found around Lekki axis of Lagos State. In a research carried out by Yousefi-Mashouf and Hashemi, (2006), out of 465 burn wound infections 73.1% of isolates were Gram-negative bacilli and 26.9% were Gram-positive cocci. *Pseudomonas aeruginosa* (32.7%), *Klebsiella pneumoniae* (21.8%) and *Staphylococcus aureus* (21.2%) were the most common isolates.

Sang (1976) suggested that stressed fish could be predisposed to bacterial gill disease because gill is meant for the exchange oxygen and the fish will be stressed because of difficulty in the exchange due to heavy load of microbial in water then the gill is to be infected. In support of this Horsley (1977), listed *Bacillus* and *Pseudomonas* as part of the normal gill flora in temperate fishes, but in this study that was carried out in the tropics it was discovered that the gill of *P. elongatus* has the highest load of *Bacillus* and *Psedomonas aeruginosa* although the morphometric properties revealed that the gill length is shorter than that of *S. barracuda* which *Pseudomonas* was not part of the isolated organism but has lower percentage of *Bacillus*.

Salmonella spp. was only found in the buccal cavity of *L. agennes* but absent in *P. elongatus* and *S. barracuda*. This was in accordance with the argument that *Salmonella* spp. preferred the intestinal tract of man and animals (including fish).

All the isolated organisms were all found in at least one out of the three spots where samples were taking except for *Staphylococcus epidermidis, Vibrio parahaemolyticus* and *Clostridium perfringenes*. *Bacillus* spp. *Escherichia coli, Salmonella* spp. *Streptococcus* spp. *Staphylococcus aureus* and *Pseudomonas* have been involved in pathogenicity of both fish and man (Babu, 2000). Staphylococcus and Streptococcus organisms were isolated from the gut, gills, heart and liver of the dead fish. The Staphylococci were Gram (+ve), non-motile cocci, catalase and urease positive, negative for oxidase and showed hemolytic growth on blood agar but no growth on McConkey`s Lactose Agar (MLA). The Streptococci were Gram (+ve), non-motile cocci, catalase and oxidase negative and showed hemolytic growth on blood agar but no growth on MLA (Saxena *et al.*, 2006). All this were isolated from the fish samples and the water sample which could establish disease of human if not properly cooked fish is consumed particularly pathogenic Salmonella that has been found to be more established inside the intestine than any other niche.

The microbial flora richness of this water may be suggested to be due to high inflow of water effluent waste and sediments into the lagoon.

CONCLUSION

This study provided information on the bacteria flora found in gill, buccal cavity and skin of *L. agennes, P. elongatus* and *S. barracuda* including the water sample of Falomo section of Lagos lagoon, the major lagoon in Nigeria. This study confirms the existence of pathogenic bacteria organisms in fish sample as well as the water sample which are of public health importance. The finding shows the diversity of microbial organism in water and fish sample. The different bacteria found in water were also found on three different spots of the fish that analysis was carried out. Stress is induced on fish when the environment becomes unfavourable for fish but on the other hand becomes favourable for microorganisms. Microorganism exists in water but effort must be made to make sure that their concentration does not go beyond the bearable limit of the fish by controlling the environmental factors. Naturally fish has immunity for diseases but their immunity drops when the concentration of the microorganism is on the increase.

Based on the findings of this study, the following recommendations are suggested:

- The lagoon should be protected against indiscriminate disposal of refuse, sewage and industrial effluents as this will increase the microbial load in water and consequently inducing stress on fish and other aquatic organism present there
- Good hygienic practices should be carried out when fish from the lagoon are purchased for consumption purposes. Bacteria in fish not properly cooked could be transferred to man as they establish themselves in the intestine particularly those that are pathogenic causing a lot of problems including diseases of various kind

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