# Microbial Assessment of Selected Smoked Fishes in Akokoland, Ondo State, Nigeria

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## Abstract

*The density and diversity of microflora of cold smoked Clupea harengus and Trachurus trachurus in Akoko area of Ondo State for public health purposes were investigated. Various parts of the fish (skin, muscle, gill and intestine) were sampled for isolation and characterisation of its microbial contaminants. The results of isolation revealed the presence of Escherichia coli, Klebsiella mobilis, Serratia marcescens, Citrobacter spp, Staphylococcus aureus, Shigella spp., Bacillus cereus and Proteus vulgaris, others are Aspergillus, Rhizopus, Penicillium, Fuasrium, Neurospora spp and Candida albicans. There were observable differences in the microbial load of the different parts analysed; specifically the skin and intestine had greater microbial load. There were also differences in the range of microbial load among the local government areas studied. Akoko Northwest had the highest bacteria count of 3.90 X $10^6$ cfu/g while the least bacteria count of 0.009 X $10^6$ cfu/g was recorded in Akoko Southeast local government. Highest fungal count of 31 x $10^5$ was from Akoko Northwest and the least was from Akoko Northeast. It was observed that some parts of fish had more than acceptable limit of bacteria and fungi load for wholesome food. Environmental factors could also account for differences in load and diversity but handling during sales might also have contributed. Hence, there is need for environmental health education in the area. Consumers should therefore be encouraged to soak cold smoked fishes for about 30 minutes in boiled water before consumption to minimize the risk of food poisoning.*

**Key Words**: Microbial Assessment, Smoked Fish, Microflora, *Clupea harengus*, *Trachurus trachurus*

## Introduction

Fish has been one of the main foods for humans for many centuries and still constitutes an important part of the diets in many countries (Obemeata *et al.*, 2011). Nigerians are large fish consumers with a total consumption of more than 1.36 million metric tonnes per year, with fish imported making up about three fifth (740,000 metric tonnes of the supply) valued at $594.4 million (FMAWR, 2008).

Sizeable proportions of the fish imported into Nigeria are usually processed to prevent economic losses, fish being highly susceptible to deterioration immediately after harvesting. The prominent fish preservation method in Nigeria is smoking (Falaye, 2013). Processing by smoking enhances the nutritive values and promotes digestibility of protein. Furthermore, the changes in colour, odour, flavour and textures which are usually produced in fish by this process are also desirable (Eves & Brown, 1993).

Smoking is usually of two types; hot smoking and cold smoking. Cold smoked fish will last longer than fresh fish but hot smoked fish last longer than cold smoked fish. Cold smoked fish should be eaten within a few days (1-3 days) after smoking. The climatic condition of an area plays a vital role in the shelf life of smoked products of such area. Cold smoked fish remains in good shape within a period of 1-2 weeks in the refrigerator and months in the freezer (Fafioye, 2009).

*Clupea harengus* which is locally called ―Sawa‖ with the common name Sardine and *Trachurus trachurus* locally called ―Kote‖ and common name Horse mackerel are the most available smoked fish in Akokoland of Ondo State, Nigeria. Others are *Tilapia, Sardinella, Clarias, Heterobranchus* spp. The affordability, taste, flavour of cold smoked *Clupea harengus* and *Trachurus trachurus* make them the choice fish to the majority of the populace in Akoko area of the State. The presence of bacteria and fungi on processed fish are a clear evidence of contamination from exogenous sources that may be harmful to the consumers (ICMSF, 1986). Therefore, the study focused on investigating the distribution and prevalence of microorganisms in different parts of cold smoked fish in Akokoland, Ondo State, Nigeria and to give background information for the improvement of handling, processing, and storage of cold smoked fish.

## Materials and Methods

### Fish Procurement

Eight samples of fish's species of *Clupea harengus* and *Trachurus trachurus* were obtained from markets in the four local governments. The local governments were Akoko South West, Akoko South East, Akoko North West and Akoko North East. Samples of these fish species were obtained from two different markets in the same local government. The samples were handled aseptically, kept in separate sterile polythene bags and transported to the Department of Microbiology Laboratory, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria for further microbial analyses.

### Dissection of Fishes

The fishes were dissected under aseptic condition at room temperature of about $27^0$C in the laboratory. One gram of the skin and muscle of the fish were excised using sterile dissecting knife and the same amount of the gills and intestines were taken for analyses.

### Serial Dilution

Nine millilitres of distilled water was dispensed into four MacCartney bottles of for the serial dilution of the fish's skin, muscle, gill, and intestine using a pipette. The various fish parts (One gram) of the skin, muscle, gill and intestine were weighed and blended/macerated in a sterile mortar. It was poured into different bottles of sterile distilled water, and was shaken vigorously for uniform distribution of microorganisms.

### Pour Plate

This process involves arranging specific number of clean sterilized Petri dishes. A pipette was used to dispense 0.2ml of each labelled diluents into corresponding Petri dishes. The different media, namely PCA, EMB, SDA (Oxoid, UK), was poured into sterile plates and allowed to gel. The plates were inoculated and incubated at $37^0$C for 24 hours while the plates for fungal growth (SDA) were kept in the incubator at $27^0$C for 5 days. The isolates were obtained in pure culture before identification.

**Identification of Fungal and Bacterial Isolates**

Fungal isolates were identified microscopically using Lactophenol in cotton blue stain according to the methods of (Treagen & Pulliam, 1982). Standard biochemical tests were conducted to identify bacterial isolates into species according to the description in Bergey‘s Manual of Systematic Bacteriology (Krieg & Holt, 1984).

**Statistical Analysis**

The data were subjected to statistical analysis using one-way analysis of variance (ANOVA), in the SAS statistical package for Windows (SAS, 2009).

## Results

The total bacterial count on the cold smoked fishes showed that the ones procured from Akoko North West Local Government had the highest bacterial count of 3.9 X $10^6$cfu/g while the least count of 0.008 X$10^6$ cfu/g was recorded in Akoko South West Local Government. The highest total bacterial count on *Clupea harengus* was 2.9 X $10^6$cfu/g recorded in Akoko North West Local government, the least being 0.01 X $10^6$ cfu/ml recorded in Akoko North East Local Government. The highest total bacteria count on *Trachurus trachurus* was 3.9 X $10^6$cfu/g recorded in Akoko North West Local Government while the least count of 0.008X$10^6$cfu/g was recorded in Akoko South West Local Government (Table 1).

The recommended microbiological limits for smoked seafood are: 5 ×$10^5$ limit per gram for bacteria counts (ICMSF, 1986). The average total bacteria counts on the two species of fishes (*Clupea harengus* and *Trachurus trachurus*) showed that the fish skin and intestine had more than the acceptable limit for bacteria. The fish intestine of both fish species were observed to be contaminated as it contained about 5 to 8 times the acceptable limit for bacteria, making it unsafe for consumption. However, the fish muscle and gills were observed to carry less than the recommended microbiological limit for smoked seafood.

The microbiology of fish skin and gastrointestinal tract has been the focus of many studies. Fish spoilage can occur from both outer and inner surfaces as fish stomach contains digested and partially digested foods, which can pass into the intestine. The present result revealed that higher population of microorganisms on the

skin and gastrointestinal tract are similar to the observation of Emikpe *et al.* (2011) and Akinjogunla *et al.* (2011) who reported higher intestinal microorganisms in *C. gariepinus* and smoked Bonga fish respectively.

**Table 1. Average Total Bacteria Count (TBC) across the Four Local Governments (CFU/g)**

| | | | LOCAL GOVERNMENT AREAS | | | |
|---|---|---|---|---|---|---|
| **S/N** | **FISH TYPE** | **FISH PART** | **AKSW X $10^6$ CFU/g** | **AKSE X$10^6$ CFU/g** | **AKNE X$10^6$ CFU/g** | **AKNW X$10^6$ CFU/g** |
| 1 | C.H | Skin | 0.165 | 0.95 | 14 | 25 |
| 2 | C.H | Muscle | 0.031 | 0.009 | 0.1 | 0.23 |
| 3 | C.H | Gill | 0.026 | 0.013 | 0.17 | 0.38 |
| 4 | C.H | Intestine | 0.27 | 0.195 | 20 | 29.5 |
| 5 | T.T | Skin | 0.16 | 0.75 | 9 | 25 |
| 6 | T.T | Muscle | 0.008 | 0.011 | 0.12 | 0.21 |
| 7 | T.T | Gill | 0.019 | 0.015 | 0.49 | 0.45 |
| 8 | T.T | Intestine | 2.5 | 0.195 | 38 | 39 |

Keys:

C.H – *Clupea harengus*, T.T - *Trachurus trachurus*

AKSW - Akoko South West Local Government, AKSE - Akoko South East Local Government, AKNW - Akoko North West Local Government, AKNE - Akoko North East Local Government

The total *E.coli* count showed that Akoko North East Local Government had the least count of 0.005X$10^6$cfu/g while the highest count of 3.3 X$10^6$cfu/g was recorded in Akoko North West Local Government, although there was no growth recorded on the *E.coli* count from Akoko South West Local Government (Table 2). *E.coli* count on *Clupea harengus* was highest in Akoko North West local government area with count of 1.7X$10^6$cfu/g while the least count of 0.013X$10^6$cfu/g was recorded in Akoko North East Local Government. *E.coli* count on *Trachurus trachurus* was highest with count of 3.3X$10^6$cfu/g in Akoko North West Local Government while the least was recorded in Akoko North East local government area with count of 0.005X$10^6$cfu/g. The *E.coli* count on *Clupea harengus* showed that only the fish skin and intestine exceeded the *E. coli* limit for smoked fish (Table 2.) Also *E.coli* count on *Trachurus trachurus* showed that fish skin from Akoko North West local government exceeded *E.coli* limit, against

the recommended microbiological limit for smoked seafood which is placed at: $11\times10^5$ limit per gram for *E. coli* counts (ICMSF, 1986). According to the statistical analysis, there was no significant difference across the four local governments in the microbial count, but were significantly different ($P > 0.05$), across the various parts of the fishes (where fish skins and intestines differ significantly from fish gills and muscles).

Bacterial infection in smoked fishes may influence human health by inducing diseases such as abdominal pain, acute gastroenteritis, bloody/mucoid diarrhoea, nausea, vomiting, and fever, upon ingestion of insufficiently heat treated fish or products contaminated during the processing. The presence of these microorganisms in man generally indicates poor sanitation in handling and processing of vended foods (Essein *et al.*, 2005). *E. coli* was isolated from the fish samples which is similar to (Olayemi *et al*., 1991) who reported that presence of faecal coliform in fish for human consumption may constitute a potential danger not only in causing disease but also because of the possible transfer of antibiotic resistance from aquatic sources.

**Table 2. Average *E.Coli* Counts across the Four Local Governments (CFU/g)**

| | | | LOCAL GOVERNMENT AREAS | | | |
|---|---|---|---|---|---|---|
| **S/N** | **FISH TYPE** | **FISH PART** | **AKSW x10$^6$ CFU/g** | **AKSE X10$^6$ CFU/g** | **AKNE X10$^6$ CFU/g** | **AKNW X10$^6$ CFU/g** |
| 1 | C.H | Skin | NG | 8.5 | NG | 17 |
| 2 | C.H | Muscle | NG | NG | 0.13 | 0.15 |
| 3 | C.H | Gill | NG | 0.15 | 0.24 | 0.29 |
| 4 | C.H | Intestine | NG | 10.5 | 14 | 17.5 |
| 5 | T.T | Skin | NG | 5.5 | 5 | 16 |
| 6 | T.T | Muscle | NG | 0.09 | 0.05 | 0.1 |
| 7 | T.T | Gill | NG | 0.12 | 0.29 | 0.26 |
| 8 | T.T | Intestine | NG | 14.5 | 30 | 33 |

Keys

C.H – *Clupea harengus*, T.T - *Trachurus trachurus*

AKSW - Akoko South West Local Government, AKSE - Akoko South East Local Government, AKNW - Akoko North West Local Government, AKNE - Akoko North East Local Government, NG - No Growth.

The total fungi count showed that fishes procured from Akoko North East had the least fungi count of $0.01X10^6$cfu/g while the highest count of $31X10^6$cfu/g was recorded in the Akoko North West Local Government (Table 3). Total fungi count on *Clupea harengus* showed that samples procured from Akoko North West had the highest count of $2.85X10^6$cfu/g while the lowest was recorded in Akoko North East with total fungi count of $0.001X10^6$cfu/g. Total fungi count on *Trachurus trachurus* was highest in Akoko North West Local Government with count of $31X10^6$cfu/g while the least was recorded in Akoko South West Local Government with count of $0.011X10^6$cfu/g. The fungi counts varied significantly by Local Governments.

The variable mould counts of the smoked fishes from the various markets in all the local government areas may also be attributed to the continuous contamination of the item through frequent handling and the poor sanitary condition of many Nigerian markets as observed by (Essein *et al.,* 2005) that fish samples from the market centre also harboured more genera and species. The level of fungal growth in the analysed fish exceeded the acceptable microbial counts ($10^2$/g for moulds), based on microbial recommendation of the Food and Agriculture Organization (FAO, 1992).

**Table 3. Average Total Fungal Counts (TFC) across the Four Local Governments (CFU/g)**

| | | | LOCAL GOVERNMENT AREAS | | | |
|---|---|---|---|---|---|---|
| **S/N** | **FISH TYPE** | **FISH PART** | **AKSW X10$^6$ CFU/g** | **AKSE X10$^6$ CFU/g** | **AKNE X10$^6$ CFU/g** | **AKNW X10$^6$ CFU/g** |
| 1 | C.H | Skin | 0.6 | 10.5 | 15.5 | 15 |
| 2 | C.H | Muscle | 0.013 | 0.65 | 0.1 | 0.14 |
| 3 | C.H | Gill | 0.023 | 0.19 | 0.3 | 0.32 |
| 4 | C.H | Intestine | 1.15 | 10.5 | 26 | 28.5 |
| 5 | T.T | Skin | 0.95 | 9.5 | 12.5 | 11.5 |
| 6 | T.T | Muscle | 0.011 | 0.15 | 0.18 | 0.21 |
| 7 | T.T | Gill | 0.034 | 0.20 | 0.26 | 0.31 |
| 8 | T.T | Intestine | 0.15 | 23.5 | 24 | 31 |

Keys

C.H – *Clupea harengus,* T.T - *Trachurus trachurus*

AKSW - Akoko South West Local Government, AKSE - Akoko South East Local Government, AKNW - Akoko North West Local Government, AKNE - Akoko North East Local Government.

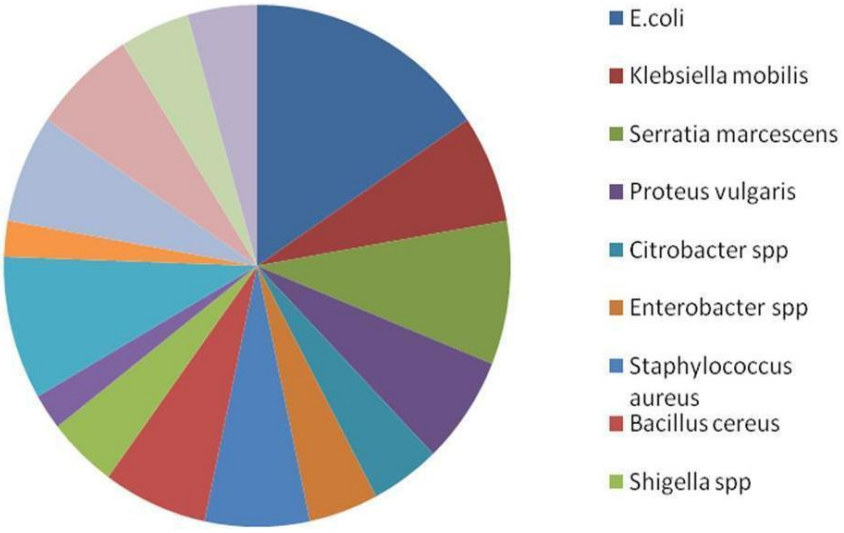


**Figure 1. Percentage Distribution of Microbial Isolates from Fish Samples**

## Discussion

The bacteria species isolated from Akoko South West Local Government were *E.coli, Klebsiella mobilis, Serratia marcescens* and *Proteus vulgaris*. The bacteria isolated from fishes procured in Akoko North East were *Citrobacter spp, Staphylococcus aureus, Enterobacter spp, E.coli, Bacillus cereus, Shigella spp, Serratia marcescens, Klebsiella mobilis* and *Proteus vulgaris. E.coli, Serratia marcescens, Staphylococcus aureus, Enterobacter spp, Bacillus cereus, Micrococcus luteus* were isolated from species procured from Akoko North West Local Government while *Proteus vulgaris, Klebsiella mobilis, Staphylococcus aureus, Citrobacter spp, E.coli, Bacillus cereus* and *Shigella spp* were isolated from Akoko South East Local Government (Table 4). Two Gram positive bacteria were isolated; they are *Bacillus cereus* and *Staphylococcus aureus*.

*E.coli* was the highest bacteria isolated (n=7) representing 15.56% of the isolates followed by *Serratia marcescens* (n=4) with 8.89%. *Klebsiella mobilis, Bacillus cereus, Proteus val*garis, *Staphylococcus aureus* accounted for (n=3) 6.6.67% respectively.

*Enterobacter spp. Citrobacter spp* and *Shigella spp* (n=2) represented 4.44% of the isolates. The least was *Micrococcus varians* (n=1) which represented 2.22% of the total isolates (Figure 1). The degree of analysis on the most frequently encountered bacteria in the fishes from all local government can be described as *E .coli> Serratia marcescens> Bacillus cereus> Shigella spp> Micrococcus varians.*

The species of fungi isolated and identified vary slightly from each Local government. Fungi isolated were *Aspergillus spp, Rhizopus spp, Penicillium spp, Fusarium spp, Neurospora spp, Candida albicans* (Table 4). The most prevalent fungi were *Aspergillus flavus*, which was isolated from fishes in all the four local governments. The most frequently encountered fungi on the fishes from all local government can be described as *A.flavus> A. fumigatus> Candida albicans> Penicillium chrysogenum*.

Seventeen genera of microorganisms were isolated from the fish samples in this study, *Staphylococcus aureus* was found in the different parts of the fish analysed. This is similar to the presence of *Staphylococcus sciuri* found in the tegument, gut and flesh fillet of *Orechromis niloticus* (Boari *et al.,* 2008). The bacterial isolates were also similar to those reported (Obemeata *et al.,* 2011) who found *Staphylococcus, Proteus, Bacillus and Micrococcus* species in stored *Tilapia guineensis* and similar to eleven genera of bacterial consisting of both gram-positive and gram-negative that were isolated from smoked and fresh Bonga fish (Akinjogunla *et al*., 2011). While the mycotoxigenic potential of the mould genera implicated in the study have been reported (Obemeata *et al.,* 2011; Essein *et al.,* 2005) with *Aspergillus flavus* and *A. niger* as dominant mould flora in the smoked fishes. The existence of these moulds on cold smoked fish is a pointer to the potential health risk associated with consumption of such ready to eat products. This is similar to observation of (Essein *et al.,* 2005).

According to these authors such presence of microbes can indicates unhygienic conditions during the processing, because the product contamination could be the result of a combination of improper handling, storage and cross contamination.

Amusan *et al*. (2010) observe that women vendor lacked good personal hygiene, which is vital in reducing the chance of contamination of foods. It is not surprising in such poor hygiene and handling situation to isolate these microbes especially

*Staphylococcus* spp. from the majority of samples found in Akokoland, Ondo State, Nigeria.

The presence and variation in the isolates of bacterial, fungal species in this study may be due to changes in chemical and proximate composition, temperature, $p^H$ of the fresh sample and plating medium as observed by Fafioye *et al*. (2002) and FDA (1998). The average total bacteria count in the two species showed that the various samples examined exceeded the acceptable limits recommended by Food and Agricultural Organisation (FAO, 1979). This result is similar to Emikpe *et al.* (2011), but different from Amusan *et al.* (2010) who reported total bacteria count within the acceptable limits. This indicates that human health is at a higher risk due to consumption of smoked fish from the four Local Governments in Akokoland, Ondo State, Nigeria.

## Conclusion

Generally, the world is moving towards healthy eating more than ever, people are more conscious of what they eat, associating same with the health conditions presently and future. This study reveals the poor sanitary level of the smoked fishes within Akokoland, Ondo State Nigeria, which may lead to the occurrence of severe public health hazards. It is important to organize basic food hygiene training for fish mongers/vendors and consumers in Akokoland, as well as the entire Ondo State.

Consumers should therefore be encouraged to soak cold smoked fishes for about 30 minutes in boiled water before consumption to avoid food poisoning. Vendors are advised to use glass/netted box, wear latex gloves while selling smoked fishes. These will help to minimize the health risk associated with these ready to eat food. The ministry of Health should ensure proper inspection of street vended food (smoked fish) products marketed in the area and strict compliance to all hygienic standards by fish vendors at all times while distributing, displaying and marketing cold smoked fishes.

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