BACTERIOLOGICAL ANALYSIS OF FRESH FISH (CAT FISH) SOLD IN OZORO MARKET

Orogu J.O.* and Okobia B. U.

Department of Science Laboratory Technology, Delta State Polytechnic Ozoro, Delta State, Nigeria

Email- joshuaorogu4@gmail.com

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ABSTRACT

The bacteriological analysis of fresh fish (Cat fish) sold in Ozoro market was carried out to evaluate the bacteriological quality of fresh fish and also provide some relevant information on the microflora of the species commonly found in fresh fish. 13 Samples were obtained from ozoro market and were labelled A to M. The samples were cultured and sub-cultured in appropriate media and the microorganisms were identified according to their morphological and biochemical characteristics. A total of four (4) bacterial isolates were obtained; Enterococcus spp, Streptococcus pyogen, Staphylococcus aureus, and Bacillus spp. The total heterotrophic count for bacteria ranged from 1.26 x 10^3cfu/ml to 9.6 x 10^3cfu/ml. Bacillus spp. has the highest occurrence of 38.46% while Streptococcus pyogene has the least occurrence of 15.38%. The result of the study shows that fresh catfish harbor a great measure of pathogenic microorganisms and this could be as a result of improper handling during harvesting. Regular draining of ponds after specific period will reduce or help eliminate the risk of fresh catfish borne disease.

Keywords: Nil

INTRODUCTION

Fish is a very vital source of high quality protein and constitutes an important parts of man's diet. It is the most important animal protein food available in the tropics, and it represents about 14% of all animal protein on a global basic (Abolagba and Melle, 2018; Tyo, 1997; 2001; Clucas and Ward, 1996). Spoilage soon sets in which is occasioned by an increase in the ambient temperature that triggers favourable conditions for microorganisms to thrive. Thus, the quality of fish as well as its potential keeping time deteriorates rapidly leading to food loss with regards to acceptable quality. This deterioration is due to growth of microorganisms or non-microbial causes such as lipid oxidation (Martin, 1994). Essuman (1992) stated that Africa is endowed and constitute a rich source of numerous species of fresh fish. Such species include *Clarias spp*, *Baqrus spp*, *Tilapia spp* amongst others (Mabawo *et al.,* 1982 and Motwani, 1970).

Fish has become increasingly important source of protein and other element necessary for the maintenance of healthy body *(*Adebayo *et al., 2012)*. The African catfish, *Clariasgarie pinus* has been reared for about 20years in Africa with mixed success, the total farmed production of these species being only 3.978 metric tonnes or 7.46 mt in Africa (Udeze *et al.,* 2012). *Clariasgarie pinus*is highly nutritious fish that contains high amount of vitamins, proteins, minerals and little or no saturated fat and is low in carbohydrate (Udeze *et al*., 2012). Annual domestic fish supply in Nigeria stands at about 400,000 tonnes. The fishery sector accounts for about 2 percent of national GDP, 40 percent of animal protein intake and a substantial proportion of employment, especially in rural areas. The sector is a principal source of livelihood for over 3 million people (De Graaf *et al.,* 1996). Nigeria is the largest African aquaculture producer, at 15,489 tonnes per year, Egypt (5,645 tonnes) follows Nigeria and then there are only five other countries (Zambia, Madagascar, Togo, Kenya and Sudan) that each produce more than 1000 tonnes (Hussein *et al*., 2002). Fish take a large number of bacteria into their gut from water sediment and food (Adedeji *et al.,* 2011). It has been well known that both fresh water and brackish water fishes can harbor human pathogenic bacteria particularly the coliform group (Adedeji *et al.,* 2011).

Fish meat deteriorates more quickly than other muscle foods, particularly when poorly handled. This spoilage is primarily bacterial in nature but other factors such as enzymatic breakdown of the tissues contribute to spoilage. About 30% of landed fish are lost through microbial activity alone (Ghaly *et al., 2010;* Huisint *et al., 1996*). Even with the improved food safety, progress is still uneven and food borne outbreaks from microbial contamination, chemicals and toxins are still common in many countries (WHO, 2007). Among all the food borne disease out breaks reported globally, seafoods accounts for up to 8% of all outbreaks (Huss, 2003).

The microbial flora associated with fish is sometimes a reflection of their aqueous environment (Arafat, 2013). Water being a natural habitat for a wide range of microorganisms including bacterial, protozoa and fungi, fish taken in or harbour these organisms from its environment. These organisms may be pathogenic to fish as well pathogenic to humans when ingested. Bacteria such as *Pseudomonas fluorescens, Aeromonas hydrophila, Edwardsiella tarda, Vibrio spp* are ubiquitous in the aquatic environment (Gilmour *et al.,* 1976, Allen *et al.,* 1983). Pathogenic bacteria such as *E. coli, Salmonella, Shigella* are most times introduced into water bodies through human or animal feaces. When fishes from these environments are ingested they could pose a greak risk to the health of the consumers (Sumer *et al.,* 2014).

Materials and Methods

Study area

This study was conducted in Ozoro with samples of fresh catfish in Ozoro, Delta State. The samples were collected from the market in the town. This town (Ozoro) is located in Delta State South-South Nigeria.

Sample collection

Thirteen samples of cat fish were obtained from ozoro market. Samples were collected in a sterile container and were taken under aseptic condition to the laboratory for microbiological analysis.

Materials

The materials used for this research work are; Electrical thermostatic incubator, Microscope, Weighing balance, Nutrient agar, Blood agar, Sabourous Detrose Agar(SDA), Conical Flasks, Distilled water, Measuring cylinders (50ml), Spatula, Masking tape, Petri dishes, Methylated spirit, Pressure pot Gas Cylinders, Methylene blue, Wire loop, Bunsen burner, Microscopic slide and Oil Immersion etc.

Method

Isolation and enumeration of microorganisms.

The solution containing the distilled water and pounded catfish was inoculated into each already prepared agar medium (Nutrient, Blood Agar and SDA respectively). The wire loop was used to streak each petri dish containing solidifies agar medium. The inoculated plate was incubated at 37^0 for 24hours. Distinct colonies were isolated and reinoculated unto appropriate agar medium (Nutrient Agar). The sub-cultured plate was incubated for another 24 hours at 40^0 for the purpose of identification.

Identification of isolates

Gram staining: A colony from the purified subculture was isolated and emulsified in sterile distilled water and a thin preparation was made on the slide. It was evenly spread to covered an approximately are of about 15-20mm in diameter on the slide. The smear made was left to dry being protected from dust and sunlight. The smear was fixed using gentle heat by rapidly passing the slide with the smear uppermost three (3) times through the flue

of a Bunsen burner. The slide was checked with the back of the hand just to make sure too much heat was not used which can affect or even kill the microorganisms. The smear was allowed to cool before staining was done.

The microscopic slide containing the smear was placed on the staining rack and covered with crystal violet and allowed for 30-60 seconds. Wash off with distilled water slowly and gently. A drop of Lugol's iodine was added and allowed for 30-60 seconds as well; Then wash off slowly and gently with distill water.

The water was then tipped off. A drop of Acetone(Alcohol) was added off immediately again, a drop of Neutral red was added and allowed for 2 minutes then washed off with distilled water. The back of the slide was wiped clean and placed on a draining rock for the smear to air-dry, using microscope. The smear was examined with 40x objective lens to check the staining pattern (checked without oil immersion). Oil immersion was added and viewed fewer than 100x Objectives lens to observe the shape, colour and other characteristics.

Biochemical test

a. **Citrate utilization test:** For each bacterial isolate 100ml of citrate medium was dispensed into each of the fore test tubes and sterilized by the use of gas cylinder and pressure pot for 30mins. The organism was then inoculated into citrate medium and incubated at 57°C for 24-48hours. A change in colour from green to blue indicated containing only the citrate medium sterried as a control (Chessbrough, 2005).
b. **Catalase test:** A drop of 3% hydrogen peroxide was placed on a glass slide. A bit of growth each isolate was collected from the medium using a wire loop and the growth has emulsified in the drop. A positive test was indicated by bubbling and frothing; negative test did not show bubbling fronthing (Cheesbrough, 2005).
c. **Oxidase test:** A piece of fitter paper was placed on a clean sterile petri dish and 3 drops of oxidized reagent was added. The bacterial isolated were sheared on the filter paper by means of sterilized rod. Organisms indicated positive when it retains the purple colouration with five to ten seconds of the analysis.
d. **Indole test:** Bacterial Isolates were inoculated into peptone water medium contained in sterilized test tubes then incubated at 37°C for 24 hours to give optimum accumulation of indole. After the incubation period, about 0.5ml of Kovac's reagent was added 50 5m of peptone water culture. The bottles were shaken thoroughly and allowed to stand and observed for colour development. A red colouration in the uppermost layer of the tube indicated a positive result. And if the isolate is negative, the reagent layer will remain yellow or slightly cloudy(Cheesbrough 2005).
e. **Triple sugar iron agar test (TSI):** Bacterial Isolated were stabbed into TSI slant media and also streaked on the surface of the slant after which the medium was

incubated at optimal temperature of 37°C for 24hous. The TSI slant medium was used to check for the presence of the following.

i. Gas: If bubbles is present in the media (gas positive)
ii. H_2S: If black is present in the media (H_2O Positive)
iii. Lactose: If the top of the media turns from pink to yellow (lactose positive).

f. **Motility test:** A single colony of each of the organism was inoculated into labeled test tube containing peptone water (5ml) and the tube incubated at 37°C over-night.

A drop of the well-mixed organisms in peptone water incubated over-night was placed on a cover slip and the edges surrounded with oil immersion. A microscopic slide was then placed over the cover slip taking care that the slide does not touch the drop on the cover slip bur suspended by the oil immersion. The slide was then observed under the microscope for motile bacteria under X100 objective (Cheessbrough, 2005).

Results and Discussion

Results

The bacterial isolated from the catfish (Fresh) were *Enterococus spp, Streptococcus pyogene, Staphylococcus aureus,* and *Bacillus spp.* (Table 1).

Table 1 shows Cultural, Morphological and Biochemical characteristics and identification of bacterial isolates. Table 2 shows Total heterotrophic plate count. Table 3 shows Percentage of occurrence of bacterial isolates. *Bacillus spp*. has the highest occurrence while *Streptococcus pyogene* has the least occurrence.

Table 1: Cultural, Morphological and Biochemical characteristics and identification of bacterial isolates.

Morphology	Motility	Catalase	Citrate	Oxidase	Lactose	H_2S	Glucose	Indole	Organism
Gram +vecocci	+	-	+	+	+	+	-	+	*Enterococcus spp*
Gram +vecocci	-	+	+	+	+	+	+	+	*Streptococcus spp*
Gram +vecocci	+	-	+	-	+	+	+	+	*Staphylococcus aureus*
Gram +vecocci	+	+	-	+	+	+	-	+	*Bacillus spp*

Table 2: Total heterotrophic plate count

Samples	Bacteria count
A	8.0×10^3
B	9.0×10^3
C	5.0×10^3
D	9.4×10^3
E	7.5×10^3
F	9.6×10^3
G	1.26×10^3
H	4.8×10^3
I	3.9×10^3
J	1.37×10^3
K	8.6×10^3
L	9.0×10^3
M	7.9×10^3

Table 3: Percentage of occurrence of bacterial isolates

Organisms	No. of occurrence	Percentage (%) of occurrence
Enterococcus spp	3	23.08
Streptococcus pyogene	2	15.38
Staphylococcus aureus	3	23.08
Bacillus spp	5	38.48
Total number of isolates	13	100

Discussion

Result of the gram staining, the cultural and morphological characteristics of isolates revealed that *Enterococcus spp*, *Streptococcus pyogene, Staphylococcus aureus,* and *Bacillus spp were* the bacteria species present in the samples assessed (Table 1). The total heterotrophic count ranges from 1.26×10^3 -9.6×10^3(table 2). *Bacillus spp*. has the highest occurrence of 38.46% while Streptococcus *pyogene* has the least occurrence of 15.38% (Table 3).

The consumption of fresh African catfish (*Clarius garierpinus)* is on the increase in

both rural and urban centers of Nigeria (Emikpe *et al.,* 2011; Adedeji *et al.,* 2012). Fish is an important food commodity in the international trade but deteriorates rapidly when storage facilities are lacking (Adedeji *et al.,* 2012).

From the results of this study, it was discovered that bacterial loads of the catfish (fresh) showed a slight difference from other parts of the fish.

Based on the frequency of occurrence, *Bacillus spp* record the highest percentage of 38.46%. The presence of these organisms might be associated with habitat in which it was caught. (Oraser and Hill, 1990, Shinkafi and Ukwaja, 2010, 2010). *Bacillus spp is* known to cause a number of infectious disease such s septicemia, wound and food borne infections, meningitis, respiratory and urinary tract infections. (Morales *et al.,* 2004; Shinkafi and Ukwaya, 2010; Bassey *et al.,* 2015).

The detection of these pathogenic microorganisms in the catfish sample analyzed may be from the source in which it was harvested and the environment where the fishes are sold. Worthy of note in this study is the presence of both spoilage and pathogenic bacteria species that were isolated from the fish samples analyzed. The most prominent of these group of bacteria isolated include, *Bacilluss spp, Enterococcus spp, Streptococcus pyogene and Staphylococcus aureus*. These organisms are pathogenic and can be become harmful it consumed without proper processing. The pressure of these isolates from catfish could create health hazard when they are ingested or when they come in contact with the human skin. This exposed or people eating this catfish to the risk of food borne infection.

Conclusion and Recommendations

Conclusion

Based on the result from the study, it can be concluded that the fresh catfish harbours a great percentage of bacteria. This study shows the prevalence of *Bacillus spp*. In cat fish. The quality of catfish is influenced by habitat, harvesting tools and handling (display) of this fish for buyers which eventually encourage cross contamination. It is therefore suggested that adequate measure should be taken while harvesting, preserving and processing the fish before consumption, cooking properly immediately before consumption is also an effective way of reducing or eliminating risk of fresh catfish borne disease.

Recommendations

The following should be taken into consideration to help stop or eliminate microbial contamination of fresh catfish use in homes, restaurants. It is recommended that;

i. the environment where fish ponds are located should be protected from pollutants and weeds which can harbour microorganisms that can find their way into fish ponds by themselves of by passive process though wind rainfall etc.
ii. The sanitary conditions under which fishes are reared or cultured should be improved by following standards or good practices such as good quality water, use of feeds with high microbial quality, regular draining of pond water after specific period of time and closure of ponds to the public.
iii. Fish handlers with open wounds should avoid contact with water from fish ponds

and fish should be properly cooked with heat before consumption.

iv. Workers should be educated on good hygienic practices

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