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Prevalence and antibiogram of *Listeria monocytogenes* isolated from seafood sold in Rivers state, Nigeria

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Abstract
The health implication of *Listeria monocytogenes* seafood a global issue and their antibiotic resistance cannot be overemphasized. This study aimed to investigate the prevalence and antibiogram of *Listeria monocytogenes* isolated from seafood sold in Rivers State, Nigeria. A total of 126 raw and parboiled samples of *Buccinum undatum* (Whelks), *Crassostrea gasar* (Oyster) and *Penaeus monodon* (Prawn) were collected from different markets (Bakana, Creek road and Kaa) and analyzed for the presence of *Listeria monocytogenes* using standard conventional methods such as culturing on a selective medium, Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol Agar. The Kirby-Bauer disc diffusion method was used for antibiotic susceptibility pattern**.** The total *Listeria* count ($x10^2$ CFU/g) in raw and parboiled Oyster, Prawn and Whelks which ranged between 1.6±0.3 to 2.9±0.4: 1.5±0.2 to 2.3±0.2; 1.6±0.4 to 3.2±0.7: 1.2±0.2 to 1.9±0.5; 2.8±0.4 to 3.6±0.3: 1.7±0.3 to 2.6±0.3 for Bakana and Creek road markets, respectively**.** There was a significant difference ($p \leq 0.05$) in the *Listeria* count. A total of 25 (19.84%) *Listeria monocytogenes* were isolated from the seafood samples with high prevalence in *Penaeus monodon* (Prawn) (62.5%) samples. *L. monocytogenes* were (100%) resistant to Cefixime, Ceftazidime, Cefuroxime and Ciprofloxacin and sensitive to Ofloxacin (76%) and all the isolates had a MAR index ≥0.2. It was observed in this study that *Listeria monocytogenes* had a relatively high prevalence in the seafood and majority of the isolates exhibited resistance to multiple antibiotics. The findings could be used to inform public health policies and guide the implementation of preventive strategies to reduce the risk of listeriosis.

Keywords: Resistance, susceptibility, prevalence, sea foods, *Listeria monocytogenes*

Introduction
It has been on record that seafood is involved in a lot of food-borne outbreak in the world at large. These seafoods are highly nutritious with high protein and vitamin content leading to their large consumption by humans (Savichtcheva and Okabe, 2006) [15]. Microbial contamination in these seafoods are due to poor hygiene, improper handling, disposal of untreated sewage into their environment, dirt, sewage contaminated water, illegal harvesting from sewage contaminated waters, processing, storage, transportation at inappropriate temperatures, sewage runoff and flooding into the habitat of these seafoods. They accumulate several pathogenic microorganisms such as *Listeria monocytogenes* from their surrounding waters during feeding resulting in listeriosis (**(**Mozaffarian and Rimm 2006; Savichtcheva and Okabe, 2006) [13, 15]. The high level of anthropogenic activities around where the seafoods are harvested is a major problem contributing to the high prevalence of the bacteria in the seafoods. *Listeria monocytogenes* is a pathogenic bacteria transmitted to man mostly through seafoods (Genigeorgis *et al*., 2006) [8]. This pathogen is responsible for human listeriosis, a severe disease that may result in meningitis, encephalitis, septicemia or abortion, with a considerable mortality rate. It can also be found on cooked ready-to-eat (RTE) food processing equipment, due to its ability to form biofilms. Most times these seafoods are consumed raw or parboiled without adequate cooking to kill the bacteria or toxins. Foodborne listeriosis is a severe bacterial infection with high rate of fatality (20-30%) and hospitalization (More than 92%) caused by the consumption of food especially seafood contaminated with *L. monocytogenes* (Du *et al*., 2017) [7]. Listeriosis is always a public health concern as this foodborne infection is a great threat to susceptible population groups such as the pregnant women, fetuses or newborn, elderly and immune-compromised individuals (Todd and Notermans, 2011) [18]. The emergence of antimicrobial resistance in *Listeria* strains is a serious health problem worldwide (Connor and Schwartz, 2005) [5]. *Listeria monocytogenes* have been known over the decade to be resistant to most antibiotics

such as Gentamicin, Ciprofloxacin, Erythromycin, Chloramphenicol, and some Trimethoprim-Sulfamethoxazole, quinolones and extended spectrum cephalosporin, making it more complicated and expensive to treat patients with serious infections (Connor and Schwartz, 2005) [5]. Therefore, this research is carried out to investigate prevalence and antibiogram of *Listeria monocytogenes* isolated from seafoods sold in Rivers State.

Materials and Methods

Description of study Area

Three markets in Rivers State, Kaa market in Khana Local Government Area, Creek Road market in Port Harcourt Local Government Area (PHALGA), and Bakana market in Degema Local Government Area, were used for the study.

Sample Collection

One hundred and twenty-six (126) samples of raw and parboiled seafoods, including Whelks (*Buccinum undatum*), Oysters (*Crassostrea gasar*), and Prawns (*Penaeus monodon*), were purchased from three markets, placed in sterile polythene bags, then put in ice chests, and aseptically transported to the Department of Microbiology laboratory at Rivers State University, Port Harcourt, for bacteriological analysis after identification by Prof. G.C. Akani in the Department of Animal and Environmental Biology, Rivers State University.

Microbiological Analysis

Bacterial Enumeration and Isolation

Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol (PALCAM) agar supplemented with *Listeria* Selective Supplement II (FD063) was used for the enumeration of the *Listeria* counts (Cheesbrough, 2005) [3]. Preparation of the stock analytical unit was carried out by weighing ten (10) grams of the edible portion of the raw and parboiled seafoods samples and homogenized in 90 milliliters of sterile normal saline for enumeration, isolation and identification. A serial ten-fold dilution was conducted by pipetting 1 milliliter of the prepared seafoods samples into 9 milliliters of sterile normal saline. Using the spread plate technique, 0.1 aliquots from the appropriate dilution (10^{-1}) were inoculated in duplicates onto already prepared sterile PALCAM agar plates. The plates were then incubated at 37 °C for 24 hours before count within 30-300 were being counted and recorded. To obtain pure cultures, discrete colonies were characterized, sub-cultured onto freshly prepared nutrient agar plates, and incubated at 37 °C for 24 hours (Taylor, 2008) [17].

Preservation of pure culture

The pure cultures of the *Listeria* isolates were preserved in 10% (v/v) glycerol suspension at -4 °C for further analysis.

Identification of *Listeria monocytogenes*

Listeria monocytogenes were identified based on their colonial/morphological characteristics such as the size, margin, surface, colour (gray-green), elevation, texture and transparency as well as using a selective medium called Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol (PALCAM) agar supplemented with *Listeria* Selective Supplement II (FD063). Identification was performed by conducting series of biochemical tests, including Oxidase, Catalase, Coagulase, Citrate Utilization, Methyl red, Indole, Voges Proskauer and sugar fermentation tests to confirm *Listeria monocytogenes* (Cheesbrough, 2005) [3].

Antibiotic Susceptibility Technique

The antimicrobial susceptibility profiles of the bacterial isolates to conventional antibiotics were determined using the Kirby Bauer disk diffusion method on sterile Mueller-Hinton agar. The *Listeria* isolates were standardized by continued adjusting to 0.5 McFarland turbidity standards ($x10^8$ cells). The swab was deepened into suspension and streaked over the surface of the agar plates, rotating the agar plate 60º to ensure appropriate distribution of the inoculum and air dried for 3–5 min. Conventional antibiotics disk impregnated with Gentamicin (10 µg), Cefixime (5 µg), Erythromycin (300 µg), Ofloxacin (5 µg), Ceftazidime (30 µg), Cefuroxime (30µg), Ciprofloxacin (5µg) and Augmentin (30 µg), were placed aseptically on the surface of the inoculated agar plate with the aid of sterile forceps. The disc was pressed down to make full contact with the surface of the agar. The plates were then incubated for 24 hours at 33 to 35 ºC in an inverted position. The zones of inhibition were measured in millimeter (mm) and compared to (CLSI, 2017).

Determination of Multiple Antibiotic Resistance (MAR) index

Multiple antibiotic resistance refers to bacterial resistance to three or more different antibiotics. Using the formula MAR = a/b, where ''a'' represent the number of antibiotics to which the test isolates depicted resistance and ''b'' represent the total number of antibiotics to which the test isolate has been tested for susceptibility, the multiple antibiotic resistance (MAR) index was ascertained (Krumperman, 1985) [12].

Data Analysis

Statistical Package for Social Sciences (SPSS) version 25 was used to analyze the data obtained from counts and the measurement of the zones of inhibition. Analysis of variance (ANOVA) was carried out to test for significant difference ($p \leq 0.05$). Duncan multiple range test was used to separate the means were difference existed (Bewick *et al*., 2004) [2].

Results Result of *Listeria* count in Raw and parboiled seafood samples are presented on table 1. The results showed that the total *Listeria* count ($x10^2$ CFU/g) in raw and parboiled Oyster ranged between 1.6±0.3, 2.6±0.4 to 2.9±0.4: 1.5±0.2 2.3±0.2 to 2.5±0.3 for Bakana, Kaa and Creek road, markets, respectively. Total *Listeria* count ($x10^2$ CFU/g) in raw and parboiled Prawn ranged between 1.6±0.3, 1.8±0.5 to 3.2±0.7: 1.2±0.2, 1.4±0.2 to 1.9±0.5 for Bakana, Kaa and Creek road markets and total *Listeria* count ($x10^2$ CFU/g) in raw and parboiled Whelks samples ranged 2.8±0.4, 2.9±0.6 to 3.6±0.3: 1.7±0.3, 2.3±0.2 to 2.6±0.3 for Bakana, Kaa and Creek road markets, respectively with a significant difference ($p \leq 0.05$) in markets. A total of 25 (19.84%) *Listeria monocytogenes* were isolated from the seafood samples. *Listeria monocytogenes* (28.57%, 45.45%, 42.86%) had a high prevalence in samples from Creek road market in the raw samples of Whelk, Prawn and Oyster respectively than in the parboiled samples as revealed in fig 1.

Table 1: Mean *Listeria* counts ($\times 10^2$ CFU/g) of Raw and Parboiled *Crassostrea gasar* (Oyster), *Penaeus monodon* (Prawn) and *Buccinum undatum* (Whelks) from Various Markets Sampled

Markets	Oyster		Prawn		Whelks	
	Raw	Parboiled	Raw	Parboiled	Raw	Parboiled
Bakana	1.6±0.3[a]	1.5±0.2[a]	1.6±0.4[a]	1.2±0.2[a]	2.8±0.4[a]	1.7±0.3[a]
Creek Road	2.9±0.4[b]	2.5±0.3[b]	3.2±0.7[b]	1.9±0.5[b]	3.6±0.3[b]	2.6±0.3[b]
Kaa	2.6±0.4[b]	2.3±0.2[b]	1.8±0.5[a]	1.4±0.2[a]	2.9±0.6[a]	2.3±0.2[b]
P-value	0.000	0.000	0.000	0.004	0.018	0.000

*Means with same alphab*et al*ong the columns shows no significant difference ($p \geq 0.05$)

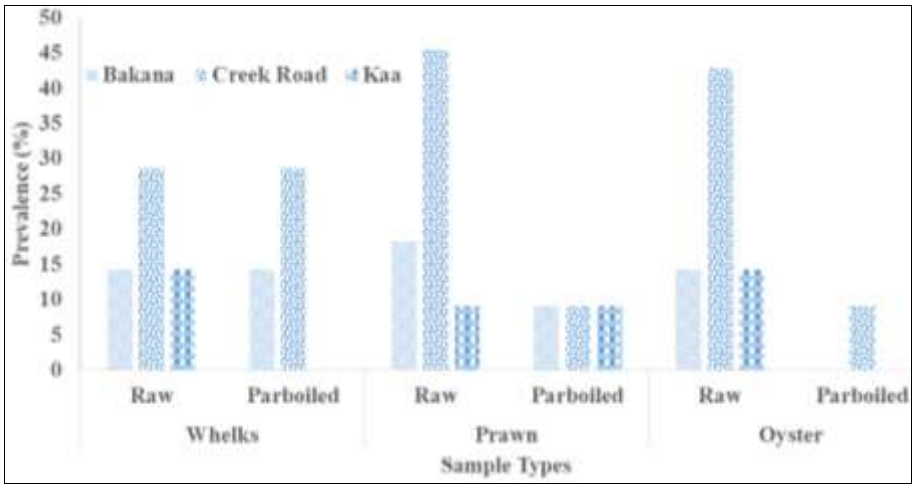

Fig 1: Prevalence of *Listeria monocytogenes* in both raw and parboiled seafood from the different samples

The susceptibility pattern of *Listeria monocytogenes* from Oyster in raw and parboiled as shown in Table 2 indicates that a greater number of the *Listeria monocytogenes* isolates were susceptible to Gentamicin and Ofloxacin (60% and 50%) and were highly resistant to Ciprofloxacin, Cefixime Augmentin, Ceftazidime and Cefuroxime (100%). *Listeria monocytogenes* from Prawn in raw and parboiled samples showed the isolates were sensitive to Ofloxacin (87.5% and 100%) and were resistant to Ciprofloxacin, Cefixime, Augmentin, Ceftazidime and Cefuroxime (100%) (Table 3).

The *Listeria monocytogenes* isolates from the Whelks samples for raw and parboiled revealed that they were susceptible to Gentamicin and Ofloxacin (100% and 50%; 66.7% and 33.3%) and resistant to Ciprofloxacin, Cefixime, Augmentin, Ceftazidime and Cefuroxime (100%) as shown in table 4. Generally, *Listeria monocytogenes* isolates were resistant to Ciprofloxacin, Ceftazidime Cefuroxime and Cefixime (100%) and sensitive to Ofloxacin (76%) as shown in table 5. Hundred percent (100%) of the isolates had a MAR index greater than 0.2 as shown in table 6.

Table 2: Susceptibility Pattern of *Listeria monocytogenes* isolated from *Crassostrea gasar* (Oyster)

Antibiotics	Conc. (µg)	Raw (n=5)			Parboiled (n=1)		
		R	I	S	R	I	S
Gentamicin	10	1 (20)	1 (20)	3 (60)	1 (100)	0 (0.00)	0 (0.00)
Ciprofloxacin	10	5 (100)	0 (0.00)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)
Erythromycin	300	1 (20)	4 (80)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)
Cefixime	5	5 (100)	0 (0.00)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)
Ofloxacin	5	0 (0.00)	1 (20)	4 (80)	1 (100)	0 (0.00)	0 (0.00)
Augmentin	30	5 (100)	0 (0.00)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)
Ceftazidime	30	5 (100)	0 (0.00)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)
Cefuroxime	30	5 (100)	0 (0.00)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)

Key: R-Resistant; I-Intermediate; S-Susceptible

Table 3: Susceptibility Pattern of *Listeria monocytogenes* isolated from *Penaeus monodon* (Prawn)

Antibiotics	Conc. (µg)	Raw (n=8)			Parboiled (n=4)		
		R	I	S	R	I	S
Gentamicin	10	4 (50)	2 (25)	2 (25)	3 (75)	0 (0.00)	1 (25)
Ciprofloxacin	10	8 (100)	0 (0.00)	0 (0.00)	4 (100)	0 (0.00)	0 (0.00)
Erythromycin	300	3 (37.5)	5 (62.5)	0 (0.00)	0 (0.00)	4 (100)	0 (0.00)
Cefixime	5	8 (100)	0 (0.00)	0 (0.00)	4 (100)	0 (0.00)	0 (0.00)
Ofloxacin	5	0 (0.00)	1(12.5)	7 (87.5)	0 (0.00)	0 (0.00)	4 (100)
Augmentin	30	5 (62.5)	1 (12.5)	2 (25)	3 (75)	0 (0.00)	1 (25)
Ceftazidime	30	8 (100)	0 (0.00)	0 (0.00)	4 (100)	0 (0.00)	0 (0.00)
Cefuroxime	30	8 (100)	0(0.00)	0 (0.00)	4 (100)	0 (0.00)	0 (0.00)

Key: R-Resistant; I-Intermediate; S-Susceptible

Table 4: Susceptibility Pattern of *Listeria monocytogenes* isolated from *Buccinum undatum* (Whelks)

Antibiotics	Conc. (µg)	Raw (n=4)			Parboiled (n=3)		
		R	I	S	R	I	S
Gentamicin	10	0 (0.00)	0 (0.00)	4 (100)	1 (33.3)	0 (0.00)	2 (66.7)
Ciprofloxacin	10	4 (100)	0 (0.00)	0 (0.00)	3 (100)	0 (0.00)	0 (0.00)
Erythromycin	300	0 (0.00)	4 (100)	0 (0.00)	0 (0.00)	3 (100)	0 (0.00)
Cefixime	5	4 (100)	0 (0.00)	0 (0.00)	3 (100)	0 (0.00)	0 (0.00)
Ofloxacin	5	2 (50)	0 (0.00)	2 (50)	1 (33.3)	1 (33.3)	1 (33.3)
Augmentin	30	4 (100)	0 (0.00)	0 (0.00)	3 (100)	0 (0.00)	0 (0.00)
Ceftazidime	30	4 (100)	0 (0.00)	0 (0.00)	3 (100)	0 (0.00)	0 (0.00)
Cefuroxime	30	4 (100)	0 (0.00)	0 (0.00)	3 (100)	0 (0.00)	0 (0.00)

Key: R-Resistant; I-Intermediate; S-Susceptible

Table 5: Overall Susceptibility Pattern of *Listeria monocytogenes* isolated from seafoods (Whelks, Oyster and Prawn)

Antibiotics	Conc. (µg)	*Listeria monocytogenes* (n=25)		
		Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Gentamicin	10	9 (36)	3 (12)	13 (52)
Ciprofloxacin	10	25 (100)	0 (0)	0 (0)
Erythromycin	300	4 (16)	21 (84)	0 (0)
Cefixime	5	25 (100)	0 (0)	0 (0)
Ofloxacin	5	3 (12)	3 (12)	19 (76)
Augmentin	30	21 (84)	1 (4)	3 (12)
Ceftazidime	30	25 (100)	0 (0)	0 (0)
Cefuroxime	30	25 (100)	0 (0)	0 (0)

Key: R-Resistant; I-Intermediate; S-Susceptible

Table 6: Multiple Antibiotic Resistance Index

MAR Index	*Listeria monocytogenes* (n=25)		
	Oyster	Prawn	Whelks
0.2	0 (0.0)	0 (0.0)	0 (0.0)
0.3	0 (0.0)	0 (0.0)	0 (0.0)
0.4	0 (0.0)	0 (0.0)	0 (0.0)
0.5	2 (8.0)	1 (4.0)	1 (4.0)
0.6	8 (32)	3 (12)	2 (8.0)
0.8	3 (12)	1 (4.0)	4 (16)
0.9	1 (4.0)	0 (0.0)	2 (8.0)
1.0	1 (4.0)	0 (0.0)	2 (8.0)

Discussion

The presence of *Listeria* in seafood samples indicates poor hygiene and inappropriate sanitary conditions, and the quality of seafoods depends on the quality of water from which the seafoods are harvested as well as sanitary conditions of the storage areas (Dons *et al*., 2011) [6]. The high *Listeria* count count in the Oyster, prawn and Whelks samples as observed in the Creek road market could also be attributed to the poor environment condition of the harvest area. This environment where the raw Oyster, prawn and Whelks were harvested are exposed to high refuse dump and was also a site for defaecation by those residing around the market zone. Low counts were observed in the parboiled samples of Oyster, prawn and Whelks probably because the high heat content involved during boiling could have reduce the microbial load in the Oyster, prawn and Whelks. This agrees with the work of Sampson *et al*. (2020) [14] in which heat reduced the level of bacteria found in edible cockles and the work of Dons *et al.* (2011) [6] which reported a reduction of moisture content by heating and consequently reduction of the number of microorganisms in the shellfish. Kramarenko *et al*. (2014) [11] also recorded similar values in their research on shellfish. The total *Listeria* count of the raw samples were above the limits specified by the International Commission on Microbiological Specifications for foods (ICMSF, 2002) [10]. The occurrence of *Listeria* could be attributed to the fact that most *Listeria* spp are psychrophiles which indicate that they can survive at freezing temperature ranging from -5 ºC to -28 °C especially *Listeria monocytogenes*. Handling and exposure to contaminants by vendors could also contribute to the high level of *Listeria* in seafoods as reported by Ajao *et al*. (2009) [1]. A greater number of the *Listeria monocytogenes* isolates were susceptible to Ofloxacin and Gentamicin and were highly resistant to Ciprofloxacin, Cefixime, Augmentin, Ceftazidime, and Cefuroxime. The sensitivity of *Listeria* to Ofloxacin and Gentamicin corroborates with the work of Shakoor *et al*. (2012) [16] and disagrees with their 100% sensitivity report for Erythromycin. Differences in the antimicrobial susceptibility pattern recorded in this study when compared to some studies done previously could be as a result of in environmental conditions which may include the exposure of the organism to antibiotic used frequently and changes in genome as a result of harsh and chemicals which in turn enable these organisms transform to strain that are able to resist antibiotics they are normally susceptible to. The high resistance to the beta-lactam antibiotics can be explained by the extensive and uncontrolled use of these antibiotics as well as their affordability and acquisition of the extent-ended-spectrum spectrum β-lactamases genes (Gourmelon *et al*., 2006) [9].

Conclusion and Recommendations

The occurrence of *Listeria monocytogenes* in seafood as revealed in this study poses a potential risk to public health. The Listerial load were high in the seafoods samples and can be inferred from this study that the risk of infection is high when raw seafoods samples are consumed. *Listeria monocytogenes* demonstrated resistance to a variety of antibiotics used in this study but its sensitivity to Ofloxacin revealed that it can be used for the treatment of infections caused by the organism. The findings from this study could help in formulating appropriate strategies to reduce the incidence of *Listeria monocytogenes* in seafoods, ensure food safety and abuse of antibiotics.

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