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INCIDENCE AND ANTIBIOGRAM OF *LISTERIA* SPECIES FROM SMOKED FISH SOLD IN ZARIA METROPOLIS, KADUNA STATE, NIGERIA

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ABSTRACT

Background: Fish and fish products are important in human nutrition worldwide as a source of protein. Therefore, microbial contaminate such as Listeria spp. especially, monocytogenes, which are of clinical significance, implicated in rhombencephalitis, meningitis and sepsis among pregnant women, children, immunocompromised and older people, could trigger public health concern and should be of interest to every stakeholder globally. **Aim**: This study assessed the incidence of *Listeria* species from smoked fish sold within Zaria metropolis and their susceptibility profile to common antibiotic in Zaria. **Methods**: A total of two hundred (200) smoked fish samples were collected from Samaru, Sabongari, Tudunwada and Zaria city markets. Proximate composition, total viable count (TABC) and total coliform count (TCC) were carried out using standard microbiological technique. *Listeria* was isolated using cultural method and was confirmed using MicrobactTM 12L. **Results**: The smoked fishes' proximate analyses revealed that protein (60.71 ±0.01) was the most abundant followed by lipid (27.67±0.00). Smoked fish sold at Zaria City had the highest TABC of $7.10x10^7$-$6.72x10^6$ CFU/g while the least TABC was observed in smoked fish sold at Tudunwada with a TABC of $3.67x10^7$-$1.36x10^7$ CFU/g.Smoked fish sold at Samaru market had highest TCC of $3.77x10^5$- $1.56x10^4$ CFU/g while smoked fish sold at Zaria City market had the least TCC of $2.20x10^5$ -$3.99x10^6$ CFU/g. The occurrence of *Listeria* species in smoked fish was found to be 7.5% (15/200). Based on market location; Zaria city had 3(6%), Tudun Wada 1(2%), Sabongari 1(2%), Samaru10(20%). **Conclusion**: Smoked fish sold in Zaria Metropolis were found to be contaminated and its consumption can be regarded as a threat to the health of consumers. As such smoking of fish should be done properly to avoid deterioration and increase in bacterial load.

Keywords: *Listeria species*, smoked fish (*clarius gariepinus*), proximate analysis,

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INTRODUCTION

Clarias gariepinus is among the species of fish that is consumed by people of Zaria and Nigeria in general. It formed a significant percentage of captured fish in River Kubanni and River Galma in Zaria, Nigeria. It is an important source of protein to the inhabitants (1). The intake of fish is beneficial to human growth and development and acts against some diseases such as rheumatoid arthritis, psychiatric disorder (2). Fish will become unfit for human consumption within one day of capture, unless it is subjected to some form of processing or preservation. Even after the fish has been processed, particularly if traditional methods have been used, the fish is still subject to many forms of deterioration and spoilage (3).

In Nigeria, fish is eaten fresh, preserved or processed (Smoked) and form a much-relished meal that spans socio-economic, age, educational and religious barriers (4). Smoking is one of the oldest food preservation technologies and can be used to achieve the characteristics taste, colour, aroma for food (especially meat and meat product, fish and fish products) (5). Besides the good benefits of fish, poor hygiene practices in food processing plants may result in the contamination of food products with pathogens, which means a serious risk for the health of consumers. Moreover, the complete elimination of pathogens from food processing environments is a difficult task, because bacteria can attach to food contact surfaces and form biofilms, where they survive even after cleaning and disinfection (6, 7). Some of the major pathogens to be controlled in the fish industry include *Staphylococcus aureus*, *Salmonella* species, *Campylobacter* species, enterohemorrhagic *Escherichia coli* (*E. coli* O157:H7), and *Listeria monocytogenes* (8). The best strategy for improving the safety of meat and fish products is adequate hygiene and the application of antimicrobial intervention technologies at processing, storage, distribution, and consumption stages. Some of these bacteria can be present in the natural environment, but many others enter the food chain as a result of poor hygienic conditions during processing and storage (9). *Listeria* species are ubiquitous, Gram positive, facultative anaerobe, non-spore-forming, rod-shaped bacteria which classically classified to six characterized species including *Listeria monocytogenes, Listeria ivanovii, Listeria innocua, Listeria seeligeri, Listeria welshimeri, and Listeria grayi* (10). Only the hemolytic species of *Listeria* such as *Listeria monocytogenes, Listeria ivanovii and Listeria seeligeri,* are associated with human pathogenicity, but *Listeria ivanovii* and *Listeria seeligeri* which respectively have been described to be involved rarely in human pathology and once to be the cause of meningitis in a non-immune compromised adult (11). One particular *Listeria* specie *Listeria monocytogene*s, can cause a serious food borne illness called listeriosis, an atypical food borne disease with a high fatality rate, in susceptible populations (12). However, there are some reports of *Listeria seeligeri* and *Listeria ivanovii* causing illness in humans (13). *Listeria monocytogenes* is responsible for *Listeria* infections that can lead to abortion, bacteremia, sepsis, and meningoencephalitis (14). The pathogen is

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transmitted via fecal-oral route directly from animals to humans. Vertical transmission from mother to neonate occurs trans-placentally or through an infected birth canal. The fatality ranges from 30%to 75% especially in high-risk groups like pregnant women, unborn or newly delivered infants, and the elderly people as well as persons with severe underlying disease conditions like immune-suppression, AIDS, chronic conditions like cirrhosis (15).

METHODS

The study was conducted in four (4) different markets in Zaria. The markets were Zaria City market, TudunWada market, SabonGari market and Samaru market. A total of two hundred samples of "smoked cat fish" scientifically known as '*Clarias gariepinus*' was purchased from Zaria City, Tudun Wada, SabonGari and Samaru markets respectively with 50 samples from each market based on convenient for a period of 5weeks, so as to ensure collection of new batches of fish samples. All the samples purchased in each market were wrapped in sterile aluminum foil, packed and labelled appropriately in sterile polythene bags and transported separately in clean polythene bags to the Food and Industry Laboratory, Department of Microbiology, Ahmadu Bello University Zaria where the samples were analyzed.

Proximate Composition of Smoked Fish (*Clarias gariepinus*)

Eight grams (8g) of the smoked fish sample was used to carry out proximate analyses at the Product Development Research Programme, Institute of Agricultural Research, Ahmadu Bello University, Zaria in order to determine the total carbohydrate content, the crude protein content, crude fat, crude fibre, ash content and moisture content as the percentage composition of the substrate according to standard methods described by Association of Official Analytical Chemists (16).

Detection of *Listeria* species from smoked fish samples

The culture method was used on the basis of International Standard. Twenty-fivegrams (25g) of each of the collected samples were transferred into 225 ml of 0.1%peptone water and incubated for 24 h followed by primary enrichment in which 1ml of the culture was transferred to a tube containing 9 ml of *Listeria* enrichment broth (Oxoid, CM0862). The tubes were incubated for 24h at 37°C. Two loops of *Listeria* enrichment broth culture were streaked onto *Listeria* selective media (Oxford formulation, Oxoid, CM0856) and incubated for 48h at 37°C after which the plates were examined for typical *Listeria* colonies, suspected colonies were transferred onto tryptic soy agar (TSA) (Difco England) and incubated for 24 h at 37°C. The suspected colonies of *Listeria* species were Gram stained and inoculated onto nutrient agar slants then stored at 4°C for further identification as described by (17).

Determination of Total Viable Count and Coliform Count of smoked fish sample

Serial dilution was carried out by pipetting 1ml of each of the stock solutions to 9ml of buffered peptone water to give a dilution of 10^1, one millilitre was aseptically dispensed into another fresh 9ml of the same diluents, the second dilution gave 10^{-2} dilution. The

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procedure was repeated until 10^{-6} dilution for each of the samples collected (18). From the 10^{-6} dilution, 0.1ml was aseptically inoculated on to the surface of freshly prepared Plate count agar (PCA) and freshly prepared MacConkey agar (Oxoid, Basingstoke, England) by the spread plate method and ensured evenly distribution of the sample on to surface of the media. The plate was incubated at 37°C for 24hrs after which the number of colonies that appeared on the surface of the Plate Count Agar were counted using a colony counter (SWARD, London) and used to determine the total viable count. Whereas the number of colonies that appeared pinkish or reddish on the MacConkey agar were considered lactose fermenters and were also counted using a colony counter.

Biochemical Identification of the suspected *Listeria* isolates

The suspected *Listeria* isolates were subjected to conventional biochemical tests (catalase, oxidase, MR-VP, motility test, aesculin hydrolysis β-haemolysis on 7% sheep's blood agar), carbohydrate fermentation test using mannitol, rhamnose, maltose, sucrose and xylose as described by Janzten *et al*., (17).

Confirmation of presumptive *Listeria* species with Microbact12L System

After subjecting the isolates to conventional biochemical tests, confirmatory test was carried out using the MicrobactTM 12L, by following the manufacturer's procedures. A single well isolated colony from 24hours culture was selected and emulsified in a vial of *Listeria* suspending medium (2.5ml). The medium was mixed thoroughly until a homogenous medium was obtained. Micro well test strips were removed from the foil pouch, placed on the holding frame and the lid removed. Using a sterile Pasteur pipette, 4drops (approximately 100µL) of the bacterial suspension was transferred to each well of the micro well test strips. As a purity check, one drop of the organism suspension was transferred onto nutrient agar plate. The plate was incubated aerobically at 37^0C for 24 hours. One drop of the haemolysin reagent was added to well 12 and the lid was placed back on the micro well test strip and incubated at 37°C for 24hours. After incubation, lids were removed from the micro well test strips and the result were recorded on the report form provided. The various octal codes were ran on Microbact 12L software version (Oxoid, MB1244A) to identify various species of *Listeria*

RESULTS

Proximate Composition of Smoked Fish Sold within Zaria Metropolis

Table 1 shows the proximate composition of smoked fish sold within Zaria metropolis. The result of the proximate analysis of the smoked fish revealed the following composition: 50.86% Protein, 26.30% Lipid, 10.75% Ash, 7.71% Carbohydrate, 4.38% Moisture and 0.00% Fibre. The difference observed was statistically significant ($p < 0.00001$).

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Table 1: Proximate Composition of Smoked Fish Sold in Zaria Metropolis

Proximate	Composition * Mean ± SE (%)
Moisture	2.27±0.05
Table	
Ash	1.52±0.02
Lipid	27.67±0.00
Protein	60,71 ±0.01
Fibre	0.00 ±0.00
Carbohydrate	7.71 ± 0.01
P value<0.0001	

*Values are mean ± SE of duplicate readings

Total Aerobic Bacteria Count of Smoked Fish Sold within Zaria Metropolis

Total Aerobic Bacterial Count (TABC) of smoked fish sold within Zaria metropolis is presented on Table 2. The lowest TABC was $1.00x10^6$ CFU/g while the highest TABC was $1.60x10^8$ CFU/g. Based on location, smoked fish sold at Zaria City had the highest TABC of $7.10x10^7±6.72x10^6$ CFU/g while the least TABC was observed in smoked fish sold at Tudunwada with a TABC of $3.67x10^7 ± 1.36x10^7$ CFU/g. The difference observed in the TABC of different location was statistically significant ($p < 0.00001$).

Table 2: Total Aerobic Bacterial Count of Smoked Fish Soldwithin Zaria Metropolis

Location	*Mean ± SE (CFU/g)	Range (CFU/g)
Zaria City	$7.10x10^7±6.72x10^6$	$4.20x10^7$-$1.00x10^8$
Tudunwada	$3.67x10^7±1.36x10^7$	$1.00x10^6$-$1.60x10^8$
Sabon gari	$3.95x10^7±6.35x10^6$	$1.20x10^7$-$8.80x10^7$
Samaru	$4.94x10^7±7.52x10^6$	$1.80x10^7$-$1.12x10^8$
F= 5758.81	P-value<0.00001	

*Mean values were significantly different between the sampling locations.

Total Coliform Count of Smoked Fish Sold within Zaria Metropolis

The Total Coliform Count (TCC) of smoked fish sold within Zaria metropolis ranged from $2.60x10^5$ CFU/g to $4.42x10^5$ CFU/g. Smoked fish sold at Samaru market had highest TCC of $3.77x10^5±1.56x10^4$ CFU/g while smoked fish sold at Zaria City market had the least TCC of $2.20x10^5±3.99x10^6$ CFU/g as shown in Table 3. The difference observed in the TCC of different location was statistically significant ($p < 0.00001$).

Table 3: Total Coliform Count of Smoked Fish Sold within Zaria Metropolis

Location	*Mean ± SE (CFU/g)	Range (CFU/g)
Zaria City	$2.20x10^5±3.99x10^6$	$4.50x10^4$-$3.60x10^5$
Tudunwada	$2.78x10^5±2.98x10^4$	$4.50x10^4$-$3.60x10^5$
Sabon gari	$3.39x10^5±1.62x10^4$	$2.60x10^5$-$4.20x10^5$
Samaru	$3.77x10^5±1.56x10^4$	$2.92x10^5$-$4.42x10^5$

F= 605.70P-value<0.00001

*Mean values were significantly different between the sampling locations

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Identification of *Listeria* species Isolated from Smoked Fish

Listeria sp. isolates were Gram positive rods, hydrolyzed aesculin, motile, catalase positive and β – haemolytic on sheep blood agar as shown in Table 4.

Table 4:Morphological and Biochemical characteristics of *Listeria* sp. Isolated from Smoked fish

Isolate code	Gram reaction	Aesculin hydrolysis	Indole	Motility	H_2S	Catalase	Oxidase	MR	VP	Haemolysis	Inference
SM7	Gram+ rods	+	-	+	-	+	-	+	+	β-haemolytic	*Listeria* sp.
SM17	Gram+ rods	+	-	+	-	+	-	+	+	β-haemolytic	*Listeria* sp.
SB3	Gram+ rods	+	-	+	-	+	-	+	+	β-haemolytic	*Listeria* sp.
SB5	Gram+ rods	+	-	+	-	+	-	+	+	β-haemolytic	*Listeria* sp.
TW3	Gram+ rods	+	-	+	-	+	-	+	+	β-haemolytic	*Listeria* sp.
TW11	Gram+ rods	+	-	+	-	+	-	+	+	β-haemolytic	*Listeria* sp.
KS2	Gram+ rods	+	-	+	-	+	-	+	+	β-haemolytic	*Listeria* sp.
KS3	Gram+ rods	+	-	+	-	+	-	+	+	β-haemolytic	*Listeria* sp.

***Listeria* species confirmed using Microbact**

The isolates were found to belong to species of *Listeria* namely *L. ivanovii* and *L. seeligeri* using Microbat as shown in Table 5. The percentage probability of species identity ranged between 52.27% and 99.99%.

Table 5: *Listeria* species confirmed using Microbact 12L

Isolate code	Octal code	%Probability	Identity
SM7, SM16, SM4, SM6, SM17, TW3	4001	96.25	*L. ivanovii*
SM13 SM18 SM8	5201	52.27	*L. seeligeri*
SM14	4041	83.81	*L. ivanovii*
KS2, SB3	4021	95.75	*L. ivanovii*
SM12	6001	90.66	*L. ivanovii*
KS3, KS1	4011	99.99	*L. ivanovii*

Keys: Sm - Samaru, Tw - Tudunwada,Sb – Sabon gari, Ks – Kasuwa (Zaria city).

Overall occurrence of *Listeria* species in smoked fish sold within Zaria metropolis

Out of the 200 samples of smoked fish screened, fifteen *Listeria* species were isolated and characterized giving an overall occurrence of *Listeria* species in smoked fish of 7.5% as shown in Figure1. Out of the 15 isolates of *Listeria* species, 3 (20.0%)

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were *L. seeligeri* while 12 (80.0%) were *L. ivanovii.* The differences observed in the distribution of smoked fish in different location was statistically significant (p = 0.0013) Figure2.

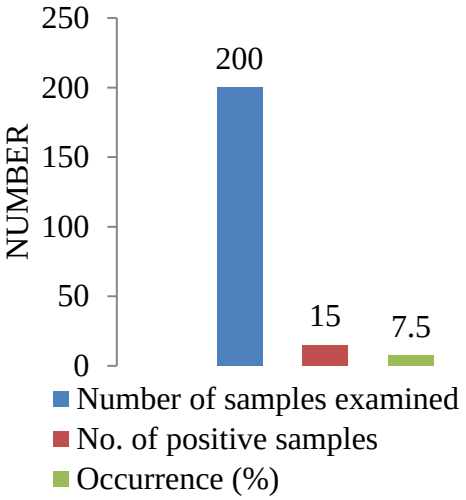

Figure 1: Overall occurrence of *Listeria* species in smoked fish sold within Zaria metropolis

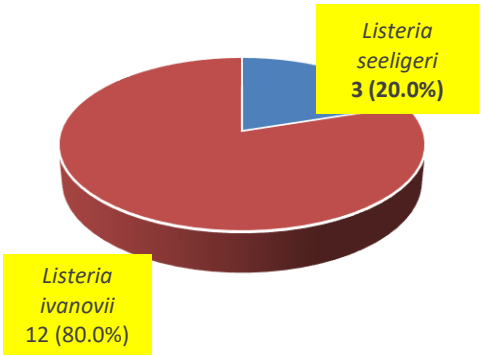

Figure 2: Percentage Distribution of *Listeria* species in Smoked Fish

DISCUSSION

The high protein content (60.71%) as well as the lipid (27.67.30%), ash (1.52%) carbohydrate (7.71%) and moisture (2.27%) contents of smoked fish observed in this study indicates that smoked fish can support the growth of bacteria that might contaminate it as a result of in appropriate preservation temperature or handling. The high protein content values as well as low ash, carbohydrate and moisture content values revealed by the proximate analysis is in line with the report of (19). The moisture content of the smoked fish was within the recommended limit considered safe for storage (6 – 8%) (19). The low moisture content could be a contributory factor of low occurrence of *Listeria* in smoked fish since lower moisture food increase the shelf life and also slow down the growth of microorganisms.

The mean Total Aerobic Bacterial Count (TABC) of smoked fish samples analyzed in this study was between $1.00x10^6$ CFU/g and $1.60x10^8$ CFU/g. These counts are higher than the acceptable limit of TABC for good quality fish products set by International Commission on Microbiological specifications for Food which is $5.0x10^5$CFU/g (20). These high counts might be linked to long storage, poor handling and lack of proper preservation (smoking temperature and time). The fact that smoked fish are usually kept at ambient temperature which is favourable for the growth and proliferation of bacteria might account for the high counts observed.

This count range is low compared to TABC range of 4.2 x 10^6CFU/g to 9.14 x 10^6CFU/g reported by (21), in smoked fish sold in Lagos metropolis. (22) reported a TABC of 1.2 ± 0.25 × 10^6 CFU/g in smoked Thai Pangus and 4.0 ± 0.56 × 10^4CFU/g in smoked Tilapia. Lower TABC ranges of 0.07±0.04 x 10^3 CFU/g to 0.35±0.11 x 10^3

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CFU/g was reported by (23) in smoked fish sold at Ibadan, Oyo State, and 4.82 x 10^4CFU/g to 4. 92 x 10^4CFU/g was reported by (24) in smoked catfish sold at Ota markets, Ogun state, Nigeria.

The range of total coliform count (TCC) in smoked fish was observed to be 2.60x10^5 CFU/g - 4.42x10^5 CFU/g. These TCCs are higher than the TCC limit of 1.0x10^3set by (20). Presence of coliform in smoked fish could be an indication of faecal contamination and poor handling of the samples. Lower mean coliform count of 4.53 x10^2 CFU/g in some dried fish species in Sabon Gari Zaria, Nigeria was reported by (25). Higher TCC range of 2.2 x 10^6 CFU/g to 5.1 x 10^6CFU/g was reported by (21) in smoked fish sold in Lagos metropolis.

The highest TABC observed in smoked fish sold in Zaria City market (7.10x10^7±6.72x10^6 CFU/g) and highest TCC of 3.77x10^5±1.56x10^4 CFU/g observed in smoked fish sold at Samaru market might be due to differences in smoking method used or handling. Smoked fish whose TABC and TCC exceed the set acceptable limit are not acceptable for human consumption as they may cause food-borne diseases. Also (26) confirmed that smoked dried catfish obtained from various markets in Kaduna exceeded the international standard limit.

Differences in counts and range of TABCs and TCCs in smoked fish sold at different markets were observed in this study. Similar differences in counts as well as TABC and TCC ranges was also reported by (23) in smoked fish sold at different markets in Ibadan, Oyo State, and (24) in smoked catfish sold at different markets in Ota, Ogun state, Nigeria.

Insufficiency of proper storage facilities as well as non-refrigeration makes smoked fish prone to contamination even before getting to the markets. This could be a contributing factor to the high microbial load observed in the smoked fish. (27) reported that coliform count may also be due to continuous exposure to dust, overstay due to poor sales in the market and constant touching by buyers and sellers.

The overall occurrence of *Listeria* species in smoked fish sold within Zaria metropolis was found to be 7.5%. This occurrence is similar to the 8.33% occurrence rate of *Listeria* species in smoked fish reported by (28). Higher occurrence of *Listeria* species (58.3%) in smoked fish sold in Sokoto was reported by Salihu *et al.*, (2008). Contrary to our finding where *Listeria ivanovii* was the dominant species of *Listeria* in smoked fish, (29) reported that *Listeria monocytogenes* (25.0%) was the dominant species of *Listeria* in smoked fish in Sokoto followed by *Listeria ivanovii* (13.0%). In study carried out by (28), *Listeria innocua* was reported to be the most frequent species of *Listeria* in smoked fish.

The occurrence of *Listeria* species was highest in smoked fish sold at Samaru market (20.0%) followed by smoked fish sold at Zaria city market (6.0%). The occurrence was least in smoked fish sold at Sabon gari market (2.0%) and Tudunwada market (2.0%). Similarly, 10% occurrence of *Listeria ivanovii*in smoked fish sold at community market of Ahmadu Bello University Main Campus, Zaria was reported by (30).

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CONCLUSION

Smoked fish in Zaria constitute mainly of protein and lipid but has no fiber. They also had significant bacteria load of public health importance, including *Listeria* species of 7.5% incidence rate. The most common Listeria spp. were *L. ivanovii* (80%) and *L. seeligeri* (20%).

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