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Bacteria Quality of Smoked *Clarias gariepinus* Sold at Opolo Market, Yenagoa, Bayelsa State

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Abstract

The bacterial load of *Clarias gariepinus* (Cat Fish) sold at Opolo Market, Yenagoa, Bayelsa State was analyzed for microbial load twenty (20) hot smoked catfish was purchased from different vendors in the Opolo Marketin Yenagoa Bayelsa state and was examined for microbial loads, under standard microbiological techniques. The Total Heterotrophic Bacterial Counts (HTBC) of the analyzed catfish samples ranged from 1.3 x10^6 – 1.3 x10^7Cfu/g. The bacterial species isolated and their hierarchy of occurrence in the fish samples were as follows: *Salmonella gallinurium* and *Salmonella pollorum* (20.0%) > *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (13.0%) > *Klebsiella pneumonia*, *Salmonella choleraesuis* and *Proteus vulgaris* (7.0%). The antimicrobial sensitivity profile of the bacterial isolates from the catfish samples demonstrated using gentamicin, cefixime, augmentin, nitrofurantoin, ciprofloxacin, ceptazidine, erythromycin and ceftriaxone antibiotics, revealed that the isolates were susceptible to gentamicin, cefixime and ciprofloxacin, whilst demonstrating varied degrees of resistance to augmentin, nitrofurantoin, erythromycin and ceptazidine.The Heterotrophic Bacterial Counts obtained from the study exceeded the 10^3Cfu/g satisfactory microbial counts for safe foods stipulated by the World Health Organization (WHO), thereby making the fishes unwholesome. Antimicrobial resistance tendencies of the bacterial isolates also increase the risks in managing diseases arising from the consumption of these fishes. therefore the need to educate the fish vendors on the importance of hygiene practices during the processing and vending stages of these fish and also highlight the importance of cooking the fish properly to consumers.

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Introduction

Aquatic food is major source of protein to man on a global scale including the Niger Delta region. Fresh water food consumed in the region include finfish and shrimps other shell fish which are essential sources dietary protein. animal protein consumed by an average Nigerian compared to meat is about 40% (Adebayo-Tayo *et al.*, 2008). Despite its nutritious value, fish as food is greatly challenged by infectious agents because of the various stages of handling and processing. Food-borne diseases continued to be a major public health problem on a global scale, especially in countries were safeguarding food from cross contamination is difficult (Zige *et al*., 2013). Aquatic foods contain millions of microorganisms; the most prevalent ones is enteric bacteria and fungi, this is due to pollution of water bodies from industrial sewage, home refuges etc. Pollution in many cases vary and could include faecal contamination as well. This act may result to a high number of coliforms in post harvested food (Amakoroma *et al.*, 2013).

The microbial flora of any fish depends solely on the content of the water bodies in which they are harvested as the slime that covers the surface of fish has been found to contain a great variety of bacteria genera. Therefore, if a fish is harvested from polluted water and eaten raw or not properly cooked possess a anger. This is also the cause of the rapid spoilage of freshly harvested fish. The nutrients present in fish provide a good medium for the proliferation of microbs. Various methods have been developed to preserve fish, however, the techniques employed depend on the technological advancement of the people (Adebayo-Tayo *et al.*, 2008). In Bayelsa and any states in Nigeria, smoking is the major way of fish preservation. In smoke–drying the source of heat is firewood. After processing, the products are placed in

locally made baskets or jute sacs ready for transportation to various markets in the country. Often, the products are not properly packaged and stored (Amakoroma *et al.*, 2013). Consequently, reabsorption of moisture and post-processing contamination of fish occur. Sikoki and Aminigo (2002) reported that changes in the moisture content of smoked fish were most significant during the first 3week of storage and that bacterial population increased during this period. Thus, the quality of fish as well as its potential keeping time, deteriorates rapidly leading to food loss with regard to acceptable quality. Furthermore, some processing techniques are in operation in Nigeria. These include chilling, freezing, salting, canning, drying, and smoking. However, smoking is the most popular method of fish processing (Eyo, 2000) who reported that: smoking involves heat application to remove water and inhibit bacterial and enzymatic action on fish. The study was aimed at determining the microbial load of the smoked *C. gariepinus* old in Opolo Market, Yenagoa, Bayelsa State.

Materials and Methods

Study Area

This study was carried out in Yenagoa Metropolis, Bayelsa state, while facilities at Valdalis Medical Laboratory were used for laboratory analysis.

Collection of Samples

A total of twenty (20) hot smoked catfish was purchased at random from five (5) different retailers in Opolo Market in Yenagoa Metropolis, Bayelsa State. The fish samples were collected in sterile polythene bags and transported to the microbiology laboratory on ice-packed coolers. All samples obtained were ground with an electric blender to obtain a fine texture of the fish samples.

Serial Dilution

The fish samples were serially diluted before inoculation into the media. Ten-fold serial dilution was carried out by transferring 1g of each of the fish samples into a test tube containing 9 ml of peptone water using a sterile pipette nd mixed to obtain 10^{-1} dilution. One millilitre of the dilution (10^{-1}) was then transferred into another test tube (10^{-2}) containing 9ml of peptone water. Using separate 1ml pipette, these transfers were repeated until dilution 10^{-5} was achieved (Cheesbrough, 2010)

Microbiological Analysis of Fish Samples

Total Heterotrophic Bacterial (THB) Counts

Total heterotrophic bacteria counts were enumerated using the Nutrient Agar (NA) and MacConkey agar (MAC) by spread plate method. Aliquot of 0.1ml of 10`², 10-3 and 10-4 dilutions of the fish samples were inoculated in duplicates and spread a sterile hockey stick. The plates were incubated at 37°C for 24 hr. Thereafter the mean counts of the bacteria colonies were taken. The number of bacteria in the fish samples were obtained by multiplying the mean count of the sample by the dilution factor and expressed in Cfu/g (colony forming unit per gramme of the fish samples) with the formula: Cfu/g = x dilution factor

Isolation and Identification of Bacteria

The method adopted by (Zige *et al.*, 2013) was used with slight modifications for the isolation of food-borne bacteria. Ten (5g each) of each food sample were homogenized with sterile mortar and pestle; the resulting homogenate was aseptically added to 9 ml prepared nutrient broth. Streaking on the media directly from the overnight broth culture was done aseptically on EMB, SSA, chocolate agar, nutrient agar, and Macconkey agar and incubated at 37°C for 24-48 hours. The streaked plates after incubation were examined for colonies that showed dissimilar cultural characteristics and subcultured on respective media. Pure colonies were obtained by subculturing on nutrient agar. All presumptive isolates were further identified using conventional biochemical methods (Esha *et al.*, 2009) These characteristics of differentiation for the isolated strains were read as described by (Cheesbrough, 2000) and (WHO, 2003)

Results

Total Heterotrophic Bacterial (THB) Counts for the Smoked-Catfish Samples

The Total Heterotrophic Bacterial Counts (THBC) of the fish samples is shown in Table 1. ranging from $1.3x10^6$ – 1.3×10^7cfu/g.

Frequency of Isolation of Various Bacterial Isolates from the Smoked Catfish Samples

The frequency of isolation of the bacterial isolates from the Smoked catfish samples is shown in Table 2. gives a comparison of the isolated bacterial species, which were as follows: *S. gallinarium, S. pollorum* (20.0%) > *E. coli, P. mirabilis, P. aeruginosa* (13.0%) > *K.*

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Table 1: Total Heterotrophic Bacterial Count of the fish samples

Sample	Dilution	Average colony counts	Cfu/g	Log_{10}(Cfu/g)	Mean Log_{10}(Cfu/g)
CF.MAC 1A	10^{-3}	242	2.4×10^6	6.4	
	10^{-4}	106	1.1×10^7	7.0	6.7
CF.MAC 1B	10^{-3}	183	1.8×10^6	6.3	
	10^{-4}	114	1.1×10^7	7.0	6.6
CF. SSA 1A	10^{-3}	159	1.6×10^6	6.2	
	10^{-4}	100	1.0×10^7	7.0	6.6
CF. SSA 1B	10^{-3}	125	1.3×10^6	6.1	
	10^{-4}	98	9.8×10^6	6.9	6.5
CF. NA 1A	10^{-3}	204	2.0×10^6	6.3	6.7
	10^{-4}	131	1.3×10^7	7.1	
CF. NA 1A	10^{-3}	130	1.3×10^6	6.1	6.6
	10^{-4}	97	9.7×10^6	7.0	

pneumonia, S. choleraesuis and *P. vulgaris* (7.0%).

Table 2: Frequency of identified Bacteria Isolates from the Microbiological Analysis of the Smoked Catfish Samples

Bacterial isolates	Number of occurrences	Percentage prevalence (%)
Escherichia coli	2	13.0
Proteus mirabilis	2	13.0
Pseudomonas aeruginosa	2	13.0
Salmonella gallinarium	3	20.0
Salmonella pollorum	3	20.0
Klebsiella pneumonia	1	7.0
Salmonella choleraesuis	1	7.0
Proteus vulgaris	1	7.0
TOTAL	**15**	**100**

Antimbial Sensitivity Profile of the Bacterial Isolates from the Smoked Catfish Samples

The antimicrobial sensitivity profile of the bacterial isolates from the smoked catfish samples is shown in Table 3. *Proteus* species were susceptible to (gentamicin) (GEN) and Ofloxacin (OFL), but demonstrated degrees of resistance activity against the other antibiotics. This was also the trend for *Escherichia coli*, *Salmonella*, *Pseudomonas*, and *Klebsiella* species with varied susceptibility as follows: Ofloxacin (OFL) and gentamicin (GEN), Gentamicin (GEN) and Ofloxicin (OFL), ceptazidine (CAZ) and gentamicin (Gen) and Ofloxicin (OFL) and cefixime (CXM), respectively.

Table 3: Antibiotic Sensitivity Profile of the Bacterial Isolates from the Smoked Catfish Samples

Bacterial isolates	Number Resisted antibiotics	Resisted Antibiotics	Susceptible Antibiotics
Proteus spp	5	CXM, AUG, NIT, CTR, CAZ	GEN and OFL
Escherichia coli	5	CXM, AUG, NIT, CPX, CAZ	OFL and GEN
Salmonella spp	5	CXM, AUG, CTR, CAZ, NIT	GEN and OFL
Pseudomonas spp	5	OFL, CXM, AUG, CPX, NIT	CAZ and GEN
Klebsiella spp	6	CAZ, GEN, AUG, CPX, NIT, ERY	OFL and CXM

KEY: GEN = Gentamicin, CXM = Cefixime, AUG = Augmentin, NIT = Nitrofurantoin, CPX = Ciprofloxacin, CAZ = ceptazidine , ERY = erythromycin CTR = ceftriaxone

Discussion

The study obtained Total Heterotrophic Bacterial counts which ranged from 1.3 x 10^6 – 1.3 x 10^7Cfu/g. It also isolated species of bacteria, whose percentage prevalence and identity were as follows: *S. gallinarium*, *S. pollorum* (20.0%) > *E. coli*, *P. mirabilis*, *P. aeruginosa* (13.0%) > *K. pneumonia*, *S. choleraesuis* and *P. vulgaris* (7.0%). The study attributes the high microbial counts and prevalence of bacterial species in the fish samples, to the poor hygiene surrounding the smoking processes of the fish, its storage/packaging and transportation and vending in the market environments (Ameko *et al.*, 2012). The Total Heterotrophic Bacteria Counts (THBC) obtained from this study were not in alignment with the regulations of the World Health Organization (WHO), (2002), and the International Commission on Microbiological Specifications for Food (ICMSF) (2004), which unanimously specified that THB Counts in the range of 10^3cfu/g for ready-to-eat foods were satisfactory, while those above this range would be unsatisfactory and unsafe for final consumption (Amponsah-Doku *et al.*, 2010; Ameko *et al.*, 2012). The bacterial counts obtained in this study also rose above the guidelines of the Hazard Analysis and Critical Control Points-Total Quality Management (HACCP-TQM), which rates the microbial quality of foods containing <4 log cfu/g of organisms as "good" and those containing approximately >8 log cfu/g as spoilt (Food Safety Authority of Ireland (FSAI) (2014). Findings from this study is slightly similar to those of Daramola *et al.* (2020), who obtained a Mean Total Plant Count of Bacteria in the range of 4.82 x 10^4cfu/g – 4.92 x 10^4 cfu/g, from smoked catfish (*C. gariepinus*) sold at Ota Market, Ota, Nigeria. They also reported the presence of *Escherichia coli*, *Salmonella*, and *Shigella* species from the fish samples, among other bacterial species they isolated. The findings are also similar to those of Ineyougha *et al.* (2015), who reported total heterotrophic bacteria counts in the range of 6.384 - 6.608 log Cfu/g, and also isolated *E. coli*, *Salmonella*, *Shigella*, *Pseudomonas* and *Proteus* species from their assessment of the microbial quality of smoked *Trachurus trachurus* (mackerel fish) sold in three selected markets of South-South States in Nigeria.

The results of this study agree with the findings of Oku and Amakoromo (2013), who reported bacterial counts of smoked fish in the range of 1.8 x 10^4- 2.5x 10^7Cfu/g. They also reported *Klebsiella* species to be present in the smoked-dried fish samples, among other bacterial species they isolated. Kingdom *et al.* (2018), also reported Total Heterotrophic Bacterial Counts of 5.13 x 10^6 $\pm$ 0.55 x 10^6 Cfu/g from processed freshwater clam (*Galatea paradoxa*). Each of the bacterial isolates demonstrated various degrees of resistance and susceptibility to the tested antibiotics (Gentamicin, Cefixime, Augmentin, Nitrofurantoin, Ciprofloxacin, ceptazidine, erythromycin, and ceftriaxone). *Proteus* species were susceptible to (gentamicin) (GEN) and Ofloxicin (OFL), but demonstrated degrees of resistance activity against the other antibiotics. This was also the trend for *Escherichia coli*, *Salmonella*, *Pseudomonas*, and

Klebsiella species with varied susceptibility as follows: Ofloxicin (OFL) and gentamicin (GEN), Gentamicin (GEN) and Ofloxicin (OFL), ceptazidine (CAZ) and gentamicin (Gen) and Ofloxicin (OFL) and cefixime (CXM), respectively. These results were similar to the findings of Imarhiagbe *et al.* (2016), who reported the resistance activity of *Escherichia coli* and *Pseudomonas species* to ciprofloxacin (CPX). Adesoji *et al.*, (2019) also reported high resistance of *Escherichia coli* to Cefuroxime, cefttazidime and ciproflaxin.

Conclusion

The study determined the microbial load of smoked catfish (*C. gariepinus*) sold in Opolo Market, Yenagoa, Bayelsa State. The Total Heterotrophic bacteria counts of the fish samples were in the range of $1.3 \times 10^6 - 1.3 \times 10^7$ Cfu/g, which exceeded the standards of 10^3cfu/g, microbial counts of safe foods set by the World Health Organization (WHO, 2002). This It study isolated species of bacteria, whose percentage prevalence and identity were as follows: *Salmonella gallinarium*, *Salmonella pollorum* (20.0%) > *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* (13.0%) > *Klebsiella pneumonia*, *Salmonella choleraesuis* and *Proteus vulgaris* (7.0%). The occurrence of the bacterial species in the smoked catfish samples was attributed to the poor hygiene practices surrounding the smoking, storage/packaging, transportation, and vending of the fish in the market environments (Ameko *et al.*, 2012). Since these bacteria are of public health importance, there is therefore need for consumers to exercise caution in the consumption of smoked fish sold in the Opolo Market of Yenagoa, Bayelsa State. The fish should be subjected to proper cooking before they are served on the table for final consumption.

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