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RESEARCH PAPER

Microbiological study of Sciaenid species collected from coastal waters of Niger Delta, Nigeria

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Abstract

Microbial load in Sciaenid fishes *(Pseudotholithus. typus, P. senegalensis,* and *P. elongtatus*) collected from the coastal waters in Rivers State, Nigeria were compared. Fish samples were purchased from local fishermen and transported in ice packs to the microbiology laboratory. The mean bacterial counts on the skin of the three fish species using nutrient agar for general purposes showed that *P. typus* had the highest loads (2.560 x 10^3 CFU $g^{-1)}$ followed by *P. elongtatus* with 1.360 x 10^3 CFU g^{-1} and *P. senegalensis* (8.00 x 10^2 CFU g^{-1}). Mannitol Salt Agar (MSA) used for the isolation and identification of *Staphylococcus aureus* revealed that *P. senegalensis* had 3.0 X 10^{-1} CFU g-1, and *Staphylococcus aureus* was detected on the skin of *P. typus* and *P. elongtatus.* On SSA agar, the bacterial counts for the species were highest in *P. senegalensis* (6.326 x 10^2 CFU g^{-1}), followed by *P. elongtatus* and *P. typus,* with mean values of 5.90 x 10^2 CFU g^{-1} and 5.350 x 10^2 CFU $g\text{-}^1$ respectively. Bacterial counts on TCBS were highest in *P. senegalensis* with a mean value of 3.15 x $10^2 10^1$ CFU g^{-1}, *P. typus* had 2.925 x 10^2 CFU g^{-1} and *P. elongtatus* had 2.26 x 10^2 CFU g^{-1}. Fungi isolated on TCBS were low in all three fish species, with the highest mean value of 1.60 x 10^1 CFU g^{-1} recorded on *P. typus,* followed by *P. elongtatus* (1.50 x 10^1 CFU g^{-1}) and *P. senegalensis* (1.25 x 10^1 CFU g^{-1}). The prevalent bacteria species in this study were *Shigella, Aeromonas, Pseudomonas flourescens, Vibrio*, *Staphylococcus*, *Salmonella, Bacillus*, and *Micrococcus*. Fungal counts found on the skin of the three fish species include *Aspergillus flavus, Aspergillus niger,* and *Rhizopus stolonifera.* All these organisms isolated in this study are of public health significance, and their presence suggests that foodborne illness could arise if these fish are consumed in an undercooked state.

Introduction

Microorganisms live from the deepest trenches to the highest tide pools, and they are the most important primary producers, directly and indirectly feeding most marine animals. Microorganisms occur when they find food, moisture, and a suitable environment for their growth and multiplication. Fishes are constantly exposed to microorganisms from the surrounding waters. Fish living in tropical waters are more susceptible to microbes than those living in temperate waters because tropical waters provide favourable conditions for the introduction and spread of microorganisms because of temperature and other water quality parameters. Some of these microbes are indigenous to water; others are transient, entering the water from air, soil, or individuals. Microflora found in fish are related to various factors such as the developmental stage of the fish, digestive tract structure, water temperature, site, food availability, and physiological state of the organism (Sugita et al., 1985). The fish digestive tract houses many diverse microorganisms, including bacteria, archaea, and fungi, which together create a complex microbial ecosystem (Wei et al., 2018).

The quality of fish varies considerably depending on the location of the fishing ground, species, water quality, and harvesting techniques. A variety of fish consumed regularly are prone to pathogenic spoilage, especially by Vibrio spp., Shigella spp., Salmonella spp., streptococci, staphylococci, coliforms, Listeria spp., and Clostridium spp. (Rahman et al., 2012), which may get into the fish from their habitat or during fish transportation and storage (Frazier and Westhoff, 1995). Pathogenic bacteria in marine fish constitute the majority of these pathogenic agents (Feldhusen, 2000). Most bacteria responsible for causing disease in fish are gram negative rods, but some are gram-positive rods or cocci, and a few that are acid-fast rods cause disease in aquatic animals. Bacterial and fungal contamination of fish is considered the main cause of signs of spoilage such as off-flavour and unpalatable taste, and may constitute a public health hazard an many economic losses (Zaky and Salem, 2015).

The microflora of marine fish varies according to the genus, the water it lives in, the season, and the development period (Ogur, 2022). The growth of pathogenic microorganisms in a food is certainly undesirable, as it can make that food unsafe to eat, but other microbial induced changes in food, such as decreased nutritional content and altered taste, odour, colour, and texture, can also make a food undesirable for human consumption. The species of the genus Pseudotolithus (family Sciaenidae) (croakers) constitute an abundant and commercially important fish throughout the Atlantic coast of West Africa (Nunoo et al., 2013), and considered to be one of the most important commercial fish in Nigeria. Fish species like croakers, catfish, tilapias, and threadfins account for about 90% of Nigeria’s fishery (Osibona, 2011). There is a dearth of information on the public health importance associated with fish caught and sold in Nigeria. Considering the level of pollution and other human activities in the marine waters of Nigeria, there is an urgent need to establish the microbial quality of selected commercially important fish species. Therefore, it is crucial to estimate the rate of microbial spoilage and establish a preventive strategy to ensure general food safety. The purpose of this study was to assess and compare the microbial load in the skin of three croaker fish species (*Pseudotolithus senegalensis, P. elongatus,* and *P. typus)* caught from the coastal waters of Nigeria.

Materials and Methods

Fresh fish samples (*P. senegalensis, P. typus, and P. elongtatus)* were collected from the Nigerian south coast artisanal fishermen landings at Creek Road Market (longitude 5° 10’ N and latitudes 4° 55’’ and 4° 60’ E) and splashed with clean potable tap water for the removal of attached sand particles before gutting. During gutting, a bilateral incision was made to expose the stomach contents,

which were removed, and the stomach cavity was washed with clean potable tap water. The fish samples were transported in an ice pack to the Microbiology Laboratory in the Department of Microbiology, University of Port Harcourt for microbial examination on the same day.

Preparation of media: The media used in the experiment were weighed out according to the manufacturer's specifications; these include Nutrient agar, Salmonella-Shigella agar, Mannitol salt agar, Lactose broth, Potato dextrose agar, and TCBS, and distributed in a conical flask containing distilled water and boiled gently to complete dissolution. The conical flasks were plugged with cotton wool, covered with aluminium foil, and sterilized in the autoclave at 121°C for 15 minutes at 1.06 mmHg. Agar media were allowed to cool to 45°C before dispensing aseptically into sterile petri dishes.

Preparation of the serial dilution: 1ml of the original stock solution was aseptically poured into 9ml sterile distilled water and mixed thoroughly to give 10^{-1} dilution of the original stock solution.1ml of the dilution 10^{-1} of the original stock solution was aseptically poured into another tube of 9ml sterile distilled water to give 10ml of 10^{-2} dilution of the original stock culture. Repeating the above procedure, dilutions of 10^{-3} and 10^{-4} were obtained. The dilutions 10^{-1}, 10^{-2} and 10^{-4} were used.

Viable bacteria count on nutrient agar: The pour plate technique was employed. 1ml of dilution 10^{-2} and 10^{-4} of the stock culture was introduced into each Petri dish. Sterile molten nutrient agar at about 30°C was added and then, agitated gently and allowed to set undisturbed. The set agar plates were incubated at 37°C for 24 hours. Finally, an automatic colony counter counted the number of colonies per plate and the count was expressed by as colony forming count unit (CFU g^{-1}). Colonies of bacteria growing on the plates were observed, isolated and separated on a fresh media until pure culture was obtained.

Isolation of microorganisms from stock culture: A loopful each of the stock culture was inoculated into a sterile nutrient agar plate. The nutrients agar plate was incubated at 37°C for 24 hours for microbial growth.

Fungal isolation: 1 ml of the dilution 10^{-1} was poured on a plate, and molten Potato dextrose agar was added. The mixture was thoroughly agitated to give an even distribution of the sample. The plate was incubated at room temperature for between 48-72hrs after which observation and sub culturing were done and recorded.

Purification of bacteria:

Distinct colonies from the original culture on nutrient agar plates were picked by a sterile platinum wire loop and streaked to isolate on sterile nutrient agar and tryptone soy agar. These plates were incubated at 37°C for 24 hours for microbial growth. This process continued until pure bacterial colonies were obtained.

Identification of isolates

All isolates were sub-cultured and Gram staining was carried out. The identification of isolates was carried out based on the method described by Cheesbrough (2002). The Gram staining was aimed at differentiating Gram reactions, sizes, shapes, and arrangements of cells in the isolates. For the Gram-staining of the various isolates, glass slides were washed and air-dried. A drop of normal saline was placed on the slide. Using a flame inoculated wire loop, a small amount of inoculum was taken and smeared on a drop of normal saline on the slide. The smear was allowed to air dry and heat fixed by passing over a flame three times. The fixed smear was flooded with crystal violet for a minute and then rinsed with clean water. Lugols iodine was added for another minute, and this served as a mordant. This was later rinsed and cleaned with distilled water. Acetone alcohol was added as a decolorizer and rinsed immediately with clean water. A counterstain, safranin, was added and allowed to stand for a minute before being rinsed with clean water. The slide was

allowed to dry before being observed under an oil immersion objective microscope (100 objective lens). The formation of a very deep purple colouration indicated a positive reaction, while pink colouration indicated a negative reaction.

Slant Preparation: These isolates were transferred to their appropriate agar slants, labelled, and incubated at 37°C for 24 hours for growth, after which they were kept in the refrigerator at 4°C for identification.

Biochemical tests

Oxidase test: A piece of filter paper was placed into a clean petri dish, and 2 or 3 drops of freshly prepared oxidase reagent were added. A small portion of the organism to be identified was smeared on the filter paper using a glass rod (not an oxidising wire loop). A blue purple colour indicated a positive result (AOAC, 1998).

Catalase test: A drop of 3% hydrogen peroxide was placed at the centre of a slide, and a sterile wire loop was used to pick a small portion of the microorganisms to be identified from the nutrient agar plate into the hydrogen peroxide for immediate gas bubble formation or foaming, which indicates a positive result (AOAC, 1998).

Citrate utilization test: A slant of citrate agar was aseptically inoculated with an organism to be identified using a sterile wire loop. The inoculated citrate agar slant was incubated at 37°C for 24 hours and observed for colour change daily for up to 4 days. Turbidity and a blue colour indicate a positive test (AOAC, 1998)

Hydrogen sulphide production: Filter paper was cut into slips of about 5–10 mm wide and 50–60 mm long. These were impregnated with a hot, saturated aqueous lead acetate solution, dried at 50°C–60°C, and stored in a tightly closed container before use. The lead acetate paper was then inserted between the cap or the plug of the tube containing the organism in nutrient broth or peptone water. This was examined for seven days. A brown or black colour indicated a positive result (AOAC, 1998).

Indole reaction: The microorganism to be identified was inoculated into tryptophane broth for 48 hours at 37 °C. Five drops of Kovac's reagent were then added. A deep red ring formation indicate a positive result (AOAC, 1998).

Sugar fermentation: This is the ability of the isolates to utilize carbon sugar as the only source of energy. The sugar fermentation test was carried out using the method of Nair and Surrendran (2005). 1% solution (1g of the sugar in 100 ml of distilled water) was prepared separately in Maccartney bottles. 1 ml of 1% solution was added to 9 ml of phenol-red peptone water in test tubes and mixed gently. The test tubes were covered with cotton wool and sterilized at 121°C for 15 min. On cooling, the tubes were inoculated with bacteria isolate and incubated at 37°C for 48 hours. Colour change from red to yellow indicated acid production during fermentation.

Fish sample analysis: The parts of fish collected (fish skin) measuring 1 g each were cut and weighed out aseptically and introduced into 10 ml of sterile peptone water in a test tube. It was properly shaken to homogenise the sample. A 10-fold serial dilution of each of the samples was carried out using peptone water as the diluent. Appropriate dilutions (10^{-2} and 10^{-3}) of 0.1 ml of the sample were plated in sterile plates of nutrient agar plates, eosin-methylene blue, and MacConkey agar plates for the culture of bacteria. All culture plates were incubated at 37 °C aerobically for 24-48 hours. Developed colonies were counted to obtain a total viable count and coliform count (Sharmin et al., 2014). Discrete colonies were obtained by sub culturing into nutrient agar plates and were subsequently identified using standard methods. Identification of the bacterial isolates was accomplished by the observation of colony characteristics, Gram reaction, and biochemical tests (Cheesbrough, 1984). The characterization of the isolates was performed by employing Gram staining reaction and the catalase test.

Data analysis

The analysis was performed in triplicate, and the results were expressed as mean ± standard deviation using the Statistical Package for the Social Sciences (SPSS).

Results

Descriptive statistics on the length (cm) and weight (g) measurements of fish are presented in Table 1. The total length ranged from 29.3 to 42.5 cm with a mean value of 37.56 for *P. typus*, 29.7 to 41.7cm with a mean of 38.18 for *P. senegalensis,* and 28.5 to 39.1cm with a mean of 35.26 for *P. elongtatus.* The body weight ranged from 280 to 610.27 g, 290 to 605.73 g, and 270 to 583.71g for samples *P. typus, P. senegalensis,* and *P. elongtatus,* respectively. From the table, the weights of *P. typus, P. senegalensis,* and *P. elongtatus* are not significantly different from each other at P<0.05. Also, the total lengths of the three species were not significantly different at P<0.05.

Tablo 1. Summary of morphometric characteristics of the three Sciaenid fishes

Fish species	TL (Cm) Min	Max	Mean	SD	BW (g) Min	Max	Mean	SD
P. typus	29.3	42.5	37.56^a	4.95	280	610.27	502.47^b	130.81
P. senegalensis	29.7	41.7	38.18^a	4.94	290	605.72	522.50^b	133.63
P. elongtatus	28.5	39.1	35.26^a	4.30	270	583.71	465.80^b	126.27

Means with same superscripts along same column shows no significant difference (P>0.05)
TL, Total length; BW, Body weight; SD, Standard deviation.

The mean microbial counts of the skin of the three fish species on nutrient agar for general purpose are shown in Table 2. The results revealed significant differences between the three Sciaenid fishes (p<0.05) with the highest loads for *P. typus* (2.560×10^3 CFU g^{-1}) followed by *P. elongtatus* (1.360×10^3 CFU g^{-1}), and *P. senegalensis* (8.00×10^2 CFU g^{-1}). However, the results with other media apart from MSA showed no significant differences among the three Sciaenid fishes (p>0.05). Mannitol Salt Agar (MSA) is used as a selective and differential medium for the isolation and identification of *Staphylococcus aureus.* No bacterial load was detected on the skin of *P. typus* and *P. elongtatus,* while *P. senegalensis* had 3.0×10^{-1} CFU g^{-1}. On SSA Agar, the bacterial counts between species were highest in *P. senegalensis* (6.326×10^2 CFU g^{-1}), followed by *P. elongtatus and P. typus,* with mean values of 5.90×10^2 CFU g^{-1} and 5.350×10^2 CFU g^{-1}, respectively. Thiosulfate-Citrate-Bile Salts-Sucrose Agar, or TCBS Agar, is a selective and differential culture medium that is used for the isolation and cultivation of *Vibrio cholerae* and other *Vibrio* species. Bacterial counts on TCBS were highest in *P. senegalensis* with a mean value of 3.15×10^2 CFU g^{-1}, *P. typus* had 2.925×10^2 CFU g^{-1}, and *P. elongtatus* had 2.26×10^2 CFU g^{-1}. Potato Dextrose Agar (PDA) contains dextrose as a carbohydrate source, which serves as a growth stimulant, and potato infusion, which provides a nutrient base for the luxuriant growth of most fungi. Fungi isolated on TCBS (Table 2) were low in all three fish species, with the highest mean value of 1.60×10^1 CFU g^{-1} recorded on *P. typus,* followed by *P. elongtatus* (1.50×10^1 CFU g^{-1}) and *P. senegalensis* (1.25×10^1 CFU g^{-1}).

Table 2: Total microbial counts of the skin of the three Sciaenid fishes using bacteria and fungi agar media

Media	*P. typus*	*P.senegalensis*	*P. elongtatus*	p-value
NA	2.560×10^3	8.00×10^2	1.360×10^3	0.45
MSA	-	3.0×10^{-1}	-	-
SSA	5.350×10^2	6.326×10^2	5.90×10^2	0.67
TCBS	2.925×10^2	3.15×10^2	2.26×10^2	0.90
PDA	1.60×10^1	1.25×10^1	1.50×10^1	0.64

Keys:
- SSA- Salmonella Shigella agar for culturing salmonella and shigella
- NA- Nutrient agar for total viable count of bacteria.
- MSA- Mannitol Salt Agar selective for staphylococcus.
- TCBS- Thiosulfate citrate bile salt agar for isolation of vibrio. = negative
- PDA- Potato dextrose agar for isolation of fungi

The pattern of bacterial reactions to Gram and biochemical tests is shown in Table 3. The results showed that a total of eight bacterial isolates were identified from the croaker fish species during the sampling period, with the following bacterial species: *Shigella* species, *Aeromonas* species, *Pseudomonas flourescens, Vibrio* species, *Staphylococcus* species, *Salmonella* species, *Bacillus* species, *and Micrococcus* species. Only *Shigella* species and *Vibrio* species were Gram-negative, and the other six were Gram-positive.

Table 3: Biochemical and tentative identification of the bacteria isolated from croaker species

Gram reaction	TRIPLE SUGAR IRON TEST			Catalase	Citrate	Indole	Suspected organism
	Dextrose	Lactose	Sucrose				
-	+	-	-	+	+	-	*Shigella sp.*
-	-	-	-	+	+	+	*Aeromonas sp.*
-	+	+	+	+	+	-	*Pseudomonas flourescens*
-	-	-	-	-	-	+	*Vibrio* sp.
+	+	+	+	-	+	+	*Staphylococcus* sp.
-	+	+	+	+	+	+	*Salmonella sp.*
+	+	+	+	+	+	+	*Bacillus* sp.
+	-	-	-	+	+	+	*Micrococcus* sp.

Keys: - negative, + positive reaction

The number of occurrences of fungal isolates (Table 4) revealed the fungi found on the skin of the three fish species, including *Aspergillus flavus, Aspergillus niger, and Rhizopus stolonifera.* In terms of frequency of occurrence, *Rhizopus stolonifera* occurred most, with 45.45% for both *P. senegalensis* and *P. elongtatus,* followed by *Aspergillus flavus* with 36.36% for *P. typus* and *P. elongtatus,* while *Aspergillus niger* occurred least on the three croaker fish species.

Table 4: Percentage frequency of fungal isolates during the sampling period on the croaker fish species

Sample	Fungal isolates	Frequency	Percentage (%)
P. typus	*Aspergillus flavus*	4	36.36
	Aspergillus niger	3	27.27
	Rhizopus stolonifera	4	36.36
	Total	**11**	**100.00**
P. senegalensis	*Aspergillus flavus*	3	27.27
	Aspergillus niger	3	27.27
	Rhizopus stolonifera	5	45.45
	Total	**11**	**100.00**
P. elongtatus	*Aspergillus flavus*	4	36.36`
	Aspergillus niger	2	18.18
	Rhizopus stolonifera	5	45.45
	Total	**11**	**100.00**

Discussion

The mean bacterial counts on the skin of the three fish species using Nutrient Agar for a general-purpose count showed that *P. typus* had the highest loads (2.560 x 10^3 CFU g-^{1}), followed by *P. elongtatus* with 1.360 x 10^3 CFU g-^{1}, and *P. senegalensis* (8.00 x 10^2 CFU g^{-1}). The total viable count, which fell between 1.0 x 10^1 cfu/g and 4.5 x 10^3 cfu/g, was also in tandem with the work of Adeboyejo et al., (2021), who had a total viable count of between 5.8 x 10^2 cfu/g and 4.9 x 10^3 cfu/g. Mannitol Salt Agar (MSA) used for the isolation and identification of *Staphylococcus aureus* revealed that *P. senegalensis* had 3.0 x 10^{-1} CFU g-1, and *Staphylococcus aureus* was detected on the skin of *P. typus* and *P. elongtatus.*

The value recorded for *P. senegalensis* was lower than the findings from Ndiaye (1998). However, *Staphylococcus aureus* causes intoxications by forming toxins (Kutlu et al., 2011), and the enterotoxin produced by *S. aureus* leads to Staphylococcal poisoning. The enterotoxins produced by *S. aureus* are of major public health importance because they can make several types of toxins, many of which are responsible for food poisoning, and cause gastroenteritis after consumption of fish products. On SSA agar, the bacterial counts between species were highest in *P. senegalensis* (6.326 x 10^2 CFU g^{-1}) followed by *P. elongtatus* and *P. typus,* with mean values of 5.90 x 10^2 CFU g^{-1} and 5.350 x 10^2 CFU g-^{1} respectively.

Bacterial counts on TCBS were highest in *P. senegalensis* with a mean value of 3.15 x $10^2 10^1$ CFU g^{-1}, *P. typus* had 2.925 x 10^2 CFU g^{-1}, and *P. elongtatus* had 2.26 x 10^2 CFU g^{-1}. The values recorded in this study were lower than the threshold recommended by NAFDAC for public health, which is between 5.0 × 10^5 and 61.0 × 106^6 CFU g-^{1}. According to the International Commission on Microbiological Specification for Food (ICMSF), the maximum recommended bacterial count for a good-quality product is 5.0 x 10^5 CFU g^{-1} (5.7 log10 CFU/g^{-1}) (ICMSF, 1998) and the maximum for marginal acceptable quality products is 1.0 x 10 (7 log CFU g^{-1}). Fungi isolated were low in all three fish species, with the highest mean value of 1.60 x 10^1 CFU g^{-1} recorded on *P. typus,* followed by *P. elongtatus* (1.50 x 10^1 CFU g^{-1}) and *P. senegalensis* (1.25 x 10^1 CFU g^{-1}). The values observed in this study were within the approved safety standard by NAFDAC.

The prevalent bacteria in this study were *Shigella* species, *Aeromonas* species, *Pseudomonas flourescens, Vibrio* species, *Staphylococcus* species, *Salmonella* species, *Bacillus* species, and *Micrococcus* species. The most prevalent bacterial pathogens affecting fish worldwide are Aeromonas and Pseudomonas (Kayansamruaj et al., 2017), in addition to the genus Vibrio (Kannapiran et al., 2009). The microorganisms isolated from this research were similar to those reported by Okonta and Ekelemu (2005) and Bankole et al. (2005). The fungal count found on the skin of the three fish species includes

Aspergillus flavus, Aspergillus niger, and *Rhizopus stolonifera.* The fungi isolated in this study are all opportunistic pathogens (Job et al., 2016) of medical and veterinary importance.

The presence of toxigenic fungi, for example, some species of *Aspergillus,* in foods as contaminants increases the risk for mycotoxins production, which could induce gastrointestinal and metabolic disturbances when contaminated foods like fish are consumed (Martin, 2008). The microorganisms isolated from this study were similar to the microorganisms reported by Oranusi et al. (2014). The microorganisms isolated and identified from the fresh fish samples can be said to be normal flora of the fish, e.g., *Bacillus sp*ecies (Ola and Oladipo, 2004). The normal microbial flora of the fish is not initially harmful, as they even help in preventing the invasion of the fish flesh by other microorganisms, but they become pathogenic when there is an enabling environment that promotes their growth, e.g., bad handling, which can lead to bruises, poor hygiene, and delayed processing and preservation of the fish after harvest.

Conclusion

The results of the current study confirmed the existence of contaminating microorganisms in the three croaker fish. The isolated bacteria in this study were *Shigella* species, *Aeromonas* species, *Pseudomonas flourescens, Vibrio* species, *Staphylococcus* species, *Salmonella* species, *Bacillus* species, and *Micrococcus* species. Some of the fungi species identified in this study were *Aspergillus flavus, Aspergillus niger,* and. *Rhizopus stolonifera.* The organisms isolated in this study are of public health significance, and their presence suggests that foodborne illness could arise if these fish are consumed in an undercooked state. Since these microorganisms could contaminate fish and therefore be a source of food poisoning, cooking of fresh fish, especially croaker fish species, should be done properly to eliminate zoonotic infections from fish, especially in this area.

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Ethical approval

The author declares that this study complies with research and publication ethics.

Informed consent

Not available.

Conflicts of interest

There is no conflict of interests for publishing of this study.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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Author contribution

Olaniyi Alaba Olopade contributed to actual design of the study. Olaniyi Alaba Olopade, Henry Eyina Dienye, and Jose-Maria Akpofure Sharta contributed significantly to the investigation. Adedayo Alidu Aranyo and Olugbojo Joseph contributed to the design of the data. Olaniyi Alaba Olopade helped with editing and revising. Olaniyi Alaba Olopade and Henry Eyina Dienye, contributed to supervision and validation of the study.

References

Adeboyejo, A.O., O.E.D. Clark and O. P. Fajana. (2021). Microbial load in five demersal marine fin and shell fishes from Nigerian Coastal Waters. *Advances in Oceanography & Marine Biology* 2(4): 1-7 DOI: 10.33552/AOMB.2021.02.000543

AOAC. (1998). Bacteriological analytical manual, 8th ed. Association of official analytical chemists, Washington, DC.

Bankole, M.O., D. S. Oladimeji and A. M. Omemu. (2005). Microorganisms associated with the palms of food under in Abeokuta Metropolis. In: The Book of Abstracts of the 29th Annual Conf. Act Lienural Meeting (Abeokuta, 2005 on by Nigerian Society for Microbiology (NSM), University of Agriculture, Abeokuta, from 6-10th pp: 17.

Cheesbrough, M. (1984) Medical Laboratory Manual for Tropical Countries. Volume Two, Cambridge University Press, Cambridge, 294-299

Cheesbrough, M. (2002). Medical Laboratory Practice in Tropical countries. Part 2, Cambridge University Press, United Kingdom, 157 – 200.

Feldhusen, F. (2000). The role of seafood in bacterial foodborne diseases. *Microbes and Infection*, vol. 2, no. 13, pp. 1651- 1660. https://doi.org/10.1016/S1286-4579 (00)01321-6

Frazier, W. C. and D. C Westhoff. (1995). Contamination, preservation, and spoilage of fish and other seafoods. In Food Microbiology, p. 243. 4th edn. New Delhi: Tata Mcgraw-Hill publishing company ltd

ICMSF (1998). (International Commission on Microbiological Specification for food). Fish and fishery products. Microorganisms in Food 6. Microbial Ecology of Food Commodities. London: Blackie Academic and professional. Pp 130-189.

Job, M. O., S.E. Agina and H.S Dapiya. (2016). Occurrence of Aflatoxigenic Fungi in Smoke-dried Fish Sold in Jos Metropolis. *British Microbiology Research Journal*, 11, 1-7. https://doi.org/10.9734/BMRJ/2016/21465

Kannapiran, E., J. R. Ravindran, R. Chandrasekar and A. Kalaiarasi. (2009). Studies on luminous, *Vibrio harveyi* associated with shrimp culture system rearing *Penaeus monodon*. *J. Environ. Biol*., 30: 791-795. PMID: 20143707

Kayansamruaj, P., D. H. Dong, I. Hirono, H. Kondo, S. Senapin, et al., (2017). Genome characterization of piscine 'Scale drop and muscle necrosis syndrome'- associated strain of *Vibrio harveyi* focusing on bacterial virulence determinants. *J. Applied Microbiol.*124: 652-666. DOI: 10.1111/jam.13676.

Kutlu, S., A. F. Yesilsu, and S. Firidin. (2011). Aquatic products as a source of contamination. *Yunus Research Bulletin*, vol. 3, pp. 20-25.

Martin, A. (2008). Fish Processing: Biochemical Applications. Chapman and Hall, London.

Ndiaye, A. (1998). Contribution à l'évolution de la qualité bactériologique des produits de la pêche destinés à l'exportation en 1996 et 1997. *Thèse de Médecine Vétérinaire*, Dakar. pp. 73.

Nunoo, F. K. E., E. Sossoukpe, A. Adite and E.D. Fiogbe. (2013). Food habits of two species of Pseudotolithus (Sciaenidae) off Benin (West Africa) near shore waters and implications for management. *International Journal of Fisheries and Aquaculture* 5 (6): 142-151.

Ola, J.B. and A. E. Oladipo. (2004). Storage life of croaker (*Pseudotholitus senegalensis*) in ice and ambient temperature. *African Journal of Biomedical Research* 7 (1): 13-17.

Ogur, S. (2022). Pathogenic bacteria load and safety of retail marine fish. *Brazilian Journal of Biology*, vol. 82, e262735 https://doi.org/10.1590/1519-6984.262735

Okonta, A.A. and J.K.S. Ekelemu. (2005). A preliminary study of micro-organisms associated with fish spoilage in Asaba, Nigeria. Proceedings of the 20th Annual Conference of FISON Port Harcourt. 14-18th NOV. 2005/ 557-559.

Oranusi, S., T. U. Obioha, T. U. and B.T. Adekeye. (2014). Investigation on the microbial profile of frozen foods: Fish and Meat. *International Journal of Advanced Research in Biological Sciences*. 1(2): 71-78.

Osibona, O. A. (2011). Comparative study of proximate composition, amino and fatty acids of some economically important fish species in Lagos, Nigeria. *African Journal of Food Science* 5 (10): 581-588.

Rahman, M. M., F. Rahman, F. Afroz, F. Yesmin, K. K. Fatema, K. K. Das and R. Noor. (2012). Prevalence of pathogenic bacteria in shrimp samples collected from hatchery, local markets and the shrimp processing plant for export quality frozen shrimp. *Bangladesh Journal of Microbiology* 29(2): 7-10.

Sharmin, M., Nur, I.T., Acharjee, M., Munshi, S.K. and Noor, R. (2014). Microbiological profiling and the demonstration of in vitro anti-bacterial traits of the major oral herbal medicines used in Dhaka Metropolis. SpringerPlus, 3, 739. https:// doi.org/10.1186/2193-1801-3-739

Sugita H., Tokuyama K., Deguchi Y. 1985 - The intestinal microflora of carp Cyprinus carpio, grass carp *Ctenopharyngodon idella* tilapia *Sarotherodon niloticus - Bull. Japan. Soc. Sci. Fish.* 51: 1325-1329.

Wei, J., X. Guo, H. Liu, Y. Chen and W. Wang. (2018). The variation profile of intestinal microbiota in blunt snout bream (*Megalobrama amblycephala*) during feeding habit transition. *BMC Microbiology*, 18(1), 99 10.1186/s12866-018 1246-0.

Zaky, M.M.M. and M. A. M. Salem. (2015). Environmental Factors Influencing Antibiotic Resistant Bacterial Pathogens in Polluted Lake Manzala, Egypt. *Journal of Bacteriology & Parasitology*, 6, Article ID: 1000249. DOI: 10.4172/2155-9597.1000249