Original Article

Journal of Food Safety and Hygiene

Journal homepage: http://jfsh.tums.ac.ir

Prevalence and antibiotic susceptibility of *Listeria innocua* in seafood from selected markets of Lagos, Nigeria

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ARTICLE INFO

Article history:
Received 19 Apr. 2023
Received in revised form
18 Sep. 2023
Accepted 27 Sep. 2023

Keywords:
Infections;
Non-pathogenic;
Seafood samples;
Public health

ABSTRACT

Listeria is a bacterial genus that is widely distributed in fish and fishery products and is a vehicle for food-borne bacterial infections and intoxications. *Listeria innocua*, though considered non-pathogenic, is a close relative to *L. monocytogenes* a known food-borne pathogen. It has been implicated in the transfer of antimicrobial-resistant genes. Therefore, this study investigates the prevalence of *Listeria innocua* and its antimicrobial susceptibility pattern in seafood found in Badagry, Iyana Ipaja, Liverpool, Makoko and Mushin, Nigeria. A total of 500 samples comprising of fresh and smoked blue whiting, croaker and shrimps were collected aseptically from retail outlets across Lagos. Culture, biochemical and sugar tests were carried out to identify *L. innocua*. 16S rRNA gene sequencing was conducted to confirm the isolates as *L. innocua*. The antimicrobial susceptibility testing was determined by the disk diffusion assay. Out of 500 seafood samples analysed, 36 (7.2%) were positive for *Listeria innocua*. Raw croaker had the highest occurrence of 13.0%. The antimicrobial susceptibility test revealed that all isolates were resistant to ceftazidime and cloxacillin. However, high sensitivities to ofloxacin (83.3%) and erythromycin (72.2%) were exhibited by the isolates. The recovery of these antimicrobial-resistant *Listeria innocua* strains in the seafood samples analysed warrants the need for suitable control procedures as this could constitute a great risk to public health.

Citation: Amusan EE, Sanni A, Onipede G. **Prevalence and antibiotic susceptibility of *Listeria innocua* in seafood from selected markets of Lagos, Nigeria.** J food safe & hyg 2023; 9(3): 175-184

1. Introduction

In soil, water, and animal guts, there are rod-shaped Gram-positive bacteria called Listeria species. It has been discovered that this genus' members can infect a variety of food types and the related food handling environments consequently addressing a danger to general well-being (1).

Listeria has been recorded in 20 different species (2,3). The only one of them, *L. monocytogenes*, is thought to be capable of causing listeriosis in both people and animals (4). *Listeria innocua* was previously regarded as

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a species that was not infectious and had a closer evolutionary connection to *L. monocytogenes* (5,6). Unlike *L. monocytogenes*, they usually do not spread diseases. In any case, the nearly high genomic likeness between both every so often, and their concurrence in comparable biological niches might introduce the chance for resistance or virulence gene transfer (7).

These organisms are broadly dispersed in various normal and metropolitan conditions and in food (2). *Listeria innocua* is a direct relation to *Listeria monocytogenes*. The food-borne pathogen *L. monocytogenes* is the etiological agent that causes listeriosis in humans. Listeriosis is an uncommon yet regularly lethal infection (8).

L. innocua is not pathogenic to warm-blooded animals, unlike *L. monocytogenes*, albeit exorbitantly uncommon instances of septicemia and meningitis contaminations arising from *L. innocua* have been accounted for in humans (8,9) and cattle (10). A characteristic of *L. innocua* is non-hemolytic, yet all the same abnormal isolates of *L. innocua* are hemolytic. These have also been recognized in fish and other animal proteins from Asia, Northern America and Europe (11-13), recommending that abnormal isolates of *L. innocua* that are hemolytic are really spreading around the world. In 2004, the foremost unusual strain of *L. innocua* (PRL/NW 15B95) was identified (11).

Despite the low occurrence of listeric infections, the significant mortality rate associated with this contamination makes it a serious concern for the health of the public. However as drugs are used in both human and veterinary medicine, the antibiotic sensitivity profile of Listeria species is altered in various topographical regions. Improper utilization of antibacterial medication is a significant reason for acquired resistance in species of Listeria (14). Strains of *Listeria* spp. isolated from raw and prepared-to-eat fish samples have been found to exhibit multiple antibiotic resistance (15). Hence, a nonstop spotlight on antibiotic-resistant Listeria isolate is fundamental to bypass future dangers to the human populace (16,17).

In Lagos, fresh and smoked seafood is usually eaten by individuals, shockingly no outbreaks of listeriosis related to fish utilization have been accounted for. However, the existence of Listeria in seafood products may cause significant public health concerns. Therefore, this study aimed to investigate the prevalence and antibiotic susceptibility of *Listeria innocua* isolated from seafood in markets of Lagos, Nigeria.

2. Materials and Methods

2.1. Sample collection

Five hundred samples in total comprising 100 samples each of fresh and smoked blue whiting and croaker and 50 samples each of fresh and smoked shrimps were purposively collected monthly for a period of 12 months from retail five outlets (Badagry, Makoko, Liverpool, Iyana-Ipaja and Mushin) across Lagos. The smoked samples were collected in clean sample bags while iceboxes were used for fresh samples only) and conveyed to the Microbiology Laboratory of the Department of Fish Technology of the Nigerian Institute for Oceanography and Marine Research, Lagos where analyses were carried out on them.

2.2. Isolation and identification of *Listeria innocua*

Listeria innocua were isolated using culture methods based on selective enrichment and plating. For each

sample, there was an addition of approximately 25 g aseptically into 225 mL of Listeria broth (one-broth, Oxoid), which was mixed together and incubated at 37°C for 24 h. A loopful from the one-broth Listeria (Oxoid), was streaked onto Brilliance Listeria agar (Oxoid), for the purpose of carefully separating colonies of Listeria. Agar plates with its inoculations were put into the incubator at a temperature of 37°C for 24-48 h. Blue-green colonies with or without halos observed on the plates were reported to be Listeria. Typically, 3-4 typical colonies were confirmed by gram staining, motility, catalase test and oxidase test. The Oxoid Listeria Latex Agglutination Test is an additional test that was carried out. This was used to confirm the existence of Listeria spp. in culture. MICROBACT Listeria 12 L system containing 12 tests comprising 11 tests for sugar utilization and a quick test for hemolysis was utilized to fully identify *L. innocua*.

2.3. PCR identification of *Listeria innocua* isolates using 16S rRNA

The amplification of the 16S rRNA gene for all *Listeria innocua* isolates in this study was carried out using 16S F (5′-CAGCAGCCGCGGTAATAC- 3′) and 16S R (5′-CTCCATAAAGGTGACCCT-3′) universal primers (18). It was performed in a 25 µL reaction with a master mixture of 4.75 µL (PCR buffer, deoxynucleoside triphosphate (dNTP) and Taq DNA polymerase), 0.25 µL for all the primers used, 14.75 µL nuclease-free water and 5 µL of template DNA. The protocols for PCR were 95°C for 3 min, 35 cycles of 94°C for 1 min, 60°C for 2 min and 72°C for 1 min. The final extension was performed for 10 min at 72°C. The amplified PCR products were analyzed by gel electrophoresis using 1% agarose in 1x TAE buffer. The electrophoresis was run for 30 min at 100 V and 500 mA using ethidium bromide staining and viewed under the ultraviolet (UV) transilluminator. The sequencing of the amplicons was carried out at GATC Biotech in Constance, Germany. Using BLAST, the DNA sequences were identified and a comparison was made with related sequences in the GenBank (NCBI, USA).

2.4. Antibiotic susceptibility

Listeria innocua isolates were examined with the use of disc-diffusion for the assessment of antibiotic susceptibility. Isolated organisms were cultivated separately in Tryptone soy broth (Oxoid) for 18-24 h. Growing bacterial cultures were uniformly distributed over the outward area of a Mueller Hinton agar plate (Oxoid). Typical Gram-positive antibiotic discs (Erythromycin 30 µg, Cefuroxime 30 µg, Cloxacillin 5 µg, Gentamicin 10 µg, Ceftriaxone 30 µg, Ofloxacin 5 µg Ceftazidime 30 µg and Amoxycillin/Clavulinate 30 µg) (Rapid Labs) were placed on plates using sterile forceps. They were put in the incubator at 37°C for 24-48 h. Dimensions of individual regions of inhibition were observed and defined as sensitive, intermediate, or resistant using the Clinical Laboratory Standards Institute (CLSI) guidelines (19).

3. Results

The findings of the study are summarized in the following Tables and are explained. The phenotypic characterization of *Listeria* spp. isolated from selected seafood is shown in Table 1. A total of 500 seafood samples were analyzed out of which 36 (7.2%) were positive for *Listeria innocua*. Raw croaker had the highest occurrence of 13.0%. This is shown in Table 2. The antimicrobial susceptibility test as shown in Table

3 revealed that all isolates were resistant to ceftazidime and cloxacillin. However, high sensitivities to ofloxacin (83.3%) and erythromycin (72.2%) were exhibited by the isolates.

Table 1. Phenotypic characterisation of *Listeria* isolates

Sample Code	Gram Reaction	Shape	Catalase	Latex Agglutination	Motility	OBIS	CAMP (*Staph. aureus*)	CAMP (*R. equi*)	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Glucode-1-Phosphase	Methyl-D-Glucose	Methyl-D-Mannose	Haemolysis	Probable Organism
FC_{1-9}	+	SR	+	+	+	+	-	-	+	-	-	+	-	+	+	-	-	+	+	-	*L. innocua*
FC_{10}	+	SR	+	+	+	+	-	-	+	-	-	+	-	+	+	-	-	+	+	-	*L. innocua*
FC_{11}	+	SR	+	+	+	+	-	-	+	-	-	+	+	+	+	-	-	+	+	-	*L. innocua*
FC_{12}	+	SR	+	+	+	+	-	-	+	-	-	+	+	+	+	-	-	+	+	-	*L. innocua*
FC_{13}	+	SR	+	+	+	+	-	-	+	-	-	+	-	+	+	-	-	+	+	-	*L. innocua*
SC_{1-3}	+	SR	+	+	+	+	-	-	+	-	-	+	-	+	+	-	-	+	+	-	*L. innocua*
FB_{1-8}	+	SR	+	+	+	+	-	-	+	-	-	+	+	+	+	-	-	+	+	-	*L. innocua*
SB_{1-7}	+	SR	+	+	+	+	-	-	+	-	-	+	+	+	+	-	-	+	+	-	*L. innocua*
SB_{8-10}	+	SR	+	+	+	+	-	-	+	-	-	+	+	+	+	-	-	+	+	-	*L. innocua*
FS_1	+	SR	+	+	+	+	-	-	+	-	-	+	-	+	+	-	-	+	+	-	*L. innocua*
FS_2	+	SR	+	+	+	+	-	-	+	-	-	+	-	+	+	-	-	+	+	-	*L. innocua*

+ = Positive;
- = Negative;
SR = Short Rod;
FC= Fresh Croaker;
SC= Smoked Croaker
SB= Smoked Blue Whiting
FB= Fresh Blue Whiting
FS= Fresh Shrimp
1-13= Isolates numbers
OBIS = Oxoid Biochemical Identification System;
CAMP = Christie–Atkins–Munch-Petersen

Table 2. Prevalence of *Listeria innocua* in seafood

Samples	Number of samples examined	Number of positive samples (%)	Number of negative samples (%)
Fresh Croaker	100	13 (13.0)	87 (87.0)
Smoked Croaker	100	3 (3.0)	97 (97.0)
Fresh Blue Whiting	100	8 (8.0)	92 (92.0)
Smoked Blue Whiting	100	10 (10.0)	90 (90.0)
Fresh Shrimp	50	2 (4.0)	48 (96.0)
Smoked Shrimp	50	0 (0.0)	100 (100.0)
Total	**500**	**36 (7.2)**	**464 (92.8)**

Table 3. Antibiotic susceptibility and resistance (%) of *L. innocua* strains isolated from seafood

Antibiotic Class	Antimicrobial Agent	Sensitive n= 36, (%)	Intermediate n= 36, (%)	Resistant n= 36, (%)
Cephalosporin	Ceftazidime	0 (0.0)	0 (0.0)	36 (100.0)
	Cefuroxime	13 (36.1)	2 (5.6)	21 (58.3)
	Ceftriazone	7 (19.4)	0 (0.0)	29 (80.6)
Fluoroquinolones	Ofloxacin	30 (83.3)	5 (13.9)	1 (2.8)
Macrolides	Erythromycin	26 (72.2)	6 (16.7)	4 (11.1)
Aminoglycosides	Gentamicin	15 (14.7)	8 (22.2)	13 (36.1)
Penicillin	Cloxacillin	0 (0.0)	0 (0.0)	36 (100.0)
	Amoxycillin/Clavulanate	15 (41.7)	9 (25.0)	12 (33.3)

4. Discussion

Listeria spp. are commonly found in a range of food groups, including seafood products, and in several food processing environments. This is well-documented in many nations and seems to support the idea that these organisms are ubiquitous bacterial pathogens. Due to their ubiquity in nature, introduction into food-processing environments may result in the contamination of food products (20). In the present study, 7.2% of the seafood samples tested were contaminated with *L. innocua*. The occurrence of *L. innocua* observed in our study is in accordance with published data in Kerala, India, which reported the existence of *L. innocua* in 7.56% of seafood samples (16). Jamali et al. (15) and Sanlibaba et al. (21) reported 8.1% and 8% prevalence of *L. innocua* from Iran and Turkey respectively. A lower prevalence of 0.66% was recorded in Iran from marine foods (22).

Since the same surroundings where food is processed frequently harbor both *L. innocua* and *L. monocytogenes*, their existence may be a sign of possible *L. monocytogenes* contamination (23,24). In addition, there are still biofilms of Listeria in the working environment's equipment, tools, flooring, and drainage systems and this may have contributed to the contamination.

According to a worldwide viewpoint, antimicrobial resistance has been seen as a substantial threat to the well-being of the populace around the world. Antimicrobial abuse and impulsive usage, including in human and veterinary medicine, have been blamed for the development of bacterial resistance from the use of antibiotics (25). Treatment for *Listeria* spp. infections have typically involved combining an aminoglycoside antibiotic, such as gentamycin, with a β-lactam anti-infection drug, such as amoxicillin, penicillin, or ampicillin (26).

Despite the fact that *Listeria* spp. is innately impervious to certain antibiotics — basically cephalosporins, oxacillin, and fosfomycin — it has generally remained vulnerable to the majority of the antimicrobial agents that are utilized in the treatment of Gram-positive microscopic organisms (27, 28). In this study, *L. innocua* isolates showed 100% resistance against two antibiotics ceftazidime and cloxacillin. The result is comparable to that of Eneh et al. (29), who recorded resistance of ceftazidime and cloxacillin at 96.67% and 90% respectively from various food groups in Enugu, Nigeria. *Listeria* spp. in the present study was sensitive to gentamicin. This supports the work of Enurah *et al*. (30) and Wu et al. (31) but does not support the work of Kawo and Bello (32) whose Listeria isolates were resistant to gentamicin. *Listeria* spp. of this study showed sensitivity to erythromycin and this agrees with the results of Enurah et al (30), Wu et al. (31) as well as Moreno et al. (33). Cloxacillin resistance detected amongst Listeria species in this work was high and is in consonance with results validated in prior reports (30,34,35). However, this was not the case in the study of Kawo and Bello (32), whose isolates of *Listeria* remained susceptible to cloxacillin. Akano et al. (36) found that all Listeria isolates obtained from abattoir effluent in Lagos were susceptible to ofloxacin, which is similar to the results obtained in this study.

The widespread usage of antimicrobials to promote development in farm animals, or in the clinical management of people or livestock has significantly increased selective pressure, which has been definitively linked to the manifestation of antibiotic resistance (37). The rate at which the species of Listeria

bacteria develops resistance due to antimicrobials differs greatly among the strains, depending on where they were isolated from, the period (date, time, season) of the isolation, the usage of antibiotics by both humans and animals, as well as geographic variables (38). Since variations in antibiotic resistance influence Listeria species, including *L. monocytogenes*, it is imperative to monitor these changes. This is because resistant strains have the potential to seriously harm people's health. It is significant to remember that numerous studies of antimicrobial resistance involving Listeria species have relied on human isolates; however, it is essential to expand using observational data from numerous samples, such as food and animals utilized in agriculture, the environment where food production occurs, and animal manure (39). Due to its virulence, ease of environmental spread, and transmission through workers, and raw materials with machinery in the setting of food preparation, the microbe is given the necessary conditions to enable long-lasting colonization. As a result, Listeria species are able to thrive in a range of habitats and are present in samples of food, surroundings of farms, and locations where food is processed or produced (40). The creation of biofilms, efflux pumps, and the horizontal gene transfer of antibiotic resistance features with other bacterial species are all adaptive processes that this bacterium uses to become resistant to antibiotics, rendering them ineffective (41). The rise of microbes resistant to antibiotics in the food chain is one of the major problems the food industry faces. The research's isolates were all resistant to multiple antibiotic classes. This corroborates the results obtained by Odu *et al*. (42) who had similar outcomes from studies on Listeria species in tilapia in Port Harcourt. According to Bertsch et al. (43), the number of resistant bacteria harming both human and animal health can only be decreased through avoidance and/or reduction of antimicrobial usage/prevention in livestock.

5. Conclusion

The microbiological analysis of seafood samples in this study revealed the presence of *Listeria innocua* in varying prevalence with the exception of smoked shrimp. Furthermore, the recovery of antimicrobial-resistant *Listeria innocua* strains in the seafood samples analysed warrants the need for suitable control procedures as this could constitute a great risk to public health.

Conflict of interest

No conflict of interest, according to the authors.

Acknowledgment

The Nigerian Institute for Oceanography and Marine Research provided part funding for this work.

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