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Listeria Species in Seafoods from two Major Fish Markets in Lagos, Nigeria

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Abstract: *Listeria* is a bacterial genus thatis widely distributed in our environment. Its most economically important species is *Listeria monocytogenes*. Listeriosis is a serious infection caused by eating food contaminated with *Listeria monocytogenes*. The contamination of seafoods with *Listeria* species may occur during processing, handling and packaging due to poor quality control measures. The aim of this study was to isolate and identify *Listeria* spp. from seafoods sold at Liverpool and Makoko fish markets in Lagos. A total of 193 seafood samples including Blue Whiting (*Micromesistus poutasou*), Croaker (*Pseudotolitus elongatus*) and Pink Shrimp (*Penaeus notialis*) were screened for the presence of *Listeria* spp. The isolation and identification of *Listeria* species were carried out using the Oxoid *Listeria* Précis method. Forty-nine samples were positive for *Listeria* spp. and were identified as *Listeria ivanovii*,*L. grayi*, *L. welshimeri*, *L. monocytogenes* and *L. innocua*. Fresh croaker had the highest prevalence of 58.8%. The results of this study indicate the presence of *Listeria* spp. in seafoods in Liverpool and Makoko fish markets. It also revealed the possibility that these seafoods could contribute to food borne infections. Therefore, the improvement of seafood quality is of utmost importance.

Keywords: Seafoods, *Listeria* species, contamination, fish markets.

Introduction

Listeria is a bacterial genus thatis widely distributed in our environment. They are motile Gram-positive short rods, non-spore formers, catalase positive and oxidase negative [1]. The genus *Listeria* currently consist of 17

recognized species: *Listeria monocytogenes, L. seeligeri, L. ivanovii, L. welshimeri, L.marthii, L. innocua, L. grayi, L. fleischmannii, L. floridensis, L. aquatica, L.newyorkensis, L. cornellensis, L. rocourtiae, L. weihenstephanensis, L. grandensis, L. riparia* and *L. booriae*. Of all these, *L. monocytogenes* and *L. ivanovii* are considered pathogens [2]. *L. monocytogenes* is of major concern for public health authorities and the food industry, as the cold-tolerant organism is known to cause human infections and has been associated with a large number of foodborne disease outbreaks [3-6]. It is endowed with numerous adaptive physiological traits that enable it to survive under a wide range of environmental conditions [7]. Implicated foods include milk products, vegetables, salads, seafoods (especially ready-to-eat seafoods) and meat products [8]. Listeriosis is a serious infection caused by eating food contaminated with *Listeria monocytogenes*. The disease affects primarily pregnant women, newborns and adults with weakened immune systems [9]. It has mortality rate of 20-30% and hospitalization rate of 91% [10]. Consumers' awareness of nutrition and food quality has led to the increased consumption of seafood products. Seafood is recommended by nutritionists because of its high nutritional value [11]. However, along with the nutrients and benefits gotten from seafood consumption come the potential risks of eating contaminated seafood [12]. Seafood are susceptible to several food poisoning organisms as well as to some that are unique to marine products such as *Clostridium botulinum, Yersinia enterocolitica, Listeria monocytogenes* and *Vibrio parahaemolyticus* [13]. The occurrence of *Listeria* species in seafoods have been investigated in several countries but little has been reported about it in Nigeria. The contamination of seafoods with *Listeria* species may occur during processing, handling and packaging due to poor quality control measures. The aim of this study was to isolate and identify *Listeria* spp. from seafoods sold at Liverpool and Makoko fish markets in Lagos State in order to generate information on the prevalence of this pathogen so as to provide baseline information for Nigerian regulatory authorities to allow the formulation of a regulatory framework for controlling *Listeria* spp. and ensuring seafood safety.

Materials and Methods

Sample Collection

A total of 193 seafood samples including Blue Whiting (*Micromesistus poutasou*), Croaker (*Pseudotolitus elongatus*) and Pink Shrimp (*Penaeus notialis*) were screened for the presence of *Listeria* spp. The samples were obtained monthly for a year from two fish markets (Liverpool and Makoko) in Lagos State.

Isolation and identification of *Listeria* species

The isolation and identification of *Listeria* species was carried out using the Oxoid Listeria Précis method. The identification of *Listeria* was carried out according to the methods described in the Bacteriological Analytical Manual [14]. Some rapid methods such as Oxoid Biochemical Identification System (O.B.I.S.), Oxoid Listeria Test Kit and MICROBACT Listeria 12L system were also used for identification of *Listeria* isolates.

Statistical analysis

A test of significance of the prevalence of *Listeria* spp. in seafoodwas carried out using the one sample t-test computed using SPSS package. A difference of 95% was used in the analysis, $p<0.05$ level was considered to be statistically significant.

Results

Phenotypic characterisation of *Listeria* isolates showed that all isolates were Gram positive short rods, catalase positive and oxidase negative. They were all motile and tested positive for *Listeria* latex agglutination test. The species of *Listeria* identified by Microbact *Listeria* 12L system were *Listeria ivanovii*, *L. grayi*, *L. welshimeri*, *L. monocytogenes* and *L. innocua*. Furthermore,of the 193 seafood samples, 49 (25.4%) were found positive for *Listeria* spp. which were identified as *Listeria ivanovii* (16, 8.3%), *Listeria welshimeri* (12, 6.2%), *Listeria monocytogenes* (12, 6.2%), *Listeria grayi* (5, 2.6%) and *Listeria innocua* (4, 2.1%). Fresh croaker had the highest prevalence of 58.8%. Species wise, 16 isolates of *L. ivanovii* were all from fresh croaker; 12 *L. welshimeri* isolates included six recovered from fresh shrimp, four from smoked blue whiting and two from fresh croaker; 12 *L. monocytogenes* comprised of eight isolates from fresh croaker, three from smoked blue whiting and one from smoked shrimp; 5 *L. grayi* isolates that consisted of three smoked shrimp and two smoked blue whiting and 4 isolates of *L. innocua* recovered from fresh croaker (Table1). *Listeria* species were not isolated from smoked croaker and frozen blue whiting. In Liverpool market, *L. monocytogenes* were recovered from fresh croaker (20%), smoked blue whiting (12%) and smoked shrimp (10%) (Table 2). *L. welshimeri* was isolated from fresh croaker (9.5%), smoked blue whiting (13.3) and fresh shrimp (20%) in Makoko market (Table 3). The statistical analysis conducted showed that the prevalence of *Listeria* spp. in seafoods was significant at 95% confidence limit ($p<0.05$).

Table 1: Prevalence of *Listeria* species in seafoods

Seafood Samples	Number of Samples Analyzed	*Listeria* spp. positive, n (%)	*L. grayi* n (%)	*L. innocua* n (%)	*L. ivanovii* n (%)	*L. welshimeri* n (%)	*L. monocytogenes* n (%)	
Fresh Croaker	51	30 (58.8)	0 (0.0)	4 (7.8)	16(31.4)	2 (3.9)	8 (15.7)	
Smoked Croaker	41	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Frozen Blue Whiting	3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Smoked Blue Whiting	55	9 (16.4)	2 (3.6)	0 (0.0)	0 (0.0)	4 (7.3)	3(5.5)	
Fresh Shrimp	24	6 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (25.0)	0 (0.0)	
Smoked Shrimp	19	4 (21.0)	3 (15.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)	0 (0.0)
Total	193	49 (25.4)	5 (2.6)	4 (2.1)	16 (8.3)	12(6.2)	12(6.2)	

Key: n: number of positive samples

Table 2: Prevalence of *Listeria* species in seafoods from Liverpool market

Seafood Samples	Number of Samples Analyzed	*Listeria* spp. positive, n (%)	*L. grayi* n (%)	*L. innocua* n (%)	*L. ivanovii* n (%)	*L. welshimeri* n (%)	*L. monocytogenes* n (%)	
Fresh Croaker	30	18 (60.0)	0 (0.0)	2 (6.7)	10 (33.3)	0 (0.0)	6 (20.0)	
Smoked Croaker	24	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Frozen Blue Whiting	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Smoked Blue Whiting	25	3 (12.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (12.0)	
Fresh Shrimp	9	3 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (33.3)	0 (0.0)	
Smoked Shrimp	10	2 (20.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)
Total	98	26 (26.5)	1(1.0)	2(2.0)	10 (10.2)	3 (3.1)	10 (10.2)	

Key: n: number of positive samples

Table 3: Prevalence of *Listeria* species in seafoods from Makoko market

Seafood Samples	Number of Samples Analyzed	*Listeria* spp. positive, n (%)	*L. grayi* n (%)	*L. innocua* n (%)	*L. ivanovii* n (%)	*L. welshimeri* n (%)	*L. monocytogenes* n (%)	
Fresh Croaker	21	12 (57.1)	0 (0.0)	2 (9.5)	6 (28.6)	2 (9.5)	2 (9.5)	
Smoked Croaker	17	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Frozen Blue Whiting	3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Smoked Blue Whiting	30	6 (20.0)	2 (6.7)	0 (0.0)	0 (0.0)	2 (6.7)	2(6.6)	
Fresh Shrimp	15	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (20.0)	0 (0.0)	
Smoked Shrimp	9	2 (22.2)	2 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	95	23 (24.2)	4 (4.2)	2 (2.1)	6 (6.3)	7 (7.4)	4 (4.2)	

Key: n: number of positive samples

Discussion

The results from this study have shown that there is a significant difference in the prevalence of *Listeria* spp. in seafoods. Also, prevalence of *Listeria* spp. in seafoods from Liverpool and Makoko markets are significant with the exception of smoked shrimp from Liverpool market. The occurrence of *Listeria* spp. isolated from seafood as shown in Table1 is in accordance with earlier reports in Nigeria and other countries [15-19]. *Listeria ivanovii, L. grayi, L. welshimeri, L. monocytogenes* and *L. innocua* were isolated from Liverpool and Makoko markets which are primary fish markets in Lagos (Tables 2&3). This is similar to the results of Modaresi *et al*. [16] who also isolated these five *Listeria* spp. from seven fish species in Urmia fish markets in Iran. The results in this study has shown that severe controls need to be undertaken on the hygienic quality of these seafood so as not to lead to an outbreak since these markets are major fish markets. Furthermore, food safety and quality standards need to be adhered to in order to control the growth of *Listeria* during fishing, collection, transmission, distribution and storage [20]. Liverpool fish market in Lagos State, Nigeria, plays a prominent role in the distribution of fishery products across the state.

The market lacks social amenities such as water and sanitary facilities. The only available water used was taken from the

Lagoon [21].The same scenario also occurs in Makoko fish market. The coexistence of several *Listeria* species on the same food is not unusual and often the prevalence of *Listeria* species is higher than that of *L. monocytogenes* [22]. The differences in prevalence of *Listeria* spp. in seafood might be attributed to the type of seafood, source of samples, number of samples, method of sampling, sampling season, isolation method, human activity, geographical area, climate of area and sensitivity of bacteriological detection methods [18,20,23]. Also, the presence of non-pathogenic species such as *L. innocua* may indicate potential contamination with *L. monocytogenes* [24]. Apart from *Listeria monocytogenes*, other *Listeria* spp. such as *L. ivanovii, L. seeligeri, L. innocua, L. welshimeri* are regarded as non-pathogenic to human, and have been implicated in human infections. Also, it is believed that the occurrence of non-invasive listeriosisis underestimated because *L. monocytogenes* is not among the pathogens routinely investigated in the outbreaks of gastro-intestinal diseases [25,26].

The results of the current study demonstrated that 25.4% of seafood samples tested was contaminated with *Listeria* spp. and 6.2% were contaminated with *L. monocytogenes*. Contamination with *L. monocytogenes* may occur long before the raw material reaches retail trade or processing factories. The main sources of *L. monocytogenes* are contamination from water and ice, soiled surfaces and boxes, as well as contamination from human and avian sources [27]. Not much is known about the potential *Listeria* contamination of fish and fish products at the retail level. Products that are purchased in large quantities and re-packaged prior to sale may be at risk to *L. monocytogenes* contamination. Despite the occurrence of *L. monocytogenes* in raw and frozen seafood, these products do not pose a threat to the majority of people as they undergo some processing before being eaten. However, they still pose risk to susceptible populations when consumed raw or lightly cooked [27].The occurrence of *Listeria* spp. in cooked Ready-To-Eat seafood samples could be from the cross-contamination during fish handling, incomplete cleaning and disinfection procedures, and incomplete implementation of HACCP principles in processing plants.

Conclusion

The results of this study indicate the presence of *Listeria* spp. in seafoods in Liverpool and Makoko fish markets. The presence of *Listeria* spp. particularly *L. monocytogenes* in smoked and uncooked products could be a potential risk for consumers. Also, the findings in this study has provided basic information on the occurrence of *Listeria* spp. in seafoods sold at Liverpool and Makoko fish markets in Lagos State. This information can be used by the Nigerian food safety authorities to formulate a regulatory framework for controlling *Listeria* spp. and ensuring seafood safety.

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