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ASSESSMENT OF THE MICROBIAL CONTAMINATION OF SOME FROZEN, SMOKED AND CANNED FISH SOLD IN KADUNA NORTH L.G.A. WITH *LISTERIA* SPECIES

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ABSTRACT

This study was carried out to investigate the occurrence of *Listeria* species in frozen, smoked and canned fish in Kaduna North Local Government Area of Nigeria. A total of one hundred and eighty (180) fish samples comprising of 60 frozen, 60 smoked and 60 canned were purchased from the fish mongers at the three major markets in Kaduna North L.G.A (Kawo, Abubakar Gumi and Unguwar Rimi markets). Sampling was done once a weekly. *Listeria* species were isolated using pre-enrichment selective medium and were identified by conventional biochemical tests and confirmed with Microbact ID-System. Results shows that from the 180 samples, 24 (13.3%) were found to be contaminated with *Listeria* species. Out of the 24 isolates, Microbact 12L –ID system showed that 9(37.5%) of *L.grayi* and 15 (62.5%) of *L. ivanovii* were positive isolates respectively. Distribution of the occurrence of *Listeria* spp by sample type showed that smoked fish had the highest occurrence of 19 (31.7%) when compared with frozen fish which had 5 (8.3%) whereas canned fish had 0 (0%). Distribution of *Listeria* isolates by location showed that Unguwar Rimi market had the highest 15 (25%) followed by Kawo market 5(8.3%) and the least contaminated was Abubakar Gumi market 4(6.6%).The prevalence of Listeria species in this study showed that there is a potential threat to health and safety of the public. Therefore it is recommended that good hygiene practices should be implemented when handling fish.

Keywords: Determination, Microbial, Contamination, Fish, Kaduna North, *Listeria* Species

INTRODUCTION

Food safety is a major public health concern worldwide. During the last decades, the increasing demand for fish consumption has been recommended because it is a good source of high quality protein, minerals, vitamins and omega-3 poly unsaturated fatty acids. The later composite protects consumers against coronary heart disease, reducing arrhythmias and thrombosis and risk of fatal heart attack (FAO, 2014). The intake of fish is beneficial to children's growth and development and against some diseases such as rheumatoid arthritis, psychiatric disorders and lung diseases (WHO, 2016).

Beside good health benefits of fish, there are many reports about fish contamination by microbes and chemicals in the environment (Habeb *et al.,* 2009).Fish will become unfit for human consumption within 24 hours of capture, unless it is subjected to some form of processing or preservation. Even after processing, particularly if traditional methods have been employed, the fish is still subject to many forms of loss and spoilage (Shewan, 2000).

Fish, because of their soft tissues and aquatic environment are extremely susceptible to microbial contamination. Millions of bacteria, many of them potential spoilers, are present in the surface slime, on the gills and in the intestines of live fish, although the flesh itself is normally sterile. Bacterial growth and invasion of the fish are prevented by the body's natural defense system during life but after death the defense system breaks down and the bacteria multiply and invade the flesh. Immediately a fish dies, it remains in first class quality only for a short while (Center for Food Safety, 2014). However, spoilage soon sets in which is occasioned by an increase in the ambient temperature that triggers favourable conditions for microorganisms to thrive.

The Gram-positive bacterium *Listeria monocytogenes* was probably first recognized in two rabbits in Sweden 1910. Over a decade later, Murray *et al.* (1926) in the United Kingdom and Pirie (1927) in South Africa recognized a disease in laboratory rabbits, guinea- pigs and gerbils caused by a Gram-positive bacillus. Recognition of *L. monocytogenes* as a significant food borne pathogen occurred only in the early 1980s, with demonstration of food borne listeriosis outbreak (Schlecht *et al.*, 1983). *L. monocytogenes* is widely distributed in the environment and occurs in almost all food raw materials from time to time. The disease listeriosis usually occurs in high-risk groups, including pregnant women, neonates and immune compromised adults, but may occasionally occur in persons who have no predisposing underlying condition.

Listeriosis is one of the most severe food borne infections, with low morbidity but high mortality of up to 30% (Rocourt *et al.,* 2001). *L. monocytogenes* is able to multiply in high salt concentrations even at refrigeration temperatures with or without oxygen. It is resistant to diverse environmental conditions and it can survive in industrial environments for years regardless of cleaning procedures (Rocourt *et al.,* 2001; Hoffman *et al.,* 2003). *L. monocytogenes* is ubiquitous in nature and therefore, aquatic organisms are potential bacterial sources.

Apart from seafood products which undergo various processing steps that inactivate the bacterium, if present in the raw product, *L. monocytogenes* cross-contamination of products, presents a major problem especially for ready-to-eat products. Furthermore, there are seafood products that are eaten raw, without any listericidal step, such as cold-smoked and cold-salted fish. A lot of work has been done to study the sources of *L. monocytogenes* as well as means to control its growth and contamination in different food sectors (Chasseignaux *et al.,* 2002; Pak *et al.*, 2002; Gudbjörnsdóttir *et al.,* 2004; Thimothe *et al.,* 2004). Frozen, smoked and canned fish are frequently consumed in Kaduna State, however, reported cases of *Listeria* species contamination is scarce, therefore, the objective of this study was to determine the microbial contamination of some frozen, smoked and canned fish sold in Kaduna North L.G.A. with *Listeria* species.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in Kaduna North Local Government Area of Kaduna state. It is located at Latitude 10°20'N and Longitude 7° 45' East. The city is located in the North West Geo-political zone of Nigeria. Kaduna North has an area of 72 km Sq. and a population of 364,575 according to 2006 census (KDSG, 2008). Kaduna North falls within the Sudan savannah region; it's characterized by rainy and dry seasons with a little period of harmattan. Its headquarters is located at Magajin Gari in the heart of Kaduna town. It has (3) districts; Doka, Kawo and Gabasawa respectively (KDSG, 2008).The inhabitants are mostly traders in various businesses and civil servants.

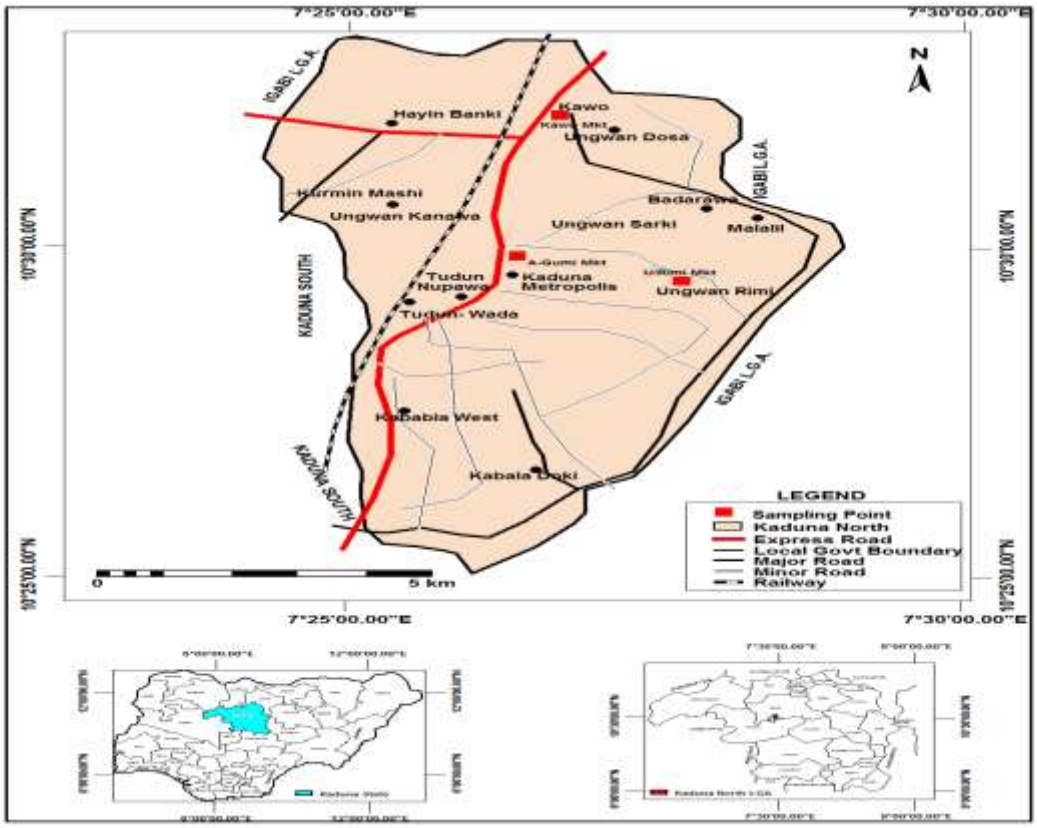

Figure 1: The Map of Kaduna North L.G.A, Nigeria showing the sampling areas.
Source: nigeriazipcodes.com Retrieved October 10 2015. 11:36GMT

STUDY DESIGN

A cross sectional study was carried out. Frozen, smoked and canned fish were purchased from three major markets in the study area for a period of 10 weeks (October, 2015-January, 2016).The required sample size was determined according to the formula as described by Campbell (1997) at 95% confidence interval using 9.67% (Shinkafi and Ukwaja, 2010).

$$n = \frac{t^2 \times p\,(1-p)}{m^2}$$

$$n = \frac{1.96^2 \times 0.0967(1-0.0967)}{0.0025} = \frac{3.8416 \times 0.08735}{0.0025}$$

$$n = 134.22$$

Therefore, n = 135; p was derived from the formula, and p-value of 9.67% (Shinkafi and Ukwaja, 2010), but for precision 45 samples were added and (n) was adjusted to 180.

DESCRIPTION

n = required sample size. t = confidence interval = 95 % (1.96). p = prevalence of the disease,

m = allowable error = 5 % (0.05)

A total of One hundred and eighty (180) samples of fish (60 frozen fish; 60 smoked fish and 60 canned fish) were used for this study.

Sample Collection and Transportation

A total of one hundred and eighty (180) samples which comprised of 60 frozen, 60 smoked and 60 canned fish were purchased from fish vendors and retail outlets of the major markets in three (3) districts of Kaduna North L.G.A. namely; Kawo Market, Abubakar Gumi market and Unguwar Rimi market, based on convenience. Sampling was done once weekly so as to ensure collection of new batches of fish samples.

Each sample was wrapped in sterile aluminum foil, packed and labeled appropriately in sterile polythene bags. Frozen fish were transported in a Coleman box containing ice packs to the Bacterial Zoonoses Laboratory in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria for microbiological analysis. Information on the container/labels was recorded to include National Agency for Food and Drug Administration and Control (NAFDAC) number, manufacture and expiry dates, batch number and Manufacturer's address where available. Canned containers were examined for evidence of defects before purchase; those with defects were excluded from sampling.

Enrichment and Isolation and Identification of *Listeria* Species

The International Standard Organization (ISO) 11920-1(1996) method for qualitative isolation and identification of *L. monocytogenes* was used as described by Indrawattana *et al.* (2011). About 10 grams from each fish sample was weighed and suspended into 90 ml of 0.1 % peptone water. Homogenization was done using a Stomacher (Stomacher Lab Blender 400) for one minute, the sample was allowed to settle for 2 to 3 minutes. One ml of the homogenized sample was suspended into the 9ml of prepared *Listeria* enrichment broth incorporated with *Listeria* selective supplement and incubated at 37°C for 18 to 24 hours.

A loop-full of the overnight culture was streaked on Oxford Listeria agar (Oxoid, Basingstoke, UK), and incubated at 37°C for 24-48 h. The colonies were identified by colonial morphology for *Listeria* colonies viz grayish colonies

surrounded by black halos with sunken center (Hitchins, 1995; Aygun and Pehlivanlar, 2006). Colonies showing black to brown colonies surrounded by black halos with sunken center on *Listeria* selective agar were sub-cultured onto Tryptone Soya Agar (Oxoid, M290) supplemented by 0.6% of Yeast Extract Powder (Oxoid, LP0021) (TSYEA) and incubated at 37°C for 24 hours. The colonies were subjected to gram staining and catalase test. The suspected colony of *Listeria* species was inoculated into nutrient agar slant and incubated at 37°C for 24 hours and then stored at 4°C for further identification as described by Janzten *et al.* (2006), and Jemmi and Stephan (2006).

Prior to inoculation into the Microbact^{TM} 12L, isolates were checked to ensure they are *Listeria* species. The suspending broth was brought to room temperature after storage at -4^{0}C before inoculation of micro well test strips. A single well – isolated colony from an 18-24hours 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, 4 drops (approximately 100µL) of the bacterial suspension was transferred to each well of the micro well test strips.

As a purity check, 1 drop of the organism suspension was transferred onto nutrient agar plate. The plate was incubated aerobically at 35-37^{0}C for 18-24 hours. One drop of the haemolysin reagent was added to well 12 and the lid was replaced onto the micro well test strip and incubated at 37° C for 24hours. After incubation, lids were removed from the micro well test strip and the result was 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*

DATA ANALYSES

Data was subjected to descriptive statistics using Statistical Package for Social Science (SPSS) version 20.0 (Ieren *et al.,* 2013). Probability less than 0.05 was considered statistically significant ($p < 0.05$). Prevalence was calculated using the formula:

$$\text{Prevalence} = \frac{\text{Number of positive samples}}{\text{Total number of samples collected}} \times 100$$

RESULTS

Determination of Occurrence of *Listeria* Species in Frozen, Smoked and Canned Fish in Kaduna North L.G.A., Nigeria

Out of the 29 isolates from 180 samples of fish examined and identified by biochemical tests twenty four (24) met the 80% cut-off point criteria for species identification by the Microbact 12L -Identification System. Among the 29 of the positive isolates, one (1) (*L. grayi*) gave 69.8% ; four (4) isolates were *L. ivanovii* with (2) 59% and (2) 74.3% each which were below the 80% cut off point for probable identification while the remaining 24 isolates had above 80% level of probability. Distribution of the 24 (100%) positive *Listeria* species based on Microbact 12L-ID system showed that 9 (37.5%) of the positive isolates were *L.grayi*,while 15 (62.5%) were *L. ivanovii* (Table 1).

Among the fish samples examined in this study, smoked fish had the highest occurrence of the *Listeria* species (31.7%) when compared with frozen fish which had 5 (8.3%) occurrence whereas canned fish showed 0 (0%) occurrence.

Table 1: Prevalence and Distribution of *Listeria* Species Based on Fish Sample Type in Kaduna North LGA, Nigeria

Occurrence of *Listeria* Species from Different Types of Fish in Kaduna North L.G.A., Nigeria

Type of Fish Sample	No. Samples Tested	*Listeria* Species Isolated		Total Positive (%)
		L. ivanovii	*L. grayi*	
Smoked	60	11	8	19 (31.7%)
Fresh	60	4	1	5 (8.3%)
Canned	60	-	-	0
Total	**180**	**15**	**9**	**24 (13.3%)**

Determination of Occurrence of *Listeria* Species in Frozen, Smoked and Canned Fish Based on Sampling Locations in Kaduna North L.G.A., Nigeria

Distribution of *Listeria* isolates by location showed that Unguwar Rimi market had the highest occurrence of *Listeria* species (25%) followed by Kawo market

(8.3%) and the least contamination with *Listeria* species was observed at Abubakar Gumi market (6.6%) (Table 2).

Only 2 species of the *Listeria* were recovered from the three markets, of which *Listeria ivanovii* was the most commonly contaminating specie with (8.9%) total prevalence (Table 2). Unguwar Rimi market had the highest occurrence of *Listeria ivanovii* isolates (16.6%) and the least occurrence of *Listeria ivanovii* (3.3%) was recorded at Kawo market. Five 5(8.3%) *Listeria grayi* isolates were recovered from Unguwar Rimi market while 3(5%) were recovered from Kawo Market (Table 2).

Table 2: Prevalence and Distribution of *Listeria* Species by Fish Sampling Locations in Kaduna North L.G.A, Nigeria

Location	**No. of Samples Tested**	**Number and Percentages (%) of Positive Samples**		
		***Listeria* species**	***L. ivanovii***	***L. grayi***
Abubakar Gumi Market	60	4 (6.6)	4 (6.6)	0 (0)
Ungwan Rimi Market	60	15 (25)	10 (16.6)	5(8.3)
Kawo Market	60	5 (8.3)	2 (3.3)	3(5)
Total	**180**	**24 (13.3)**	**16 (8.9)**	**8 (4.4)**

DISCUSSION

In this study, *Listeria* species was isolated from 31.7% smoked fish. This high prevalence could be due to the method of processing and evisceration which allows ample opportunity for contamination to occur. The unhygienic conditions of fish handling at different levels could serve as sources of contamination. The storage conditions, processing and environmental factors could also be the reason for high occurrence in smoked fish samples. Similar results have been reported by Vital *et al*. (2004) and this was attributed to the fact that *Listeria* species are pervasive in the environment.

The overall high prevalence of *Listeria* species in this study shows that human and environmental factors could be attributed to the contamination. It is also an indication of poor hygiene level in the processing and handling of fish viz-a-viz poor sanitary and hygienic practices in the fish markets and environment. The implication of this is that, the fish sold to consumers in Kaduna North Local Government Area, Nigeria could be contaminated by pathogenic bacteria which are of public health concern.

Total prevalence of *Listeria* species by sample types showed that smoked fish had a high prevalence of 10.6%. This may be due to the fact that the external surfaces of fish might act as inoculating agents that introduce *Listeria* species into processing plants; this contamination is extended by further operations such as filleting, rinsing, and brining (Eklund *et al.*, 1995). Similar result have been reported by Chukwu *et al.* (2006) who reported 9.2% prevalence. It is also likely that equipment, personnel, and surfaces might serve as secondary contamination sources of *Listeria* species.

The 8.3% prevalence of *Listeria* species in frozen fish found in this study is lower than the findings of Kuzmanovic Jelena *et al.* (2011) who reported 9.84% prevalence of *Listeria* species in Belgrade, Serbia.

In conclusion, the prevalence of Listeria species in this study showed that there is a potential threat to health and safety of the public. Therefore it is recommended that good hygiene practices should be implemented when handling fish.

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