



PREVALENCE OF *LISTERIA* SPECIES IN BLUE WHITING (*MICROMESISTUS POUTASOU*) IN LAGOS STATE, NIGERIA.

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ABSTRACT

Listeria species are ubiquitous in nature. The ubiquity of *Listeriae* enables them to colonize and persist in the food-processing environments and the food chain. Blue Whiting also known as *panla* is a pelagic fish that is widely consumed in Lagos and its environs. The present study examined the prevalence of *Listeria* spp. from Blue Whiting sold at three retail markets in Lagos State. One hundred and twenty-four Blue Whiting samples consisting of fifty-three raw fish and seventy-one smoked fish were microbiologically analyzed using the Oxoid *Listeria* Précis method, Oxoid *Listeria* Test Kit and MICROBACT *Listeria* 12L system. Genotypic identification was carried out using 16S rRNA gene sequencing. Of the 124 samples examined, 21(16.9%) were positive for *Listeria* spp. with 18 (14.5%) identified as *L. innocua* and 3 (2.4%) as *L. grayi*. Higher occurrences (14-24%) of *Listeria* spp. were observed between July and November, 2016. Fourteen out of 18 isolates selected showed amplification for the 16S rRNA region of *Listeria* spp. Sanitary conditions and personal hygiene should be adhered to during processing, distribution and marketing of Blue Whiting so as to reduce contamination by *Listeria* species since the presence of *L. innocua* serves as an indicator for *L. monocytogenes*.

Keywords: *Listeria* species, Blue Whiting, prevalence.

INTRODUCTION

Listeria species are Gram-positive, non-spore forming, rod-shaped bacteria. They are found throughout the environment, residing in soil, water, sewage, vegetation, animal feces, farms and food-processing facilities (Todd and Notermans, 2011). Its most important species, *Listeria monocytogenes* is the causative agent of one of the deadliest foodborne illnesses called listeriosis. The organism can survive and grow under several environmental conditions. They can contaminate processing plants and retail environments in which ready-to-eat foods are produced and served (Jarvis *et al.*, 2016). The main causes of listeriosis are poor microbiological quality of raw materials, cross contamination, inadequate cleaning practices, improper storage temperature, inadequate preparation processes and lack of training on food hygiene (Osimani and Clementi, 2016). This disease has developed as a result of change in eating habits and large scale industrial food production (Adams and Moss, 2010). It also leads to losses of billions of dollars yearly due to recalls of contaminated foods and patients medical treatment expenses (Camargo *et al.*, 2016). Food items that may contain *Listeria* are fruits and vegetables (Hadjilouka *et al.*, 2014; Montero *et al.*, 2015), dairy products (Dalzini *et al.*, 2015; Karthikeyan *et al.*, 2015), meat (Hadjilouka *et al.*, 2014; Montero *et al.*, 2015), fish (Wang *et al.*, 2013) and seafood products (Amusan *et al.*, 2017).

Blue whiting (*Micromesistus poutasou*) is a typical pelagic, lean fish species, which is popularly called *Panla*. It is a small codfish usually 22-30 cm. It has been recognized as a readily available and nutritious marine fish (Kolade, 2015). Although the occurrence of *L. monocytogenes* in Nigerian food processing facilities and foods has been investigated (Chukwu *et al.*, 2006, Salihu *et al.*, 2008, Eruteya *et al.*, 2014, Ajayeoba *et al.*, 2016, Amusan *et al.*, 2017), there is insufficient data regarding listeriosis cases and outbreaks directly associated with food consumption. From researches conducted in Nigeria, the prevalence of *Listeria* species in fish and fishery products has been between 6.6 and 40% (Chukwu *et al.*, 2006; 6.6%, Salihu *et al.*, 2008; 25%, Ikeh *et al.*, 2010; 40%, Amusan *et al.*, 2017; 25.4%). Thus it is important to generate information on the prevalence of this pathogen since fish and fishery products may be a vehicle for *Listeria* species. The aim of this work was to examine the prevalence of *Listeria* spp. in Blue Whiting sold at three retail markets in Lagos State, South-West, Nigeria.

MATERIALS AND METHODS

Sample Collection

One hundred and twenty-four Blue Whiting samples consisting of fifty-three raw fish and seventy-one smoked fish were collected monthly for a year from Badagry, Iyana Ipaja and

Mushin markets. These were examined for the presence of *Listeria*.

Isolation and Identification of *Listeria* species

The isolation of *Listeria* was carried out using the Oxoid *Listeria* Précis method. For each sample, 25 g was added to 225 ml of Oxoid Novel Enrichment (ONE) broth and stomached for 30 seconds to mix the sample. It was incubated at 30 °C for 24 hours. After 24 h enrichment, the broth cultures were agitated and a loopful was inoculated onto Brilliance *Listeria* agar. This was incubated appropriately (37 °C for 24 – 48 h). The plates were examined for blue-green colonies with or without opaque white halos; at least 3 suspected colonies were subcultured on Tryptone Soya Agar (TSA, Oxoid) and incubated at 37 °C for 24 h. Phenotypic characterization such as Gram's staining, Catalase and motility tests were carried out according to the methods of Hitchins and Jinneman, (2011). The Oxoid *Listeria* Latex Agglutination Test and MICROBACT *Listeria* 12L system were also used for phenotypic characterization.

Molecular Characterization

DNA Isolation

A single *Listeria* colony was grown overnight on TSA at 37 °C. One colony from the culture was inoculated into 5 ml of Trypticase Soy Broth and incubated overnight at 37 °C. The broth culture of 2.5 ml was centrifuged and the pellet washed in 1 ml of distilled water and resuspended in an Eppendorf tube containing 400 µl of phosphate buffer saline (PBS). The DNA was extracted using Qiagen DNA extraction kit according to the manufacturer's instruction.

Polymerase Chain Reaction of *Listeria* isolates using 16S rRNA specific primer for *Listeria* spp.

The PCR was carried out using C1000 Touch Thermal cycler (BioRad). The PCR mixture (25 µL) for reactions involving 16S rRNA consisted of 2.5 µl 10×PCR buffer, 2.0 µl dNTP

(deoxynucleoside triphosphate), 0.25 µl each of appropriate primer, 0.25 µl AmphiTaq DNA polymerase (TransGen Biotech, China), 14.75 µl sterile distilled water and 5 µl of appropriate DNA preparation. A reaction mixture with no DNA template was used as a negative control in each reaction. The cycling conditions were the template DNA was denatured at 95 °C for 3 min followed by 35 cycles of amplification (each cycle consisted of denaturation at 94 °C for 1 min, annealing at 60 °C for 2 min and elongation at 72 °C for 1 min). A final extension was performed for 10 min at 72 °C. The amplified products (5 µl) were analyzed by 1.5 % agarose gel electrophoresis and ethidium bromide staining. The DNA bands were observed under an ultraviolet (UV) transilluminator (Park *et al.*, 2012). The PCR products for the 16S rRNA were sequenced at GATC Biotech, Germany. The sequences obtained were blasted and compared with similar sequences in the GenBank (NCBI, USA).

RESULTS

The phenotypic identification showed that all isolates were Gram positive short rods, motile, catalase positive and gave positive reaction to *Listeria* test kit. The MICROBACT 12L was able to identify the isolates as *Listeria grayi* and *L. innocua*. The prevalence of *Listeria* species in raw and smoked Blue Whiting are shown in Tables 1&2. Ten (18.9%) of the raw samples were positive for *Listeria* spp. and these were all isolated from Iyana Ipaja market only. The smoked samples had a prevalence rate of 15.5%. A total of 124 samples of Blue Whiting were screened for *Listeria* spp. (Table 3). Twenty-one samples were positive for *Listeria* spp. with 18 (14.5%) identified as *L. innocua* and 3 (2.4%) as *L. grayi*. Higher occurrences of *Listeria* spp. were observed between July and November (Figure 1). Fourteen out of 18 isolates selected showed amplification for the 16S rRNA region of *Listeria* spp. (Plate 1).

TABLE 1: Prevalence of *Listeria* spp. in Raw Blue Whiting

Markets	Number of samples analyzed	<i>Listeria</i> spp. Positive n (%)	<i>Listeria grayi</i> n (%)	<i>Listeria innocua</i> n (%)
Badagry	15	0 (0.0)	0 (0.0)	0 (0.0)
Iyana Ipaja	23	10 (43.5)	2 (8.7)	8 (34.8)
Mushin	15	0 (0.0)	0 (0.0)	0 (0.0)
Total	53	10 (18.9)	2 (3.8)	8 (15.1)

TABLE 2: Prevalence of *Listeria* spp. in Smoked Blue Whiting

Markets	Number of samples analyzed	<i>Listeria</i> spp. Positive n (%)	<i>Listeria grayi</i> n (%)	<i>Listeria innocua</i> n (%)
Badagry	23	4 (17.4)	0 (0.0)	4 (17.4)
Iyana Ipaja	22	3 (13.6)	1 (4.5)	2 (9.1)
Mushin	26	4 (15.4)	0 (0.0)	4 (15.4)
Total	71	11 (15.5)	1 (1.4)	10 (14.1)

TABLE 3: Prevalence of *Listeria* spp. in Blue Whiting

Sample Type	Number of samples analyzed	<i>Listeria</i> spp. Positive n (%)	<i>Listeria grayi</i> n (%)	<i>Listeria innocua</i> n (%)
Raw	53	10 (18.9)	2 (3.8)	8 (15.1)
Smoked	71	11 (15.5)	1 (1.4)	10 (14.1)
Total	124	21 (16.9)	3 (2.4)	18 (14.5)

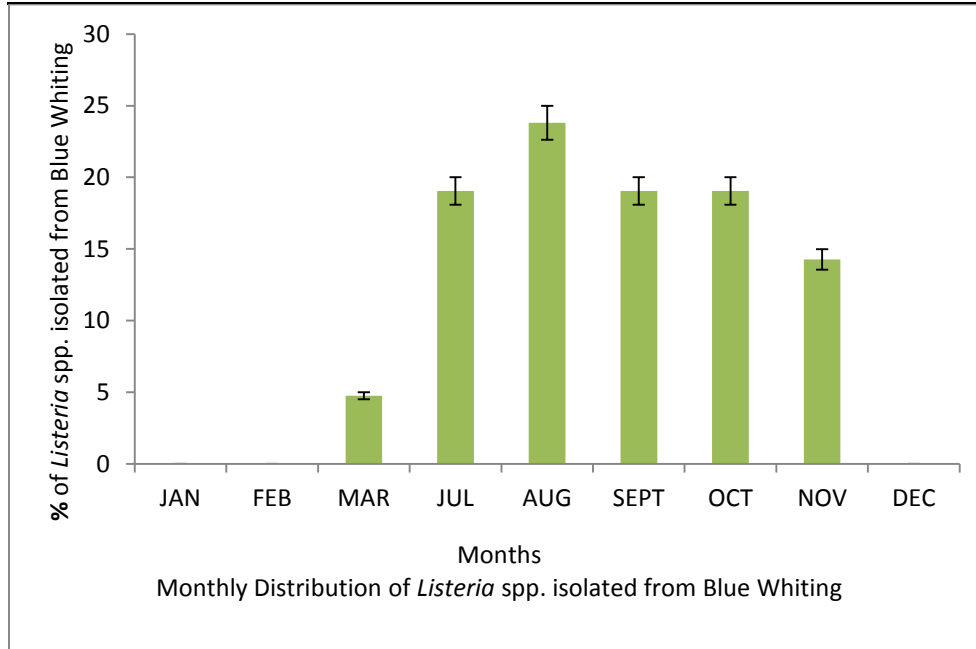


Fig. 1: Monthly Distribution of *Listeria* spp. isolated from Blue Whiting

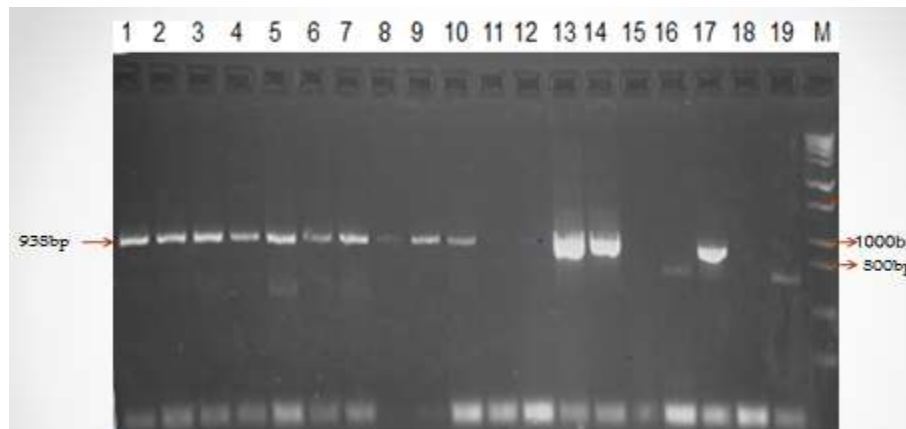


PLATE 1: Agarose gel showing Polymerase Chain Reaction amplified product of 938bp for 16S rRNA specific primer for *Listeria* spp.

Lane M: Molecular Weight Marker, Lanes 1-10, 13-14 and 17: *Listeria* spp., Lane 11: control reaction

DISCUSSION

The study has shown that *Listeria* spp. can be found in fish such as Blue Whiting. The presence of *Listeria* spp. in raw blue whiting is similar to the observation in different cities in Nigerian seafood sources where 16% was observed by Adebayo-Tayo *et al.* (2012) from Uyo; 13.3% of Adeshina and Adewale (2015) from Ilorin; 25.4% by Amusan *et al.* (2017) from Lagos. Higher prevalence of 40%, 25%, 43% and 36% for

Listeria spp. in raw fish respectively as recorded by the authors (Ikeh *et al.*, 2010; Amusan, 2012; Vinothkumar *et al.*, 2013; Rezai *et al.*, 2018). The presence of *Listeria* spp. in smoked and fried fish was reported by Chukwu *et al.*, 2006; Salihu *et al.*, 2008; Adeyeye *et al.*, 2015 and Adeshina *et al.*, 2017. In this study, *L. innocua* had the highest prevalence from blue whiting. *L. innocua* and *L. grayi* have also been isolated from different fish species in various cities across Nigeria (Shinkafi and

Ukwaja, 2010; Nwachukwu and Madubuko, 2013). *L. innocua* and *L. monocytogenes* are often found in the same food processing environments thus, the presence of this non-pathogenic spp. (*L. innocua*) may indicate potential contamination with *L. monocytogenes* (Gasnov *et al.*, 2005; Gomez *et al.*, 2012). Furthermore, the contamination of *Listeria* could have been caused by its persistence as biofilms in equipment, utensils, floors and drainage system from the processing environment.

A higher incidence of *Listeria* was observed during the rainy season than the dry season. This is similar to the results obtained by Eruteya *et al.* (2014) and Ajayeoba *et al.* (2015). Climatic conditions such as rainfall have an effect on the occurrence of *Listeria* spp. (Jami *et al.*, 2014). The rainy season in South West, Nigeria starts in March and lasts till November (Ajayeoba *et al.*, 2015). An increase in bacterial contamination occurs on surface waters during rainfall thereby causing a greater risk of *Listeria* contamination since these water contain lots of human, environmental and animal wastes in which *Listeria* is a common habitat (Jami *et al.*, 2014; Ajayeoba *et al.*, 2015).

The standard microbiological methods for identifying *Listeria* spp. are strenuous and time consuming. Thus, there is a need to use rapid and reliable molecular methods for proper identification. In general, DNA-based typing approaches have been recognized as simple and cost-effective methods that have better discriminatory power than phenotypic approaches (Mohamed *et al.*, 2016). This study showed that 16S rRNA gene sequencing analysis approach was valuable in the identification of *Listeria* spp. This is in agreement with Hellberg *et al.* (2013) who also used 16S rRNA gene sequencing for differentiation and molecular subtyping of *Listeria* spp. The 16S rRNA gene sequencing has been used for identification of the bacteria at the species level (Woo *et al.*, 2008). It is a multicopy housekeeping gene containing the highly conservative regions and hypervariable regions with considerable sequence diversity among different bacteria (Chakravorty *et al.*, 2007).

CONCLUSION

Listeria grayi and *L. innocua* were isolated from blue whiting from all the three markets examined. Although the prevalence rate was low, the severity of the illness is significant if contacted. Thus, personal hygiene and sanitary conditions should be adhered to in order to avoid infections caused by these organisms.

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