

## Original Research Article

# Public Health Implication of Microbial Loads in Smoked Mackerel, *Scomber scombrus* from Major Fish Markets in Uyo, Akwa Ibom State, Nigeria

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**Abstract:** As a way of checkmating public health implication associated with processed fish products, the study was conducted to assess the bacterial load and diversity in the fillets of smoked Atlantic mackerel, *Scomber scombrus* sold in major fish markets (Anua, Useh, Etuk, Itam and Akpan-Andem) in Uyo, Akwa Ibom State. Determination of bacterial loads, species characterization and composition in fish fillets were done using standard microbiological procedures. Results from the study revealed the highest fungal counts of  $9.0 \times 10^4$  cfu/ml in samples from Itam market. Heterotrophic bacteria count ranged from  $2.0 \times 10^6$  cfu/ml in Useh market samples to  $2.5 \times 10^6$  cfu/ml in samples from other study sites. A total of eleven bacterial and six fungal species were recovered. *Salmonella enterica* and *Shigella sonnei* accounted for the highest frequency of occurrence (100%) in all the study sites while *Escherichia coli* and *Vibrio cholera* had 80% prevalence. *Serratia liquenfaciens*, *Bacillus subtilis*, *Lactobacillus plantarum*, *Micrococcus luteus* and *Bacillus cereus* recorded 40% occurrence while the least prevalence of 20% was recorded in *Staphylococcus albus* and *Proteus mirabilis*. The isolated fungal species were *Penicillium expansum* (60% prevalence), *Aspergillus flavus*, *Mucor hiemalis*, *Rhizopus stolonifer* and *Geotriculun candidum* (40%) while *A. fumigates* (20%). Bacterial flora recovered in this study composed of potential pathogenic organisms of public health interest. This calls for intensified monitoring efforts towards controlling fish food contamination in Uyo metropolis.

**Keywords:** Atlantic mackerel, bacterial load, diversity, fungal species, contamination.

## INTRODUCTION

Fish and fishery products are important food components for humans with an estimated consumption level of about 20.1kg per capita FAO [1]. In developing countries such as Nigeria, fish products are accessible and relatively affordable. They are presented in several forms such as fresh, dried, smoked, frozen and canned. In Nigeria, preservation of fresh fish remains a problem due to lack of adequate infrastructures Anihouvi, *et al*, [2]. Thus, in order to prevent fish spoilage and post-harvest losses, various preservation methods such frying, drying, fermentation, salting and smoking are employed Ikutegbe and Sikoki [3]. Traditional fish smoking is however the main method used by fisher folks in preserving mackerel fish in Uyo. Smoking involves subjecting fish to direct or indirect action of heat which dries and preserve it from spoilage Koffi [4]. The Atlantic mackerel (*Scomber scombrus*) is the most important pelagic fish that is generally consumed in Uyo. It is also the most abundant and widely distributed migratory fish species in Nigeria. The fish is commonly seen either in fresh or smoked form in most open markets in the study area. These products are displayed on trays in selling points thus exposing them to several contaminating agents.

The presence of bacteria in fish could play diverse roles, some of which might be beneficial to the fish Egerton *et al*. [5]; Butt and Volkoff [6]. On the other hand, the presence of some bacterial species could lead to post harvest spoilage and adverse health conditions in farmed fishes Effiong and Isaac [7]. Fish and fish products are susceptible to a wide variety of bacterial and fungal invasion due to poor processing and handling methods. If allowed to proliferate, these microbes can change the physical and chemical nature of the fish leading to spoilage, food poisoning and food borne infections. In addition, these microbes had been reported to be major agents associated with diseases in human Guarner and Malagelada [8] and are known to be closely associated with the physiological status and postharvest quality

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of fish Al-Harbi and Uddin [9]. Earlier study by Feldhusen [10] stated that microbes are capable of causing diseases by passing infection to the host. Thus, these microflorae may be significant in fish spoilage as well as being able to transmit many of the established food borne microbial infections and intoxications. For instance, some of the isolated species like *Bacillus* and *Staphylococcus* had been noted to cause serious diseases in farmed fish Amande and Nwaka [11]; Effiong and Isaac [7] while others like *Pseudomonas* and *Escherichia coli* had been implicated in zoonosis to humans Babu [12]; Edun *et al.* [13]. Fish is the main source of protein for the people living in Uyo, Akwa Ibom State, and given the prevalence of water and food borne diseases in the area, it is in order that all possible infection routes of pathogens be investigated and possible mitigation measures outlined. On this note, the present study was designed to evaluate bacterial load and species diversity in the fillets of smoked Atlantic mackerel from major fish markets in Uyo.

## MATERIALS AND METHODS

### Collection of Experimental Fish

The experimental fish were procured from five major fish markets (Anua, Useh, Etuk, Itam and Akpan-andem) in Uyo, Akwa Ibom State, Nigeria. A total of 100 specimen of smoked mackerel were collected (20 samples from each market) and used for the study. Samples were transported to the laboratory of the Department of Microbiology, University of Uyo, for detailed examination and analysis.

### Sample Preparation, Culture and Bacterial Isolation

The study considered fish fillets for examination for possible presence of microbial agents. Samples from each market were separately made into fine particles using a manual blender. A 10g from each sample was homogenized with 90ml of sterile water and the homogenate was allowed to settle for 10 minute. Thereafter, 5 ml of the supernatant was transferred to make a 10-fold serial dilution. One ml of the diluted sample was inoculated using pour plate technique and a 0.5 % Nutrient agar medium was poured at 40°C on the plates. The sample and the medium were then mixed and allowed to set before incubating at 25°C for 48 hours. Colonies were sub-cultured to get pure cultures. These were further screened for the presence of indicator organisms. Microbial assay of the fillets was carried out to determine bacterial load, identification and frequency of occurrence using the method described by Cheesbrough [14]. Plates with colonies ranging between 50 – 200 were selected for determination of total bacterial count and isolation of individual bacterial groups. Total load of bacteria was estimated thus: Total load of bacteria (cfu/ml) =  $C \times D \times 10 \times V/W$  Cheesbrough [14] where: C= Number of colonies found, D= Dilution factor, V= Volume of physiological saline, W= Weight of fish sample.

### Characterization of Bacteria/Fungal Identification

Fungal isolates were identified by cultural and morphological characteristics which include surface texture, topography and pigmentation as described by Akintobi *et al.* [15]. Microscopic identification was done by placing a drop of 5% potassium manganese (KMnO<sub>4</sub>) on a slide and a small portion of representative fungi mycelium was removed and teased onto the potassium manganese stain using a sterile needle. The slide was covered, mounted and then viewed at 10x and 40x objective microscope Mailafia *et al.* [16]. Photos of identified fungal isolates were taken and compared with a documented book of fungi St-Germain and Summerbell [17].

Visual observation of morphological characteristics such as shape, size and color of the bacterial colonies were done. Shape of the individual isolate was determined by Gram staining method with the young culture. The motility test was performed by hanging drop method. Biochemical tests such as catalase activity, oxidase, indole production, gelatin liquefaction and proteinase test were performed using bacterial isolates from fresh culture according to the methods of Barrow and Feltham [18]. The pure fungal isolates were identified using both cultural and morphological characteristic texture (glabrous, powdery, granular, fluffy, downy, cottony,) surface topography (flat, raised, heaped, folded, domed, radial, grooved) surface pigmentation (white, creamy, yellow, brown, pink, grey, black) reverse pigmentation (yellow, none, brown, red, black) according to the general method by Akintobi *et al.* [15]. The procedure involved visual examination of isolates in culture (pigmentation, texture, reverse side, colony surface) and observation of stain preparation (nature of conidia, hyphae and spores) under microscope.

## DATA ANALYSIS

Data collected for microbial counts were subjected to descriptive statistics and differences in species load and diversity were analyzed using one-way analysis of variance (ANOVA) at 95% probability level using SPSS version 20. The bacterial and fungal isolates were presented in tables.

## RESULTS

The results of bacterial counts in fillets of smoked mackerel are presented in Table 1. Total heterotrophic bacterial count ranged between  $2.0 \times 10^6$ cfu/ml in Useh market samples and  $2.5 \times 10^6$ cfu/ml in samples from all other

markets. The least fungal count of  $1.0 \times 10^4$  cfu/ml was recorded in samples from Useh market while the highest ( $9.0 \times 10^4$  cfu/ml) was obtained in Itam samples. There was no *Pseudomonas* count in all the samples examined. Table 2 showed the primary characterization of different bacterial isolates. From the results, the entire bacterial flora showed negative to coagulase and haemolysis tests. The microbes were dominated by gram – negative rod shaped organisms. The results of biochemical tests for the different bacterial isolates are presented in Table 3. Here, all the bacterial species were positive to proteinase test. In all other tests, organisms showed different responses. The results of macroscopic and microscopic identification of fungal isolates are shown in Table 4. The results in Table 5 indicated that *Salmonella enterica* and *Shigella sonnei* accounted for the highest frequency of occurrence (100%). These organisms were observed in all the market samples. *Staphylococcus albus* and *Proteus mirabilis* had the least prevalence of 20% occurring only in Itam and Anua markets respectively. Among the fungal isolates, *Penicillium expansum* had the highest prevalence (60%) while *Aspergillus fumigatus* had the least value of 20%.

**Table-1: Microbial densities in smoked mackerel from major fish markets in Uyo**

Bacterial counts	Anua	Useh	Etuk	Itam	Akpan-andem
THBC (cfu/ml)	$2.5 \times 10^6$	$2.0 \times 10^6$	$2.5 \times 10^6$	$2.5 \times 10^6$	$2.5 \times 10^6$
TFC (cfu/ml)	$2.0 \times 10^4$	$1.0 \times 10^4$	$6.0 \times 10^4$	$9.0 \times 10^4$	$7.0 \times 10^4$
FCC (cfu/ml)	$8.7 \times 10^5$	$3.0 \times 10^5$	$1.7 \times 10^5$	$1.2 \times 10^5$	$1.4 \times 10^5$
TCC (cfu/ml)	$1.98 \times 10^6$	$9.2 \times 10^5$	$2.3 \times 10^6$	$1.2 \times 10^6$	$1.06 \times 10^6$
SSC (cfu/ml)	$1.84 \times 10^6$	$1.5 \times 10^5$	$2.0 \times 10^6$	$6.1 \times 10^5$	$1.1 \times 10^5$
TVC (cfu/ml)	$7.6 \times 10^5$	$1.3 \times 10^5$	$1.9 \times 10^5$	$1.4 \times 10^5$	$1.8 \times 10^5$
SC (cfu/ml)	$1.0 \times 10^5$	$7.0 \times 10^5$	$1.1 \times 10^6$	$2.9 \times 10^5$	$4.9 \times 10^5$
PC	-	-	-	-	-

Where: THBC: Total heterotrophic bacterial counts; TFC; Total fungal count FCC: Faecal coliform count; TCC: Total coliform count; SSC: Salmonella shigella count; TVC: Total vibro count; SC: Staphylococcus count; PC: Pseudomonas count

**Table-2: Primary characterization of different bacterial isolates in smoked mackerel from major fish markets in Uyo**

Characters	A	B	C	D	E	F	G	H	I	J	K
Gram stain	-	-	-	-	-	+	+	+	+	-	-
Shape	R	R	R	R	comma	cocci	R	R	cocci	R	R
Motility	+	+	+	+	+	-	+	-	-	+	+
Catalase	+	+	+	+	+	+	+	-	+	+	+
Coagulase	-	-	-	-	-	-	-	-	-	-	-
Starch	+	-	-	-	+	-	+	-	+	-	+
Urease	-	-	-	-	-	-	+	+	+	+	-
Oxidase	-	-	+	-	-	-	-	-	-	-	-
Citrate	+	-	+	-	+	+	-	-	+	-	-
Haemolysis	-	-	-	-	-	-	-	-	-	-	-
Lipase	-	-	-	-	-	-	-	-	-	+	-
Glucose	-	AG	A	A	A	A	AG	A	A	AG	AG
Maltose	AG	AG	A	AG	A	A	A	A	A	A	A
Fructose	-	-	-	-	A	AG	A	A	A	AG	A
Sucrose	-	-	-	AG	AG	-	-	-	-	AG	A
Mannitol	AG	AG	AG	AG	A	AG	-	-	A	-	-
Galactose	A	-	-	A	A	A	A	AG	A	AG	A

Where: A: *Serratia*; B: *Escherichia*; C: *Salmonella*; D: *Shingella*; E: *Vibrio*; F: *Staphylococcus*; G: *Bacillus subtilis*; H: *Lactobacillus*; I: *Micrococcus*; J: *Proteus*; K: *Bacillus aureus*; R; Rod; -: Negative; +: Positive; A: acid; AG: Acid and Gas

**Table-3: Results of the biochemical tests for the bacterial isolates**

Characters	Response by different bacterial isolates										
	A	B	C	D	E	F	G	H	I	J	K
Indole production	-	+	-	-	-	+	-	-	-	+	-
Proteinase test	+	+	+	+	+	+	+	+	+	+	+
Citrate utilization test	+	-	+	-	+	+	+	+	-	-	-
H <sub>2</sub> S production test	+	-	+	-	-	-	-	-	-	+	-
Gelatin liquefaction	-	-	+	-	-	-	-	-	-	-	+

Where: A: *Serratia*; B: *Escherichia*; C: *Salmonella*; D: *Shingella*; E: *Vibrio*; F: *Staphylococcus*; G: *Bacillus subtilis*; H: *Lactobacillus*; I: *Micrococcus*; J: *Proteus*; K: *Bacillus aureus*; -: Negative; +: Positive.

**Table-4: Macroscopic and microscopic examination of fungal isolates from smoked fish samples**

Macroscopic	Microscopic	Inference
Front black with white reverse side, filamentous soma and dense felt yellow-green Colony	Unbranched aseptate hyphae	<i>Aspergillus sp</i>
Front white with creamy reverse side, filamentous soma and grayish yellow colony	Branched aseptate coenocytic hyphae	<i>Mucor hiemalis</i>
Front brown with woolly reverse side, filamentous soma and blue-green colony	A 3-stage branched Conidiospores	<i>Penicillium expansum</i>
Front white with cotton reverse side, filamentous soma and membranous colony	Branched aseptate and very narrow hyphae	<i>Rhizopus stolonifera</i>
Front blue with white reverse side filamentous soma and white becoming membranous colony	Dichotomously branched septate hyphae	<i>Geotriculum candidum</i>

**Table-5: Prevalence of occurrence of bacterial and fungal isolates from pooled smoked fish samples**

Bacterial Isolates	% Prevalence	Fungal isolates	% Prevalence
<i>Serratia liquefaciens</i>	40	<i>Aspergillus flavus</i>	40
<i>Escherichia coli</i>	80	<i>Mucor hiemalis</i>	40
<i>Salmonella enterica</i>	100	<i>Penicillium expansum</i>	60
<i>Shigella sonnei</i>	100	<i>Rhizopus stolonifera</i>	40
<i>Vibro cholera</i>	80	<i>Geotriculum candidum</i>	40
<i>Staphylococcus albus</i>	20	<i>Aspergillus fumigatus</i>	20
<i>Bacillus subtilis</i>	40		
<i>Lactobacillus plantarum</i>	40		
<i>Micrococcus luteus</i>	40		
<i>Proteus mirabilis</i>	20		
<i>Bacillus cereus</i>	40		

## DISCUSSION

Fish being low in fat and high in protein contents play significant roles in the diets of both man and livestock. Examining factors that may contribute to their spoilage becomes necessary. Fish parasites (bacteria, fungi, virus, etc) had been reported to cause about 45% loss in fish farms Kabata [19]. Study by Cheesbrough [20] showed that serious disease outbreak had occurred in both human and animals after consuming contaminated fish. This could have been as a result of the presence of disease causing organisms such as *Escherichia coli*, *Salmonella typhi*, and *Vibro cholera*. The contamination usually occurs as a result of improper handling and indecent processing practices of ready-to-eat fish products. The present study showed that pathogenic bacterial and fungal species were present in all smoked mackerel samples collected from Anua, Itam, Etuk, Akpanandem and Useh markets in Uyo, Akwa Ibom State. A total of 11 bacterial (*Serratia liquefaciens*, *Escherichia coli*, *Salmonella enterica*, *Shigella sonnei*, *Vibro cholera*, *Staphylococcus albus*, *Bacillus subtilis*, *Lactobacillus plantarum*, *Micrococcus luteus*, *Proteus mirabilis* and *Bacillus cereus*) and 6 fungal (*Aspergillus flavus*, *Mucor hiemalis*, *Penicillium expansum*, *Rhizopus stolonifera*, *Geotriculum candidum* and *Aspergillus fumigatus*) species were isolated. Similar organisms were severally reported from street vended and open markets processed fish samples by Amusan *et al.* [21] in Street vended smoked blue whiting (*Micromesistius poutasou*); Nyarko *et al.* [22] in smoked sardine (*Sardinella aurita*) at smoking sites and market centre of Tema, Ghana; Yusuf and Hamid [23] in smoked *Clarias gariepinus* retailed in Bauchi metropolis, Nigeria; Adebayo-Tayo *et al.* [24] in fresh marine catfish (*Arius hendelotic*) from different markets in Akwa Ibom State, Nigeria; Adegunwa *et al.* [25] in smoked herring (*Sardinella eba*) from Ogun State, Nigeria; Ibrahim *et al.* [26] in smoked *Clarias gariepinus* in Minna, Nigeria.

Bacterial species had been widely reported as predominant organisms that contaminate smoked fish leading to spoilage Adegoke [27]; Yusuf and Hamid [23]; Adelaja *et al.* [28]. The route of contamination had always been poor sanitary condition and lack of adequate packaging of products since they are always seen exposed at the open markets. Herman *et al.* [29] opined that *Staphylococcus* species constitute the normal flora causing human skin and mucous membrane diseases. The species *E. coli* and *Salmonella sp* are known to be faecal borne pathogens which could occur in fish food as a result of contamination from handlers or polluted culture water. Alexander and Austin [30] earlier reported that fish harvested from contaminated water can harbor *Salmonella sp.* and other pathogenic microbes. These organisms which were dominant in all fish samples examined in this study had been linked to several health hazards in humans. For instance, Adelaja *et al.* [28] reported that *E. coli* caused diarrhea and kidney damage, as well as complicated community acquired urinary tract infection in human while *Salmonella sp* caused gastroenteritis and typhoid fever. The presence of *Aspergillus flavus* and *A. fumigatus* in smoked mackerel marketed in Uyo is of great health concern because of their mycotoxigenic potentials. The reports of Essien *et al.* [31] showed that *A. flavus* and *A. fumigatus* produced aflatoxins,

which are capable of destroying both liver and kidney in man. Other fungal species such as *Muccor sp*, *Rhizopus stolonifer* and *Geotriculun candidum* had been reported to be detrimental to human health Iwamoto *et al.* [32]. The consumption of contaminated fish could therefore cause serious health issues in man. According to Hosein *et al.* [33], seafood contributes a burden of disease to man since they are capable of transmitting many of the established food borne microbial infections and intoxications. The results of this study showed a high contamination level. Thus, smoking could be classified as a mild preservative treatment which may not achieve complete elimination of microbial load of fresh fish. Thus smoked fish should be properly cooked before consumption. Therefore, the presence of all the aforementioned pathogenic organisms in smoked *Scomber scombrus* in this study is an indication that the hygiene and safety condition of smoked fish is compromised.

## CONCLUSION

The study revealed the presence of some bacterial and fungal species in all smoked mackerel samples collected from the five different fish markets in Uyo, Akwa Ibom State. Some of the recovered species composed of potential pathogenic organisms of public health interest. The presence of these organisms may have been through improper handling of fish and fish products. Therefore, it is noteworthy that sanitary condition under which fishes are handled, processed and stored be improved upon to ensure standard and safe fish food products for consumers.

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