OCCURRENCE OF BACTERIA IN THE SKIN, GILLS AND BUCCAL CAVITY OF *Psettiae sebae* (Cuvier 1829), *Pomadasys jubelini* (Cuvier, 1830) and *Cynoglosus senegalensis* (Kaup, 1858) FROM LAGOS LAGOON, NIGERIA

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ABSTRACT

The morphometrics of *Psettiae sebae* (African moony), *Pomadasys jubelini* (Grunter) and *Cynoglossus senegalensis* (Sole) from Lagos lagoon were determined and the bacteria flora in the skin, gills and buccal cavity of the fish species were examined. There were no significant difference in the weight, head length and gill length of the fish sample but there were significant difference ($P< 0.05$) in the standard length and buccal depth of fish samples. *Psettiae sebae* recorded the highest mean body weight (148.55 ± 16.76g), *Cynoglossus senegalensis* recorded the highest mean standard length (24.80 ± 2.32cm), *Pomadasys jubelini* recorded the highest mean head length (4.53±0.93cm), *Psettiae sebae* recorded the highest mean gill length (2.83 ± 0.48cm) while *Psettiae sebae* recorded the highest mean buccal depth (1.58 ± 0.05cm). The highest mean percentage bacterial occurrence of 14.46 ± 0.26 was recorded in the buccal cavity of *Psettiae sebae* while lowest mean percentage bacteria occurrence of 9.64 ± 0.22 was recorded in the skin of *Psettiae sebae.*The number of colony forming unit per milligram ranged between $2.81\times10^4 – 3.38\times10^4$. The bacteria isolated from the water sample were similar to those of the fish samples except for *Clostridium perfrigenes* and *Vibrio parahaemolyticus*. In this study, a total of eighteen (18) bacteria species were isolated from the skin, gills and buccal cavity of *Psettiae sebae*, *Pomadasys jubelini* and *Cynoglossus senegalensis,* twelve (12) of the bacteria species were Gram negative (*Enterobacter spp*, *Pseudomonas spp*, *Proteus spp*, *Escherichia coli*, *Klebsiella spp*, *Citrobacter spp*, *Pseudomonas aeruginosa*, *Alcaligenes spp*, *Seratia spp*, *Salmonella spp*, *Shigella spp*, *Enterobacter aerogenes*) while six (6) bacteria isolates (*Staphylococcus aureus, Micrococcus spp, Bacillus spp, Streptococcus pyogen, Streptococcus spp, Staphylococcus epidermidis*) were Gram positive. This study confirms the existence of pathogenic bacteria in the fish species (*Psettiae sebae, Pomadasys jubelini and Cynoglossus senegalensis*) which are of public health significance.

Keywords: Bacteria, Morphometrics, Psettiae sebae, Pomadasys jubelini and Cynoglosus senegalensis

INTRODUCTION

Fish is a vital source of food for people. It is man's most important single source of high-quality protein, providing 16% of the animal protein consumed by the world's population, according to the Food and Agriculture Organisation (FAO) of the United Nations (1997). It is a particularly important protein source in regions where livestock is relatively scarce. Fish supplies <10% of animal protein consumed in North America and Europe, but 17% in Africa, 26% in Asia and 22% in China (FAO, 2000). The estimation was that about one billion people world-wide relies on fish as their primary source of animal protein. Fish is a reservoir for large number of microorganisms. 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 which causes food poisoning. Quality standards have been prescribed for the fish and fishery products meant for export and they are being monitored strictly (Ashokkumar, 2008) The bacteria are a large group of single-celled, prokaryote microorganisms. Typically a few micrometres in length, bacteria have a wide range of shapes, ranging from spheres to rods and spirals.

Bacteria are ubiquitous in every habitat on Earth, growing in soil, acidic hot springs, radioactive waste,(Fredickson *et al.,* 2004) water, and deep in the Earth's crust, as well as in organic matter and the live bodies of plants and animals. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a millilitre of fresh water; in all, there are approximately five nonillion (5×10^{30}) bacteria on Earth, forming much of the world's biomass (Whitman *et al.,* 1998).

Bacteria display a wide diversity of shapes and sizes, called morphologies. Bacterial cells are about one tenth the size of eukaryotic cells and are typically 0.5–5.0 micrometres in length. However, a few species–for example *Thiomargarita namibiensis* and *Epulopiscium fishelsoni* are up to half a millimetre long and are visible to the unaided eye (Schulz and Jorgensen, 2001). Among the smallest bacteria are members of the genus Mycoplasma, which measure only 0.3 micrometres, as small as the largest viruses (Robertson *et al.,* 1975). Some bacteria may be even smaller, but these ultramicrobacteria are not well-studied (Velimirov, 2001).

There are broadly speaking two different types of cell wall in bacteria, called Gram-positive and Gram-negative. The names originate from the reaction of cells to the Gram stain, a test long-employed for the classification of bacterial species (Gram, 1884). Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids. In contrast, Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. From the standpoint of microbiology, fish and related products are a risk foodstuff group. Particularly *Clostridium botulinum* type E and *Vibrio parahaemolyticus* rank among pathogenic bacteria associated with fish. Other potentially pathogenic bacteria associated with fish and shellfish include *C. perfringens*, *Staph.* spp., *Salm.* spp., *Shigella* spp., *V. cholera* and other vibrios. Outbreaks usually occur due to the ingestion of insufficiently heat-treated fish or products contaminated after or during their processing. Freezing fish and related products in the seawater, intensive handling, long-time transport or cooking in fishing containers straight on the deck contributes to their contamination with microorganisms.

This study will provide information on the morphometrics and occurrence of bacteria flora in some marine fish species such as *Psettias seba, Pomadasys jubelini and Cynoglosus senegalensis*. The presence of pathogenic bacteria groups will be ascertain by culturing, isolating and identifying suspected pathogens in order to provide information on the cultural characters, colony morphology, Gram staining, motility, sugar utilization as well as biochemical reactions and tests.

MATERIALS AND METHODS

Study Area

The study was carried out on the bacteria flora found in the skin, gill and bucal cavity of three fish species (*Psettiae sebae, Pomadasys jubelini, Cynoglosus senegalensis*) inhabiting Lagos lagoon. All samples were collected from live or dying *Psettias seba, Pomadasys jubelini, Cynoglosus senegalensis* from Lagos lagoon at Falomo fishing jetty in Victoria Island Lagos.

Collection of Samples

Live or dying fish samples from Lagos lagoon were purchased at Falomo landing centre. The bacteria isolate from each of the fish samples were obtained from the skin, gills and buccal cavity by using the swab stick to swab the respective regions (skin, gill and buccal cavity) and later corked back into the case containing 10ml of peptone water and then arranged in an ice box containing ice block in order to preserve it. Water samples were collected with macCartney bottles and then arranged in an ice box containing ice block in order to preserve it.

Morphometric Features of Fish Sample

The morphometric features (standard length, head length, gill length and buccal depth) were measured and recorded in centimetres (cm) after weighing the fish samples in grams (g) using sensitive scale (OHAUS, EB series).

Serial Dillution

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

Pour Plate Method

Unto all the disposable Petri dishes, aliquots of 1ml of different dilution of the sample type were pipette and the plates were labelled. Thereafter, sterilized media were added respectively onto the samples and swirled gently. This method allows for the growth of anaerobic and falcultative anaerobic organisms.

Total Bacteria Count (CFU/ml)

The total bacteria count for each sample was determined with the pour plate technique using the necessary agar. The agar plates were incubated for 24 hours at 37^0C. 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) of the sample.

Identification of Microorganisms

The organisms were identified using the biochemical tests to confirm the presence of the suspected microorganism by their reaction to the tests. All the isolate were transferred from the slants into appropriate agar plates, incubated appropriately and used for

identification such as cultural and morphological characteristics, biochemical tests using the procedures outlined by (WHO,1989).

Sugar Fermentation Test

The ability of the isolates to utilize certain sugar as source of energy was tested. If the organism does ferment a particular sugar, acid will be produced and gas may or may not be produced. The production of acid indicated a colour change of the medium from red to yellow, and the production of gas was indicated by a void produced in a Durham tube. Inability to ferment sugar indicated no colour change of the medium. The fermentation medium was prepared by 0.1g of peptone, 0.1g of sodium chloride and 0.1g of fermentable sugar (glucose) in 10ml of distilled water. An amount of 9ml of the medium was pipette into a test tube containing Durham's tube in replicates (based on the isolate under test). 5ml of phenol red indicator was immediately discharged into the test tubes. The test tubes containing medium were sterilized in an autoclave at 126^0C for 15 minutes. After sterilization, each isolate were inoculated into glucose medium. The test tubes were incubated at 37^0C for 24-48 hours for bacterial isolates. All un-inoculated test tubes were also incubated for glucose to serve as a control. The test was also carried out using lactose, sucrose.

RESULTS

Morphometrics of *Psettiae sebae*, *Pomadasys jubelini* And *Cynoglossus senegalensis:* *Psettiae sebae* recorded the highest mean value in weight (148.55 ± 16.76g), gill length(2.83 ± 0.84cm) and buccal depth of (1.58 ± 0.05cm) while *Cynoglossus senegalensis* recorded the highest mean value in standard length (24.80 ± 2.32cm) and highest mean value in head length (4.85 ± 0.38cm). *Psettiae sebae* recorded the lowest mean value in standard length (12.47 ± 0.49cm) and head length of (4.13 ± 0.36cm) while *Pomadasys jubelini* recorded the lowest mean value in weight (89.98 ± 47.76cm) and buccal depth (1.23 ± 0.17cm), while *Cynoglossus Senegalensis* recorded the lowest gill length (2.03 ± 0.09cm) as shown in Table 1.

Viable Bacteria Count of Bacteria Growth Isolated from the Skin, Gill and Buccal Cavity of Fish Sample (CFU/ML): *Cynoglossus senegalensis* skin recorded the highest range of viable bacteria count (3.5×10^3 - 3.38×10^4Cfu/ml) while *Psettiae sebae* skin recorded the lowest range of viable bacteria count (1.6×10^3 - 2.95×10^4Cfu/ml). *Psettiae sebae* gill recorded the highest range of viable bacteria count (2.4×10^3 – 2.96×10^4Cfu/ml), while *Pomadasys jubelini* gill recorded the lowest range of bacteria count (2.1×10^3 – 2.85×10^4Cfu/ml). *Pomadasys jubelini* buccal cavity recorded the highest range of viable bacteria count (3.3×10^3 – 2.97×10^4Cfu/ml) while *Cynoglossus senegalensis* recorded the lowest range of viable bacteria count (1.6×10^3 – 2.81×10^4Cfu/ml) in the bucal cavity. (Table 2)

Diversity and Incidence of Bacterial Flora from Skin, Buccal Cavity and Gills of *P. sebae*, *P. jubelini* and *C. senegalensis* (in percentage): The highest mean percentage bacterial occurrence of 14.46 ± 0.26 was recorded in the buccal cavity of *Psettiae sebae* while lowest mean percentage bacteria occurrence of 9.64 ± 0.22 was recorded in the skin of *Psettiae sebae. Pseudomonas spp* recorded the highest percentage occurrence of 11.1% in the skin, buccal cavity and gill of *Psettiae sebae, Pomadasys jubelini* and *Cynoglossus senegalensis* (Table 3).

Analysis of Water Sample

The bacteria isolated from the water samples were *Streptococcus pyogenes, Clostridium Perfrigenes, Bacillus spp, Micrococcus spp, Proteus spp, Alcaligenes spp, Enterobacter spp, Klebsiella spp, Salmonella spp, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Vibrio parahaemolyticus* and *Shigellae spp.* The bacteria isolated from the water samples were similar to those isolated from the fish samples, except for *Clostridium perfrigenes and Vibrio parahaemolyticus* which were not detected in fish samples. The viable bacteria count of water samples had highest range of ($2.06\times10^4 - 4.2\times10^4$Cfu/ml) and lowest range of ($2.15\times10^4 - 4.04\times10^4$Cfu/ml)

DISCUSSION

The occurrence of bacteria in the skin, gills and buccal cavity of *Psettiae sebae, Pomadasys jubelini* and *Cynoglossus senegalensis* and also in the lagoon (Lagos lagoon) in which they inhabit is in support of (Strom and Olafsen 1990, Hansen *et al.,* 1992) findings that, bacteria are abundant in the environment in which fish live and it is therefore rather impossible to avoid them being a component of their diet.In the morphometric features evaluated, the highest mean weight (g), standard length (cm), head length (cm), gill length (cm) and buccal depth were 110.10 ± 44.30^a, 24.80 ± 2.32^b, 4.85 ± 0.38^a, 2.83 ± 0.48^a, 1.58 ± 0.05^a respectively, while the lowest mean weight (g), standard length (cm), head length (cm), gill length (cm) and buccal depth were 89.9 ± 47.76^a, 12.47 ± 0.49^a, 4.13 ± 0.36^a, 2.03 ± 0.09^a, 1.23 ± 0.17^b with significant difference in the morphometrics of buccal depth of the fish samples the buccal cavity of *Psettiae sebae* (with highest buccal depth of 1.58 ± 0.05^a) recorded the highest bacteria load. In this study a total of eighteen (18) bacteria species were isolated from the skin, gills and buccal cavity of *Psettiae sebae*, *Pomadasys jubelini* and *Cynoglossus senegalensis* twelve (12) out of the bacteria specie were Gram negative (*Enterobacter spp*, *Pseudomonas spp*, *Proteus spp*, *Escherichia coli*, *Klebsiella spp*, *Citrobacter spp*, *Pseudomonas aeruginosa*, *Alcaligenes spp*, *Seratia spp*, *Salmonella spp*, *Shigella spp*, *Enterobacter aerogenes*) 67% . This confirmed the finding of Ahmed and Naim (2002) who reported that the bacteria identified from the brackish pond water sediment gills and intestines of healthy Tilapia cultured in Saudi Arabia were predominantly Gram negative rods 87%.

The similarity in the bacteria flora of water sample and fish sample observed in the study is confirmed by Osungbemiro (2005) who observed that quantitatively, the microbial flora of fish appears to be a function of the environment. Chandrasekeran (1985), also reported that animals in the aquatic environment carry bacterial flora, which is a reflection of the flora in the environment. *Psettiae sebae* recorded the highest viable bacteria count (2.96×10^4 Cfu/ml) which was recorded in the gill while the lowest viable count (1.6×10^3 Cfu/ml) was recorded in the skin. *Pomadasys jubelini* recorded the highest bacteria count (2.97×10^4 Cfu/ml) in the buccal cavity and the lowest bacteria count (2.1×10^3 Cfu/ml) was recorded in the gill.

In *Cynoglossus senegalensis*, the highest viable bacteria count 3.38×10^4 was recorded in the skin while the lowest viable bacteria count (1.6×10^3 Cfu/ml) was recorded in the bucal cavity. This implies that the bacterial load in different regions (skin, gill and buccal cavity) varies from species to species. From the result, the morphological characteristics showed

that six (6) bacteria isolates (*Staphylococcus aureus, Micrococcus spp, Bacillus spp, Streptococcus pyogen, Streptococcus spp, Staphylococcus epidermidis*) were Gram positive, while twelve (12) bacteria isolate (*Enterobacter spp*, *Pseudomonas spp*, *Proteus spp*, *Escherichia coli*, *Klebsiella spp*, *Citrobacter spp*, *Pseudomonas aeruginosa*, *Alcaligenes spp*, *Seratia spp*, *Salmonella spp*, *Shigella spp*, and *Enterobacter aerogenes*,) were Gram negative.

The sugar fermentation test showed that *Staphylococcus aureus, Enterobacter spp*, and *Escherichia coli* fully produce acid and gas while *Micrococcus spp, Pseudomonas aeruginosa, Streptococcus spp and Shigella spp* fully produced acid while *Pseudomonas spp* produced acid/gas for glucose and negative reaction for lactose and sucrose, *Proteus spp* produced acid/gas production for glucose and sucrose and negative reaction for lactose, *Klebsiella Spp* produced acid/gas for glucose and positive reaction for lactose and sucrose, *Bacillus spp, Staphylococcus epidermidis* produced positive reaction for the sugar fermentation test, while *Alcaligen spp* produced a negative reaction. *Citrobacter* spp produced acid/gas for glucose, acid for sucrose and negative reaction for lactose. *Seratia spp* produced acid/gas for glucose and sucrose while a negative reaction was observed for lactose. *Salmonella spp* produced acid for glucose, while a negative reaction was observed for lactose and sucrose. *Enterobacter aerogenes* produced acid/gas for lactose and sucrose while no reaction was observed for glucose.

In conclusion, this study confirms the existence of pathogenic bacteria in the fish species (*Psettiae sebae, Pomadasys jubelini and Cynoglossus senegalensis*) of Lagos lagoon which are of public health significance. The bacteria isolated from the fish samples are a function of bacteria found in the lagoon (Lagos lagoon) which is influenced by industrial effluence, domestic and agricultural waste emptied into the lagoon. Findings have confirmed that fish can be infected with variety of microbial species especially bacteria, which is a function of bacteria found in their habitat.

Based on the findings during this study, the following recommendations are suggested.

1. The disposal of human, agricultural and industrial waste into the lagoon should be prevented to reduce stress on fish and thereby preventing fish from bacterial infection.
2. Fish should be properly processed before consumption in order to prevent the bacteria in the fish from infecting human.
3. Further study should be carried out on the occurrence of bacteria in other fish species found in Lagos lagoon.

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Table 1: Morphometrics for *Psettiae sebae*, *Pomadasys jubelini* and *Cynoglossus senegalensis*

Morphometrics	*P. sebae*	*P. jubelini*	*C. senegalensis*	Fstatistic
Weight (g)	148.55 ± 16.76[a]	89.98 ± 47.76[a]	110.10 ± 44.30[a]	0.587
Standard length(cm)	12.47 ± 0.49[a]	12.82 ± 2.55[a]	24.80 ± 2.32[b]	12.19
Head length(cm)	4.13 ± 0.36[a]	4.53 ± 0.93[a]	4.85 ± 0.38[a]	0.35
Gill length(cm)	2.83 ± 0.48[a]	2.45 ± 0.49[a]	2.03 ± 0.09[a]	1.95
Buccal depth(cm)	1.58 ± 0.05[a]	1.23 ± 0.17[b]	1.29 ± 0.08[b]	6.19

[a,b] Means along the same row with different superscript are significantly different at (P<0.05)

Table 2: Viable Bacteria Count of Bacteria Growth Isolated from the Skin, Gill and Buccal Cavity of Fish sample (Cfu/ml).

Fish Species		Spot Viable Count Range (Cfu/ml)
Psettiae sebae	Skin	$1.6\times10^3 - 2.95\times10^4$
	Gill	$2.4\times10^3 - 2.96\times10^4$
	Buccal cavity	$6.7\times10^3 - 2.87\times10^4$
Pomadasys jubelini	Skin	$2.2\times10^3 - 2.97\times10^4$
	Gill	$2.1\times10^3 - 2.85\times10^4$
	Buccal cavity	$3.3\times10^3 - 2.97\times10^4$
Cynoglossus senegalensis	Skin	$3.5\times10^3 - 3.38\times10^4$
	Gill	$6.2\times10^3 - 2.94\times10^4$
	Buccal cavity	$1.6\times10^3 - 2.81\times10^4$

Table 3. Diversity and incidence of bacterial flora from skin, buccal cavity and gills of *P. sebae*, *P. jubelini* and *C. senegalensis* (In Percentage)

Bacterial species	*P. sebae*			*P. jubelini*			*C. senegalensis*		
	S	B	G	S	B	G	S	B	G
Escherichia coli	8.3	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
P. aerogeninosa	11.1	11.1	8.3	8.3	5.6	11.1	11.1	5.6	11.1
Proteus spp	11.1	8.3	8.3	5.6	ND	11.1	11.1	5.6	11.1
Streptococcus spp	ND	8.3	ND	2.8	ND	ND	ND	ND	2.8
Streptococcus pyogen	8.3	ND	ND	ND	ND	ND	ND	5.6	5.6
Salmonella spp	ND	11.1	ND	ND	11.	ND	ND	11.1	ND
Enterobacter spp	2.8	8.3	2.8	2.8	8.3	ND	ND	ND	ND
Micrococcus spp	ND	ND	8.3	5.6	2.8	ND	5.6	ND	ND
Klebsiella spp	ND	ND	5.6	11.1	2.8	8.3	ND	11.1	ND
Bacillus spp	11.1	2.8	ND	8.3	11.1	11.1	11.1	5.6	2.8
Shigella spp	ND	ND	ND	ND	2.8	ND	ND	11.1	ND
Serratia spp	ND	ND	ND	ND	8.3	8.3	ND	ND	5.6
Citrobacter spp	2.8	ND	5.6	ND	ND	2.8	11.1	8.3	8.3
Staphylococcus epidermidis	2.8	5.6	2.8	ND	ND	ND	ND	ND	ND
Staphylococcus aureus	2.8	8.3	8.3	ND	2.8	ND	5.6	ND	11.1
Pseudomonas spp	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
Alcaligen spp	ND	ND	2.8	ND	ND	2.8	ND	ND	ND
Enterobacter aerogenes	5.6	ND	ND	5.6	ND	ND	2.8	2.8	ND
Mean bacterial Count (cfu/ml)	10.29 ± 0.31^{a}	14.46 ± 0.26^{a}	9.99 ± 0.51^{a}	9.64 ± 0.22^{a}	12.86 ± 0.23^{b}	10.89 ± 0.32^{c}	10.96 ± 1.04^{a}	13.09 ± 0.42^{b}	10.47 ± 0.19^{ab}
F statistic	2.23			2.69			3.32		

[a, b, c] Means along the same row with different superscript are significantly different at ($P<0.05$)

Key: S- Skin B- Buccal cavity G- Gill ND- Not Detected