

BACTERIOLOGICAL PROFILE OF SELECTED FISH SPECIES AND WATER SAMPLE FROM OTUOCHA RIVER ANAMBRA STATE.

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ABSTRACT

*Attempt to ensure fish food security is a continuous process and food safety is a global concern for consumers and food industry. Fish is an important source of healthy and cheap source of animal protein that among its numerous benefit fish is widely acceptable, across socio-economic, age and religious barrier. However, supply of contaminated free fish is desirable to ensure safe consumption and a healthy consumer public. The objective of the study is to provide baseline information on the microbial quality of Otuocha river, hence establish the microbial quality of fish caught in Otuocha river. Five fish species (*Aruis gigas*, *Bagrus bayad*, *Schilbe mystus*, *Chrysichthys nigroditatus* and *Protopterus annectens*) and water sample were taken from Otuocha fish landing site and river respectively and transported to laboratory. The fish samples were cleaned with sterile distilled water and 1 g of skin, gills, intestine cut aseptically and labelled along with water sample before microbial analysis following standard method. The result obtained in water and fish samples showed high microbial load in Otuocha river and was attributed to anthropogenic sources. Nine different bacteria were isolated in fish samples while seven were isolated in water sample. The study concluded that Otuocha river has high microbial load, hence recommend that fish from Otuocha river should be properly cooked/smoked before consumption and sanitary standard of Otuocha river and its environs be improved while public enlightenment on waste disposal be undertaken.*

Key words: Microbial load, fish, water, Otuocha river

INTRODUCTION

Generally, attempt to ensure fish food security is a continuous process and food safety has been a major global concern for the consumers and those in the food service industry (Jacxsense *et al.*, 2009). Fish and fisheries products have become an increasing important source of protein and other elements necessary for balanced nutrition and maintenance of healthy body (FAO, 2012). It also constitutes the cheapest source of animal protein in Africa (Clucas and Ward, 1996). Fish have always been an important component of human food even for the poorest of the poor, particularly around lakes, rivers, deltas, floodplains, coastal areas and particularly in Island. In Nigeria, fish is the preferred source of much desired animal protein compared to other sources because it is comparatively

cheaper and highly acceptable (Adebayo-Tayo *et al.*, 2012) that fish consumption accounts for about 35%-40% of animal protein intake in Nigeria (Adedeji and Ibrahim 2013). Fish provides faster and fresher access to one of the healthiest and cheapest sources of animal protein. Hence, fish are widely acceptable on the menu card and form a much-cherished delicacy that cuts across socio-economic, age, religious and educational barriers (Adebayo-Tayo *et al.*, 2008). As a major source of food, fish provide livelihood for millions of people along the value-chain. It is a most important animal dietary protein available in the world, and represents about 14% of all animal protein on a global basis (Abolagba and Melle, 2008), while FAO (2002) submitted that most of the world population (56%) derive at least 20% of its animal protein intake from fish. Fish is regarded a healthier meat option due to the high content of long chain poly unsaturated fatty acids (LUPUFA'S) which are associated with improving health and preventing disease of old age (Kabaherda *et al.*, 2009).

However, supply of contaminated free fish is desirable to ensure safe consumption and a healthy consumer public. This is because consumption of fish may sometimes cause disease due to infection (FDA, 2011) which had been established in many of the food-borne illnesses and intoxication (Adedeji *et al.*, 2011). Some of these infections have been specially associated with pathogens which are resistant to antibiotics (Adebayo-Tayo *et al.*, 2012a). Aquatic systems that harbour the fish may be the source of contaminants due to indiscriminate deposition of faecal material and other environmental wastes discharged into natural water (Adedeji *et al.*, 2011). Thus pollution can significantly affect the quality of fish because the fish species inhabiting polluted waters have already compromised their original healthy status (Adeyemo, 2003). Fish are carriers of microbes and other health hazards (Yagoub 2009, Adebayo *et al.*, 2012) and may harbour pathogens on or inside its body after exposure to contaminated water or food (Aliyu *et al.*, 2016). Therefore, the presence or absence of pathogens in fish product is a function of the harvest environment, sanitary conditions, and practices associated with equipment and personnel in the processing environment (FAO, 2003).

Water bodies are contaminated through runoffs, sewage, agricultural wastes, and waste produce by increase human activities; these are usually high in

organic matter nutrient. Hence, they could cause increase in the microbial flora of the water bodies, thereby resulting in high heterotrophic bacteria counts. The type of microorganism found associated with fish depends on the aquatic habitat of fish and are known to be affected by certain factors like salinity level and bacterial load of the habitat (Delier *et al.*, 2000). Adebayo-Tayo *et al.* (2012) reported upon quality assessment for tropical freshwater fish species that differences exist based on location and time. In some developing nations, there is poor waste disposal which ultimately leads to pollution of bodies of water as the last point of discharge. Expectedly, the fish in such bodies of water become contaminated and a source of human infection. Since, changes in microbial population are one of the indices for assessing fish quality (Sallam, 2007) contamination concern has been on high loads of microorganisms like *Salmonella* species, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Okonko *et al.*, 2008). Though in fish, non-indigenous pathogens may not be pathogenic but could cause infection if ingested by man (Aliyu *et al.*, 2016). Few infectious agents in fish are able to infect humans, some exceptions exist that may result in fatalities (Adebayo *et al.*, 2012). However, the greatest risk to human health is due to the consumption of raw or insufficiently processed fish and fish products (Yagoub 2009, Adebayo *et al.*, 2012, Adedeji *et al.*, 2012). Novotny *et al.* (2004) had reported that human infections caused by pathogens transmitted from fish or the aquatic environment is quite common and depend on the season, patient's contact with fish and related environment, dietary habits and the immune system status of the exposed individual.

There is dearth of information on the public health importance associated with fishes caught and sold at Otuocha river in South-East Nigeria. Thus, it was the objective of this study to examine selected commercially important fish species of the Otuocha river for incidence and intensity of microbial load. Considering the level of pollution and the influx of fish consumers to Otuocha river, there is an urgent need to establish the microbial quality of fish caught in Otuocha river. Hence, this study will help to provide baseline information on biological pollution status of Otuocha river.

MATERIALS AND METHODS

Otuocha river: Otuocha river is located in Umueri, a major community of Umuobanam clan in Anambra East Local Government Area of Anambra State. Otuocha River lies on latitude 6°33'33"N and longitude 6°51'12E. It is a tributary of river Niger, on the north east by Ezu River and south east and south west by Akor River. Also close to the river is the

Otuocha market which terminated in the river and Nigerian Marine Police, to manage and see to the activities carried out in Otuocha River.

Samples collection: One sample of live *Aruis gigas*, *Bagrus bayad*, *Schilbe mystus*, *Chrysichthys nigroditatus* and *Protopterus annectens* were collected from landing site of Otuocha river. The fish samples were transported in disinfected labelled jerry cans containing the river water, to the Laboratory of the Department of Microbiology and Brewery, Nnamdi Azikiwe University, Awka. Upon arrival the samples were cleaned with sterile distilled water and one gram of their skin, gill and intestine was cut aseptically, labelled appropriately (*Aruis gigas*: sample A, *Bagrus bayad*: sample B *Schilbe mystus*: sample C, *Chrysichthys nigroditatus*: D and

Protopterus annectens: E) for microbiological analysis. Water samples for microbiological analysis were collected in a sterile amber coloured glass bottle. The samples were collected by dipping the sample bottle into the river to a depth of 30 cm below water surface, opened, allowed to fill up with water and then corked while still under the water (APHA, 1985). The water was labelled, placed in iced box and transported to the Laboratory for microbial analysis.

Water sample analysis

Total bacterial count: Ten-fold serial dilution of the water sample was done and appropriate dilutions were placed on Nutrient Agar plates and incubated for 24 hours. Thereafter, the developing colonies were counted and the Total bacterial count calculated thus:

$$TBC \text{ (CFU/ml)} = \frac{N}{VD}$$

Where: TBC = Total bacterial count

V = Volume of inocula plated

N = Number of colonies, developing on plate, that were counted

D = Dilution factor+

CFU/ml= Colony forming unit per millilitre

Determination of total coliform using membrane-filter technique: A 50 ml water sample was filtered through a membrane filter which retains the bacteria found in the sample. The filters containing bacteria were placed on an absorbent pad saturated with lactose broth and incubated at 35 °C for 2 hrs. The filters were then transferred to an absorbent pad saturated with Eosin-methylene blue and MacConkey media and incubated for another 21 hrs at 35 °C . Developing colonies were then counted under magnification and reported per 100 ml of original sample. One in ten dilution of the water sample was prepared by transferring 5 ml of the water sample into 45 ml of sterile water contained in 100 ml conical flask and filtered in the membrane filter apparatus. The calculations were thus:

No. of colonies per 100 ml

$$= \frac{(\text{No. of colonies counted on plate}) \times 100}{\text{Volume filtered}}$$

Fish sample analysis: Different parts of fish (skin, gill and intestine) 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 homogenize the sample. A 10-fold serial dilution of each of the sample was carried out using peptone water as the diluents. Appropriate dilutions (10^{-2} and 10^{-3}) of 0.1 ml of the sample were plated in sterile plates of Nutrient agar (NA) 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 total viable count and coliform counts respectively. Discrete colonies were obtained by sub culturing into Nutrient agar plates and were subsequently identified using standard methods.

Characterization and Identification of Bacteria in water and fish sample: Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests (Cheesbrough, 1984). The characterization of the isolates was performed, by employing Gram staining reaction, Catalase test, Citrate test, Sugar fermentation test, Oxidase test, Coagulase test, Motility test, Indole test, Methyl Red and Voges proskauer test as described by Bergey and Holt (1994).

RESULT

Bacteria enumeration of water sample: The result from the bacteria enumeration of water sample shows that the total bacteria count (TBC) of plate count 10^{-2} and 10^{-1} was higher (1.42×10^5 and 1.72×10^4 cfu/ml) than the World health organisation (WHO) standard (1.0×10^3 cfu/100ml) according to Atiribom and Kolndadacha (2014).

Biochemical characterization of isolates from water sample: Result of biochemical analysis shows that seven (7) isolates were identified which include, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, these are coliform bacteria while others include *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus* species as shown in table 3.

Bacteria enumeration of fish samples: The result of the bacterial enumeration of the five selected fish samples as shown in table 4 shows that the total bacteria load for serial dilution 10^{-2} ranged from 2.60×10^5 to 2.08×10^5 cfu/g for samples collected from the skin across different fish samples. But the skin of fish sample from sample D (2.60×10^5 cfu/g), recorded high bacteria load compared to other samples collected from the skin across different fish samples in this order B, C, A, E respectively. The intestine recorded the

highest microbial load in all the samples examined and the microbial load of the intestine ranged from 2.84×10^5 cfu/g in sample D to 2.30×10^5 cfu/g in sample E. The gills recorded the least microbial load which ranged from 2.16×10^5 cfu/g in sample C to 1.28×10^5 in sample B. The microbial load of the selected fish samples for serial dilution 10^2 range from 2.84×10^5 to 1.28×10^5 cfu/g. Similarly the result of the selected fish samples for serial dilution 10^{-3} follow the trend observed in serial dilution 10^{-2} . In this case, the intestinal part of the fish recorded the highest microbial load that ranged from 2.04×10^6 cfu/g in sample D to 1.96×10^6 cfu/g in sample A. While microbial load of samples collected from Skin ranged from 1.96×10^6 cfu/g in sample D to 1.48×10^6 cfu/g in sample A, and samples from the gills recorded the least microbial load among the samples. The microbial load in the fish sample range from 2.04×10^6 cfu/g to 9.40×10^5 cfu/g. Generally the microbial load of the selected fish samples was above WHO standard (1.0×10^3 cfu/g) according to Atiribom and Kolndadacha (2014). The intestine recorded the highest microbial load in all the samples (A-E) for the both serial dilution (10^{-2} , 10^{-3}) followed by skin while the gills had the least microbial load.

Biochemical characterization from the fish samples:

The result of the biochemical characterization from the five fish samples in table 5 shows the presence of the following bacteria; *Streptococcus* species, *Bacillus* species, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Micrococcus* species, *Klebsiella pneumoniae*, *Pseudomonas* species and *Serratia* species.

DISCUSSION

The result revealed high microbial load above recommended safety standard from both water and fish samples. This may be attributed to anthropogenic activities such as washing, bathing, indiscriminate dumping of refuse, open defecation in and around the river and also flood from Otuocha market which empty into the river. The difference in the bacteria load of the five selected fish samples agrees with the fact that the presence of bacteria in natural aquatic ecosystem is dependent upon the rate of contamination and the equilibrium that is established between bacteria proliferation in that environment and the rate of their elimination (Lejeune *et al.*, 2001). The total bacteria count in both fish and water samples were higher than that of WHO standard (1.0×10^3 cfu/100 ml). This may be because Otuocha people and marketers at Otuocha market use Otuocha river as dumping ground, for open defecation and due to lack of waste management and this agrees with Atiribom and Kolndadacha (2014) that most people in this part of the world see rivers as dumping ground for every kind of wastes. The result shows that both water and fish samples contain

pathogenic bacteria and this several researchers (Oyeleke and Istifanus, 2008., Raji and Ibrahim, 2011 and Novotny *et al.*, 2004) had reported could cause water-food borne infection when ingested through contaminated water or food (fish). The result reported presence of human pathogens in water and fish samples. This agrees with Mitchell (1972) who states that freshwater fish may harbour human pathogens after exposure to contaminated water or food sources. The microbial loads were higher in fish samples than water sample. This could be due to some level of additional contamination by fishermen after the fish were caught probably due to unhygienic handling practices. Fish intestines harbour more bacteria load than the skin and gills of the selected five fish species examined. This corroborate with Kasing *et al.* (1999) who observed that the intestine of all fish examined harboured most number of bacteria species. Atiribom and Kolndadacha (2014) reported lower bacteria count compared to this study in both water and fish samples. They reported that the total bacteria count of water sample ranged from 1.2×10^2 to 1.4×10^3 cfu/ml which is low compare to this study. Similarly, they also reported that the total bacteria count of the intestine of sampled fish ranged from 3.5×10^3 to 3.1×10^4 cfu/g. Such variation in total bacteria count could be associated with, diet, species of host animal and their physiological content (Sugital *et al.*, 1985). Bacteria flora of some freshwater fishes in the tropics showed that *Aeromonas* species was most predominant microorganism isolated from the skin, gills and intestine of the fish which is contrary to the findings of this study which did not detect presence of *Aeromonas* species in either water or fish samples. The high level of microbes in the water and fish samples, from Otuocha river probably revealed the attitudes of Otuocha people and marketer at Otuocha market towards waste disposal and management. This agreed with the report of Dalgaard *et al.* (2006) that pollution of the aquatic habitats with faecal matter is mostly from human activities. The result reveals that both the fish and the water samples harbour microbes above WHO standard. This agreed with the opinion of Okonko *et al.* (2012) and Adebayo-Tayo *et al.* (2012b) that the microbial composition of fish depends upon the microbial counts of water in which they live. This study isolated different bacteria from both samples analysed. However, the presence of the pathogens in the fish samples could pose a potential public health threat to consumers. Fapohunda *et al.* (1994) and Velusamy *et al.* (2010) reported that pathogens such as *Salmonella* species, *Staphylococcus* species, *Klebsiella* species, *Pseudomonas* species and *Escherichia coli* are responsible for major food-borne outbreaks worldwide. Such organism should not be present on freshly-caught fish as opined by Chattopadhyay (2000). This finding

was in agreement with the findings by Gram and Dalgaard (2002), who reported that these organisms isolated were the major organism of freshly caught fish from polluted water. Most of the organisms isolated could cause food poisoning such as Shigellosis (*Shigella* species) and *Salmonellosis* (*Salmonella* species) as reported by Acha and Szyfres (2003). Also, enterotoxins produced by *Staphylococcus aureus* as opined by Vieira *et al.* (2001) are serious cause of gastroenteritis after consumption of fish and related products. The result reveals that sample D and C recorded the highest level of microbes; this scenario was similar with the finding of Ekeocha *et al.* (2017) who reported high microbial load in *Protopterus annectens* and *Chrisitichys nigrodigitatus*. However, these they reported could be as a result of lipobacteria (fat loving bacteria) since these species are fatty fishes. Also, among the fish species, sample D harboured more bacteria than the rest of the fish species. This could be because it is a demersal species that feeds closer to the bottom where there is more organic matter in the sediment leading to a concomitant increase in the number of microbial flora. The occurrence of *Bacillus* species in both water and fish samples could be as a result of the prevalence of their spores in the environment. This was in agreement with the report of Robert (2010) that when fish are caught close to land, organisms of terrestrial origin mainly of the aerobic spore forming *bacilli* may be isolated. *Bacillus* species are implicated in causing a wide range of infectious diseases including abscesses, *Bacteremia/Septicemia*, wound and food borne infections, ear infections, endocarditis, meningitis, ophthalmitis, osteomyelitis peritonitis and respiratory and urinary infections (Morales *et al.*, 2004). The presence of *Staphylococcus aureus* might have been through handling as it is a normal flora of the human skin (Okonta and Ekelemu, 2005). It could also be as a result of its prevalence in the environment especially in soil, plants, insects, animals, human and both fresh and brackish water. The pathogenicity of food-borne *S. aureus* is associated with the ability of some strains to produce enterotoxins. The presence of bacteria isolates in different fish samples analysed agrees with report of several researchers that the normal bacteria flora of fish is a direct reflection of the bacteria flora of the water which they swim (Horseley, 1973). Showell and Ama-Abasi (2015) reported that the difference in bacteria isolates observed among the different fish species may be explain by factors such as bacteria host specificity and food type (Eddy and Jone, 2002) and water resource (Verner –Jeffreys *et al.*, 2003). Another reason for the variation in the quality of the flora could be antagonism among gut bacteria (Sun *et al.*, 2008). Olafson (2001) and Vine *et al.* (2006) also reported that both marine and freshwater fish have been shown to have a specific

indigenous gut microbiota and may change with fish age, nutritional status, and environmental conditions.

CONCLUSION AND RECOMMENDATION

The bacteriological analysis of Otuocha river reveals high load of bacteria in water and fish samples analysed and some of these bacteria are known to be pathogenic to both fish and humans. Therefore, it is apparent that greater attention must be given to the microflora of fish especially those of the gastrointestinal tract and to the natural water body holding them since most of the fish consumed in Nigeria is purchased directly from the fishermen without passing through any health safety checks. It is recommended that fish caught from the Otuocha river should be properly cooked or smoked before consumption to avoid zoonotic diseases. Also sanitation within Otuocha market and its environs should be improved by the provision of public toilets and enactment of effective policy for the collection and disposal of waste in Otuocha especially in the market. Contamination of water bodies should be discouraged through public enlightenment.

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Table 1: Total bacteria count of water sample

Water sample	Total bacteria count(10^{-1})	Total bacteria count(10^{-2})
Water sample	1.72×10^4	1.42×10^5

Table 2: Total coliform count

Water sample	Number of colonies on plate	Number of colonies per 100 ml	Nature of colonies
Water sample	32	64	Pink, without sheen
			Pink, mucoid looking colonies
			Colourless looking colonies

Table 3: Morphological and biochemical identification of the various isolates from the water sample

Key: Gram: Gram reaction, **Cat:** Catalase test **Mot:** Motility test **Ind:** Indole test, **MR:** Methyl-red test **VP:** Voges-Proskauer test **Cit:** Citrate Utilization test **Sugar Fermentation Tests Lac:** Lactose fermentation, **Glu:** Glucose fermentation, **Suc:** Sucrose fermentation, **Fru:** Fructose fermentation, **Mal:** Maltose fermentation, + : Positive, - : Negative, **A:** Acid production only, **G:** Gas production only, **AG:** Acid and Gas production

Isolate	Form	Surface	Colour	Margin	Elevation	Opacity	Gram	Cat	Mot	Ind	MR	VP	Cit	Lac	Glu	Suc	Fru	Mal	Identity
A	Circular	Smooth	White	Irregular	Flat	Rough	+	+	-	-	-	-	-	AG	AG	G	G	G	<i>Staphylococcus epidermidis</i>
B	Circular	Smooth	Yellowish	Entire	Raised	Opaque	+	+	+	-	+	-	-	AG	AG	G	G	AG	<i>Staphylococcus aureus</i>
C	Circular	Shiny	White	Entire	Convex	Moist	-	+	+	-	-	+	+	AG	AG	AG	AG	AG	<i>Enterobacter aerogenes</i>
D	Circular	Smooth	Cream	Undulate	Raised	Opaque	+	+	+	-	-	+	+	-	G	-	G	AG	<i>Bacillus species</i>
E	Circular	Glistening	Cream	Entire	Raised	Transparent	-	+	+	+	+	-	-	AG	AG	AG	A	A	<i>Escherichia coli</i>
F	Circular	Smooth	Whitish	Entire	Convex	Translucent	-	+	+	-	-	-	+	AG	AG	A	AG	AG	<i>Pseudomonas aeruginosa</i>
G	Irregular	Glistening	Cream	Entire	Raised	Opaque	-	+	-	-	+	-	+	AG	AG	AG	AG	AG	<i>Klebsiella pneumoniae</i>

Table 4: Total bacterial count of the fish samples

Isolate	Colony morphology and characteristics	Cell shape and gram stain	Motility	Catalase	Coagulase	Citrate	Methyl	Indole	V.P	Glucose	Lactose	Sucrose	Probable organism
A	Large colonies, cream to yellowish on nutrient agar	Gram positive cocci in chains	+	-	-	-	-	-	-	A+	-	G+	<i>Streptococcus species</i>
B	Whitish medium sized mucoid colonies on nutrient agar	Gram positive long rods	+	+	+	+	-	-	-	-	-	-	<i>Bacillus species</i>
C	Large colonies, cream to yellowish on nutrient agar	Gram positive cocci in clusters	+	+	+	-	-	-	-	AG+	AG+	AG+	<i>Staphylococcus aureus</i>
D	Whitish colonies on nutrient agar	Gram negative rods	+	+	+	-	-	-	-	AG+	-	AG+	<i>Proteus mirabilis</i>
E	Grey medium colonies on nutrient agar	Gram negative rods	+	+	-	-	+	+	-	AG+	AG+	AG+	<i>Escherichia coli</i>
F	Large colonies, Creamy to yellowish on nutrient agar	Gram positive cocci	+	+	+	+	+	+	+	AG+	AG+	AG+	<i>Micrococcus species</i>
G	Medium to large Grey mucoid colonies	Gram negative short rods	-	+	-	+	-	-	+	AG+	AG+	AG+	<i>Klebsiella pneumonia</i>
H	Medium to large grey mucoid colonies	Gram negative short rods	+	+	-	+	-	-	-	-	-	-	<i>Pseudomonas species</i>
I	Whitish colonies on nutrient agar	Gram negative rods	+	-	-	+	+	-	-	G+	-	-	<i>Serratia species</i>

Keys: +: Positive, -: Negative, A+: Positive and Acid produced, AG+: Positive and produces both Acid and Gas, G+: Gas production only

Table 5: Properties and identity of the bacterial isolated from fish sample

Sample	Total bacterial count(10^{-2})	Total bacterial count(10^{-3})
A Skin	2.14×10^5	1.48×10^6
A Intestine	2.64×10^5	1.96×10^6
A Gills	1.88×10^5	1.32×10^6
B Skin	2.32×10^5	1.88×10^6
B Intestine	2.46×10^5	2.00×10^6
B Gills	1.28×10^5	9.4×10^5
C Skin	2.20×10^5	1.84×10^6
C Intestine	2.48×10^5	2.00×10^6
C Gills	2.16×10^5	1.76×10^6
D Skin	2.60×10^5	1.96×10^6
D Intestine	2.84×10^5	2.04×10^6
D Gills	1.88×10^5	1.44×10^6
E Skin	2.08×10^5	1.72×10^6
E Intestine	2.30×10^5	1.98×10^6
E Gills	1.94×10^5	1.68×10^6