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LISTERIA SPECIES AND OCCURRENCE IN RETAILED SMOKED FISH SAMPLES IN SELECTED OTA MARKETS, OGUN STATE, NIGERIA

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ABSTRACT

This study investigated the prevalence of Listeria spp. in 72 samples of smoked species of codfish (Gadus morhua), herring (Clupea harengus), catfish (Clarias gariepinus) and mackerel (Scomber scombrus) sampled from Sango (SA), Oju-ore (OO), Oja-Oba, Iyana-Iyesi (II), Iju (IJ) and Atan (AT) markets, all in Ota, Ogun State, Nigeria. Adopting Oxoid Listeria Precis method, Listeria spp. were isolated and characterized morphologically and biochemically. Of the 72 samples, 8 (11.11%) tested positive. Listeria ivanovii (1.38%), L. grayi (2.78%), L. monocytogenes (2.78%) and L. innocua (4.16%) were identified in all the fish samples except Clupea harengus. Antibiotic sensitivity tests revealed all the isolates resisted Imipenem, Cefuroxime, Amoxillin and Cefexine but susceptible to Levofloxacin: 34mm (L. grayi) to 39mm (L. ivanovii), Ciprofloxacin: 25mm (L. monocytogenes) to 36mm (L. grayi) and Ofloxacin: 20mm (L. ivanovii) to 29mm (L. innocua). Occurrence of these bacteria could indict the safety of the smoked fish products for consumption. The low percentage occurrences coupled with the high antibiotic susceptibility may, however, relieve the potential consumers of the menace of the associated health risks the consumption of such fish products could pose. The required control measures to prevent the occurrence of especially Listeria monocytogenes and other species in the processing and distribution chains of these smoked fish products should be prioritized.

Keywords: *Listeria* spp., *Listeria monocytogenes*, smoked fish, consumers' safety, antibiotics resistance, antibiotics susceptibility, low occurrence, health threat, control measures.

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INTRODUCTION

Fish and fisheries products are an important part of the diet of a large portion of the world's population. Like in many parts of the world, fish and fishery products are consumed in Nigeria as a protein source practically by almost every household for which fish serves as a contingency source of nutrition (Ogunji *et al.*, 2023). Most of the outbreaks of illnesses occur in countries where seafood is eaten raw or is inadequately cooked (Bruijn *et al.*, 2018). Historically, more than 70% of fish harvested in fishing communities were smoked for preservation. Smoking fish and/or meat products is an old processing method that is still used in Nigeria today. The environment in which fish are displayed in the market is not always sanitary and this is one of the sources of microbial contamination (Mhango *et al.*, 2010). Retailers frequently display smoke-dried fish in open trays beside gutter or refuse heaps, which encourage fungal and bacterial contamination and subsequent production of toxins (Ayeloja, *et al.*, 2018). The germs that were most frequently linked to smoked fish were *Staphylococcus aureus,* Proteus, Bacillus, and Micrococcus (Daramola, *et al.*, 2020). Several outbreaks of foodborne illness have been connected to listeria

(Abdollah and Tayebe, 2019). The majority of disease outbreaks happen in nations where seafood is either consumed raw or undercooked (Bruijn *et al*., 2018). In the past, fishing villages smoked about 70% of their catch to preserve it. In Nigeria, smoking fish and/or meat products is still a common ancient processing technique. One of the origins of microbial contamination is the unhygienic conditions in which fish are presented in markets (Mhango, *et al*.2010). Retailers commonly place open trays with smoke-dried fish next to gutters or trash piles, which promotes bacterial and fungal contamination and the subsequent development of toxins (Ayeloja *et al.,* 2018). Listeria has been connected to issues with food safety in relation to a number of crop commodities. Due to its ability to thrive in harsh conditions, including low pH and high salt concentrations, as well as low temperatures, *L. monocytogenes* contamination is a significant issue in food processing facilities (Gupta & Adhikari, 2022). *Listeria* spp. are regularly detected in many different contexts and are widely spread. They widely spread in the environment and are thought to be ubiquitous organisms. They have been found all over the world, including North America, South America, Europe, Asia, Africa, and Oceania (Orsi and Wiedmann, 2016). They include *L. ivanovii* and *L. monocytogenes*, two pathogenic species. Liu (2013), reports that every species appears to infect a different host. For instance, *L. ivanovii* is believed to only infect ruminants, whereas *L. monocytogenes* appears to infect both humans and ruminants. *L. ivanovii* have infrequently been isolated from ill persons in the past, which raises the possibility that they could be dangerous to humans. In contrast to *L. monocytogenes,* Guillet *et al*. (2010) reported a case of *L. ivanovii* infection in a man undergoing kidney transplantation and suggested that this species is less common in the environment. For many years, it has been known that *L. monocytogenes* is a facultative pathogenic bacterium that can cause listeriosis, a dangerous condition that can affect both humans and animals (Thakur *et al*., 2018). Most human cases of listeriosis are seen in immunocompromised persons, elderly people, neonates, and pregnant women. Madjunkov *et al* (2017). Listeriosis affects most often the pregnant uterus, central nervous system or blood circulation (Koopmans *et al.,* 2023). Consumption of smoked fish contaminated with *L. monocytogenes* is the primary route of transmission for listeriosis (Camargo *et al*., 2017). While *L. monocytogenes* causes relatively few human disease cases, particularly compared to many other foods borne pathogens, it appears to be commonly present in raw and ready-to-eat foods (EFSA, 2018).

Several locally processed Nigerian foods have been implicated to be sources of Listeria. Ijabadeniyi (2007), cited by Olopade *et al.* (2014), carried out a microbiological survey on some fermented food products including gari and lafun made from cassava (*Manihot esculenta*) and ogiri (*Ricinus communis*). In a comparable study, Osho *et al*. (2010) used gari, elubo-isu from yam (*Dioscorea rotundata*), and iru from locust bean seed (Parkia spp.). They discovered minor quantities of listeria in only elubo-isu (yam flour). A variety of traditional drinks, including kunu (Chukwuma *et al*., 2020), wara (a soft cheese made by coagulating fresh cows' milk), and kilishi (a snack made by sun-drying beef) have all been linked to *Listeria* spp. Additionally, vegetables and frozen poultry have been connected to *Listeria* spp. (Smith *et al*., 2018; Adetunji and Odetokun, 2012).

Numerous studies have connected fish to *L. monocytogenes* (Jami *et al*., 2014). It is impossible to overstate the organism's implications for public health given that *L. monocytogenes, L. innocua, L. ivanovii,* and *L. grayii*, among other sea species, have been isolated from a variety of fish species in various parts of Nigeria, along with smoked fish (Nwaiwu, 2015). Numerous Listeria In order to ascertain the frequency of *Listeria monocytogenes* in smoked fish in Sokoto, Nigeria, Salihu *et al*. (2008) conducted experiments. Listeria spp. were also found by Musa *et al*. (2020) in smoked fish that was marketed on the Ahmadu Bello University main campus in Zaria. The prevalence of *Listeria* spp. in the smoked fish products retailed in some Nigerian markets obliges the investigation of the safety status of these products in some other parts of the country. Documentation of such study in Ota, Ogun State, is flimsy. Hence, this study investigated some commonly retailed smoked codfish (*Gadus morhua),* herring (*Clupea harengus*), catfish (*Clarias gariepinus)* and mackerel (*Scomber scombrus)* for the prevalence of *Listeria* spp. sampled from major markets in Ota.

MATERIALS AND METHODS

A preliminary survey was carried out to identify the retailing locations of the selected smoked fish species (catfish, mackerel, herring and codfish).

Location of study

The study was conducted in Ota, Ado-Odo Local Government Area of Ogun State, which is situated in a geopolitical zone in the southwest of Nigeria, between latitudes 6.38°N and 6.41°N and longitudes 3.8°E and 3.12°E (Figure 1).

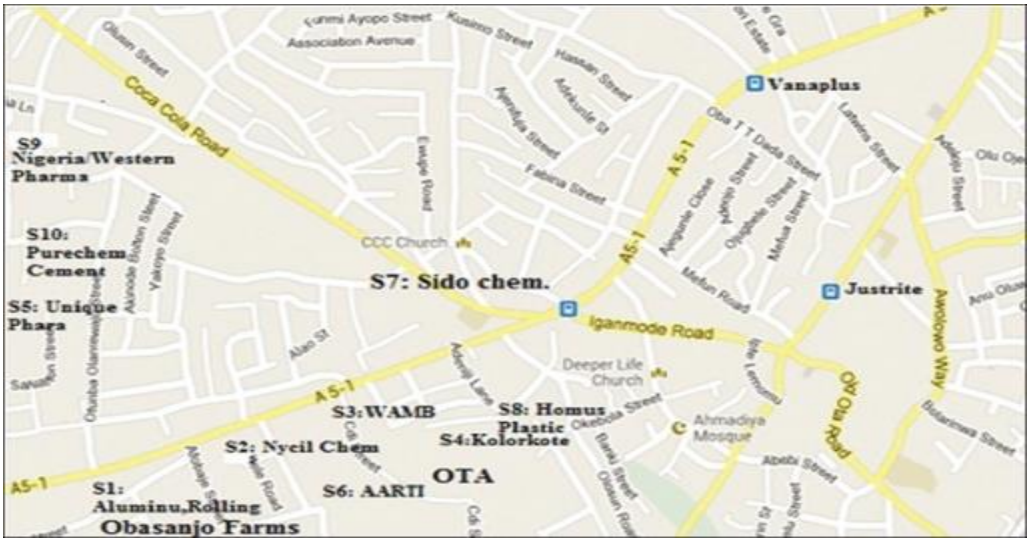

Figure 1: The map of the study area, Sango-Ota

Smoked fish samples collection

Using the stratified technique described by Amusan and Sanni (2019), a total of seventy-two (72) pieces of smoked fish were sampled from six markets in Ota (Table 1), comprising of 12 each of catfish (*Clarias gariepinus*), herring (*Clupea harengus*), mackerel (*Scomber scombrus*) and codfish (*Gadus morhua*). The samples were transported in sterile zip lock bags to the Microbiology laboratory of the Federal College of Fisheries and Marine Technology, Victoria Island, Lagos and investigated for the presence *Listeria* spp.

Materials

Amusan and Sanni (2019) and Ishola *et al*. (2016) both described the Oxoid Listeria Precis method, which was applied with minor modifications to isolate Listeria spp. from the smoked fish samples. Every media, including the Buffered Peptone water (Himedia, Mumbai), Palcalm Listeria broth, Palcalm Listeria agar (Liofilchem, Italy), Muller Hinton agar (TM Media, Delhi), Listeria Identification Broth Base (PALCALM), (HiMedia, Mumbai), and Nutrient agar (Life asible, USA), was prepared according to the manufacturer's instructions. For fifteen minutes, all of the media were sterilized at 121°C.

Isolation and identification of *Listeria* spp

The Oxoid Listeria Precis method described by Ishola *et al*. (2016) and Amusan and Sanni (2019) was slightly modified and adopted for the isolation of *Listeria* spp. from the smoked fish samples. The manufacturer's instructions were followed in the preparation of all the media which were sterilized at 121^0C for 15 minutes. Each fish sample was pounded into pieces using a sterile mortar and pestle, 25g of which was pre-enriched in 225ml of sterile buffered peptone water (Himedia, Mumbai), gently mixed for about 30 seconds and incubated at 37^oC for 24 hours. Secondary enrichment was done by transferring 2ml of the pre-enriched suspension into 18ml sterile L-Palcalm selective broth Base (HiMedia, Mumbai), and incubated at 30^oC for 24hours. The broth cultures were thereafter agitated and using spread plate technique, 0.1ml of the enriched broth base prepared from each of the fish samples was inoculated onto sterile Palcalm Listeria selective agar, LSA (Liofilchem, Italy), and incubated at 37°C for 24-48 hours. Gray-green developed colonies with black haloes were subcultured on Nutrient agar (Life Asible, USA) and incubated for a full day at 37°C. Pure cultures of isolates for characterisation were obtained by repeatedly subculturing the appropriate medium. The first identification of the isolates was done using the

morphological and microscopic features of the colonies. A battery of biochemical tests, including assays for catalase and oxidase, and a sugar fermentation test employing glucose, mannitol, sucrose, maltose, fructose, lactose, rhamnose, and xylose, were performed on the pure isolates that had undergone Gram staining. Hitchins and Jinneman (2011) described the use of phenolphthalein as an indicator.

Antibiotic susceptibility test

The isolated organisms were examined for antibiotic susceptibility using the Kirby Bauer disc diffusion technique. Spreading the refined isolates onto Muller Hinton agar plates served as the inoculation method. After gently placing the Gram-positive Antibiotic disc (Cel Tech Diagnostic, Belgium) on the Muller Hinton agar plates with sterile forceps, the plates were incubated for 24 hours at 37oC. Antibiotic susceptibility was determined by measuring the diameter of the zones of inhibition (Garedew *et al*., 2015).

RESULTS

Morphological and biochemical characteristics of the bacterial isolates

The morphological characteristics of the bacterial isolates from the smoked catfish (*Clarias gariepinus*), herring (*Clupea harengus*), mackerel (*Scomber scombrus*) and codfish (*Gadus morhua*) purchased from Sango (SA), Oju-ore (OO), Oja-Oba, Iyana-Iyesi (II), Iju (IJ) and Atan (AT) markets, all in Ota, Ogun State are summarized in Table 1. All the isolates were observed to develop as gray-green colonies with black haloes on Palcalm Listeria selective agar. With the same microscopic appearances of short rods occurring in chains, the isolates had circular shapes, entire margin, raised elevation and opaque optical characteristics.

Table 1: Morphological characteristics of *Listeria* spp. isolated from smoked fish samples

Morphological characteristics	Isolates			
	LS1	**LS2**	**LS3**	**LS4**
Pigmentation	Grey	Grey	Grey	Grey
Cell shape	Short Rod	Rods	Short rods	Rods
Cellullar arrangement	Chains	Chains	Chains	Chains
Colonial shape	Circular	Circular	Circular	Circular
Margin	Entire	Entire	Entire	Entire
Elevation	Raised	Raised	Raised	Raised
Optical	Opaque	Opaque	Opaque	Opaque
Suspected organism	*Listeria* spp.	*Listeria* spp.	*Listeria* spp.	*Listeria* spp.

Key: + = Gram positive, LS1 – LS4 = Listeria spp.

The results of the biochemical tests carried out on the isolates are presented in Table 2. All the isolates were observed to be Gram positive and motile. None of the isolates was positive to oxidase, coagulase, urease, indole and citrate tests but were all positive to catalase, MRVP and aesculin tests. They all fermented glucose, dextrose, maltose, lactose, fructose and galactose but had variable results for sucrose, xylose, rhamnose and mannitol.

Four likely species of Listeria bacteria have been identified from the morphological and biochemical characterization of the isolates found in the four smoked fish samples: mackerel (*Scomber scombrus*), catfish (*Clarias gariepinus*), herring (*Clupea harengus*), and codfish (*Gadus morhua*); the fish samples were sold in Ota, Ogun State, retail stores, and Oju-ore (OO), Oja-Oba, Iyana-Iyesi (II), Iju (IJ), and Atan (AT) markets. These four putative isolates are identified in Table 2's footnotes as LS1– *L. grayi*, LS2- *L. ivanovii*, LS3– *L. innocua*, and LS4– *L. monocytogenes*.

Frequency of occurrence of *Listeria* spp

The incidence of *Listeria* spp. in the 72 smoked fish samples varied between the six Ota markets that were investigated (Table 3). The Iyana-Iyesi market's fish samples did not show any growth, while the Atan, Ota, and Oju-Ore markets' mackerel, codfish, and catfish all showed the same frequency of occurrence (5.55%/1/72). In two samples of mackerel and one sample of catfish, Sango market samples showed the maximum occurrence of 16.6% (3/72) whereas Iju market samples showed 11.11% (2/72) in both categories. Four of the eight isolates,

shown by the respective figures in Figure 2, are *Listeria grayi*, *Listeria monocytogenes*, *Listeria innocua*, and *Listeria ivanovii*, with relatively low incidence percentages of 2.78% (2/8), 2.78% (2/8), 4.16% (3/8), and 1.38% (1/8).

Table 2: Biochemical characteristics of the isolated *Listeria* spp.

Biochemical Tests	*Listeria* spp. isolated			
	LS1	LS2	LS3	LS4
Gram stain	+	+	+	+
Cell Shape	Short rod	Rod	Short rod	Rod
Motility	+	+	+	+
Oxidase	-	-	-	-
Catalase	+	+	+	+
Indole	-	-	-	-
Coagulase	-	-	-	-
Urease	-	-	-	-
Methyl red	+	+	+	+
Voges-proskauer	+	+	+	+
Citrate	-	-	-	-
Aesculin	+	+	+	+
Glucose	+	+	+	+
Dextrose	+	+	+	+
Maltose	+	+	+	+
Lactose	-	+	+	+
Sucrose	-	+	-	-
Xylose	-	+	-	-
Fructose	+	+	+	+
Galactose	+	+	+	+
Rhamnose	+	-	+	+
Mannitol	+	-	-	-

Key: + = positive, - = negative, LS1 = L. grayi, LS2 = L. ivanovii, LS3 = L. innocua, LS4 = L. monocytogenes

Table 3: Occurrence of *Listeria* spp. in the smoked fish samples of the selected markets

Location	No Examined	M	Ct	H	Cd	Positive	(%)	Isolated organisms
SA	12	1	2	ND	ND	3	16.66	*L. innocua, L. monocytogenes L. grayi*
AT	12	1	ND	ND	ND	1	5.55	*L. innocua*
OT	12	ND	ND	ND	1	1	5.55	*L. innocua*
IY	12	ND	ND	ND	ND	0	0	NG
OJ	12	ND	1	ND	ND	1	5.55	*L. grayi*
IJ	12	1	1	ND	ND	2	11.11	*L. ivanovii, L. monocytogenes*
Total	**72**	**3**	**4**	**-**	**1**	**8**	**11.11**	

Key: SA= Sango market, AT= Atan market, OT= Oja-Ota, IY= Iyana-iyesi market, OJ= Oju-ore market, IJ= Iju market, M= Mackerel fish, H= Herring, Ct= Catfish, Cd = Codfish, ND= Not detected

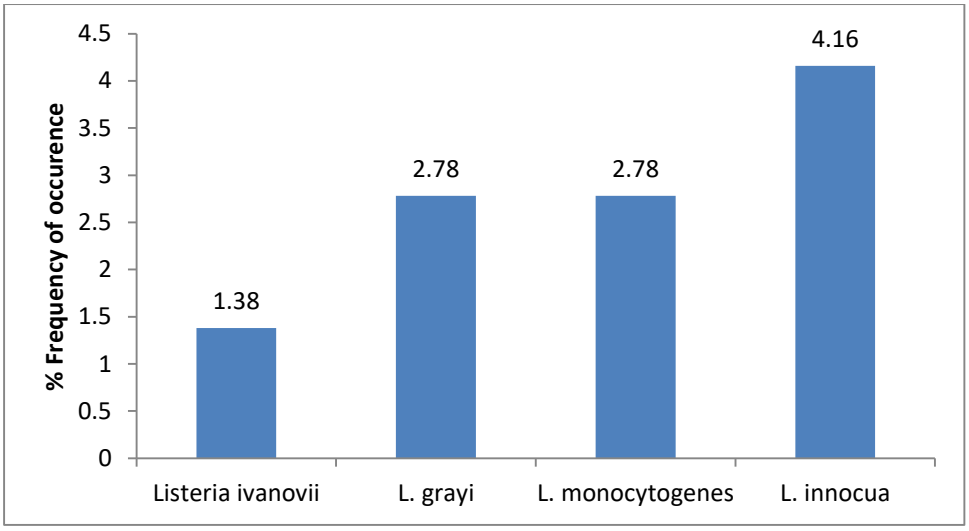

Figure 2: Percentage frequency of occurrence of *Listeria* spp. in the smoked fish samples

Antibiotics susceptibility test of the isolated *Listeria* spp

As shown in Table 4, all the isolates were observed to resist Imipenem, Cefuroxime, Amoxillin and Cefexine while they were all susceptible to Levofloxacin: *L. grayi* (34mm) to *L. ivanovii* (39mm); Ciprofloxacin: *L. monocytogenes* (25mm) to *L. grayi* (36mm) and Ofloxacin: *L. ivanovii* (20mm) to *L. innocua* (29mm). Fluoroquinolones antibiotics (ofloxacin, ciprofloxacin, levofloxacin) and aminoglycoside antibiotics (gentamicin) were shown in Table 5 to be 100% effective against the *Listeria* species isolated in this study.

Table 4: Interpretation of zones of inhibition (mm) for Kirby Bauer antibiotic susceptibility test

Antibiotics & Disk conc.	**LSI**	**LS2**	**LS3**	**LS4**
	Diameter of zone of inhibition (ZOI)			
Imipenm (10µg)	20 (S)	0 (R)	6(R)	7(R)
Cefuroxime (30µg)	20(S)	22(R)	0(R)	10(R)
Ofloxacin (5µg)	28(S)	20(S)	26(S)	29(S)
Erythromycin (15µg)	6(R)	21(R)	6(R)	25(S)
Gentamycin (10µg)	22(S)	17(S)	25(S)	24(S)
Azithromycin (15µg)	25(S)	15(R)	28(S)	27(S)
Amoxillin (30µg)	0(R)	0(R)	11(R)	37(S)
Cefotaxime (25µg)	16(I)	12(R)	17(I)	15(I)
Ceftriaxime (45µg)	20(S)	34(S)	15(I)	17(I)
Cefexine (5µg)	21(S)	16(R)	19(S)	9(R)
Levofloxacin (5µg)	34(S)	39(S)	37(S)	36(S)
Ciprofloxacin (5µg)	36(S)	32(S)	25(S)	35(S)

Key: LS1 –LS4 –Listeria spp., S-Sensitive, I-Intermediate, R-Resistant

Table 5: Antibiotic sensitivity, intermediate and resistance patterns of *Listeria* species isolated from smoked fish samples

S/N	Antibiotics	R	I	S
		Number of Isolates (Percentage)		
1	Imipenem	3 (75)	-	1 (25)
2	Cefuroxime	3 (75)	-	1 (25)
3	Ofloxacin	-	-	4 (100)
4	Erythromycin	3 (75)	-	1 (25)
5	Gentamicin	-	-	4 (100)
6	Azithromycin	1 (25)	-	3 (75)
7	Amoxycillin	3 (75)	-	1 (25)
8	Cefotaxime	1 (25)	3 (75)	-
9	Ceftriazime	-	2 (50)	2 (50)
10	Cefixime	2 (50)	-	2 (50)
11	Levofloxacin	-	-	4 (100)
12	Ciprofloxacin	-	-	4 (100)

Key: S-Sensitive, I-Intermediate, R-Resistant

DISCUSSION

Listeria grayi, *Listeria monocytogenes*, *Listeria innocua* and *Listeria ivanovii* were identified as the typical *Listeria* spp. contaminants of the smoked fish samples of this study. These species are implicated by the study of Salihu *et al.* (2008) on the different species of smoked fish sampled from various retail outlets and market places within Sokoto metropolis. Undoubtedly, smoked fish products and other foods related to these species can sustain their growth, particularly when stored for an extended length of time (Hoelzer *et al*., 2012). Although seven bacterial and six fungal species were isolated, a study conducted by Adeyemi *et al*. (2013) on the storage stability of smoke-dried African catfish (Clarias gariepinus) kept for two months revealed no presence of Listeria spp. The level of Listeria in the smoked fish samples is decided by the level of hygiene practiced during the processing and post-process handlings. The low prevalence of *Listeria* spp. recorded in the products retailed in these study markets could indicate a general good handling, storage and distribution practices adopted by the fish processors in Ota metropolis. The prevalence of these isolates of smoked fish products contrasted that of the five isolates identified by Okorie-Kanu *et al.* (2022) from raw frozen Atlantic Mackerel (*Scomber Scombrus*) fish sold in *Et al* State South-eastern Nigeria. : *Listeria innocua* (71.1%) predominated, followed by *Listeria welshimeri* (10.5%), *Listeria ivanovii* (7.9%), *Listeria grayi* (5.3%) and *Listeria monocytogenes* (5.3%). This disparity can be attributed to smoking, a preservative technique in which the heat and dry air dehydrates the smoked fish by removing moisture thereby depriving microorganisms of a prerequisite to growth (Akintola *et al.*, 2022).

Certain compounds are known to be imparted by smoke, enabling smoked foods to withstand microbial deterioration. By changing the chemistry of these spoilage organisms' surroundings, smoking either eliminates or renders harmless food poisoning and spoilage bacteria (Sathish, 2022; OB, 2020). The isolates found in the smoked fish samples used in this investigation are regarded as pollutants because, as noted by Adeyeye *et al*. (2015), they are widespread and adaptable to a wide range of environmental factors that can destroy a large number of other bacteria. Given the freshness of the smoked fish products, which appear to be in high demand in the study locations, the low incidence of *Listeria* spp. in the examined fish items may be explained. This result is consistent with the findings of Musa *et al*. (2020), who reported discovering Listeria spp. and *Staphylococcus aureus* in smoked fish marketed on the Ahmadu Bello University main campus in Samaru, Zaria. Of the 10 samples examined, only one strain of Listeria spp., identified as *Listeria ivanovii*, was found. The smoked fish samples studied by Salihu *et al.* (2008) in various retail outlets within Sokoto metropolis on the contrary recorded higher prevalence of *Listeria* spp.: *L. monocytogenes* (29, 25%), *L. grayi* (13, 11%), *L. innocua* (10, 9%) and *L. ivanovi* (15, 13%). In line with these findings, the existence of some differences in the geographic distribution or

prevalence on different types of fish and fishery products had been observed (FAO, 1999). It is, however, not impossible that the prevalence of *Listeria* spp. in the study smoked fish samples can increase with undue elongated storage period, thereby posing health threat to the potential consumers. This calls for the consideration of the need to establish control measures along the fish processing and distribution routes to minimize or eliminate the hazards associated with the occurrence of *Listeria* spp.

According to Ojo and Anibijuwon (2010), fluoroquinolones antibiotics are implicated to be of broad spectrum activities, having bactericidal effects in both replicating and resting states. Their ability to disrupt DNA functions leading to the death of the bacterial cell is remarkable. Food borne infection caused by *Listeria monocytogenes* has been linked to multi-drug resistant strains of this organism (Bhattacharjee *et al.*, 2022). The antibiotic resistance pattern of 75% displayed by the listerial isolates of this study against imipenem, cefuroxime, erythromycin and amoxycillin is similar to the findings of Kayode and Okoh (2023), in their study on antimicrobial-resistant *Listeria monocytogenes* in ready-to-eat foods: Implications for food safety and risk assessment results from previous studies. The high resistant rate of β-lactam antibiotics (cefuroxime, amoxycillin, cefotaxime, ceftriazime and cefixime) among *Listeria* spp. is in agreement with a general worldwide pattern of an increasing prevalence of multiple antibiotic resistance among many groups of bacteria (Pesavento *et al.*, 2010).

CONCLUSION

This study shows that, as compared to certain other sites tested in some selected portions of Nigeria, the occurrence of *L. monocytogenes* and other *Listeria* spp. in the selected smoked fish products popularly consumed in Ota, Ogun State, Nigeria, is comparatively lower. The low percentage of occurrences combined with the high antibiotic susceptibility of all the isolated Listeria spp. may reassure consumers of the potential health risk that consuming the smoked fish samples could pose, even though the presence of this pathogen on the samples could be an indictment on the safety of the products for consumption.

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