Molecular Characterization and Antibiotic Resistance Patterns of *Listeria* Species in Frozen Beef and Chicken Sold in Benin City, Nigeria

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Abstract

Frozen beef and chicken products were purchased from four markets in Benin City and examined for the presence of *Listeria* species using standard microbiological procedures. *Listeria* species were characterized by biochemical tests and confirmed by polymerase chain reaction (PCR). Antibiotic resistance of the isolates was carried out using the disc diffusion method. Bacterial plasmids were isolated and separated by agarose gel electrophoresis. The *Listeria* isolates in the beef samples ranged between $3.11 \pm 0.69 \times 10^3$ to $1.36 \pm 0.39 \times 10^4$ CFU/g while for chicken samples ranged between $1.56 \pm 0.24 \times 10^3$ to $1.84 \pm 0.45 \times 10^4$ CFU/g. The isolated *Listeria* species were *L. monocytogenes, L. ivanovii, L. grayi* and *L. innocua.* Among the *Listeria* isolates, *L. monocytogenes* (34.78%) and *L. innocua* (31.82%) had the highest frequency in beef and chicken respectively. Antibiotics sensitivity revealed that the most resisted drugs were ampiclox, amoxicillin and septrin while isolates were most susceptible to gentamycin and ciprofloxacin. The strains of organisms identified from DNA sequencing were: *L. monocytogenes* (KM010020.1), *L. monocytogenes* (KF588563.1), *L. ivanovii subsp. Ivanovii* (CP009577.1), *L. grayi* (JN852815.1), *L. monocytogenes* (CP013724.1), *L. ivanovii subsp. londoniensis* (CP009575.1), *L. grayi* (NR116351.1) *and L. innocua (*KU208087.1*).* The food-borne pathogens isolated from meat products of this study may be the indicator of poor hygienic practices.

Keywords: *Listeria* spp., Frozen beef product, Frozen chicken product, Antibiotic resistance, Food-borne pathogen

1. Introduction

Meat is the major source of protein and valuable qualities of vitamins for most people in many parts of the world. The nutrients contained are essential for the growth, repair and maintenance of body cells and necessary for our everyday activities (Adzitey and Huda, 2010). Owing to its chemical composition and biological characteristics, meats are highly perishable foods which provide excellent source of nutrients for growth of many potentiallyhazardous microorganisms that can cause spoilage and infection in humans (Barbuddhe *et al.,* 2000).

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Listeric infections, caused by microorganisms of the genius *Listeria*, occur worldwide and in a variety of animals including man (Border *et al.,* 1990). Cases of listeriosis arise mainly from the ingestion of contaminated food and the disease is particularly common in ruminants fed on silage and poultry animals (Border *et al.,* 1990). The genus Listeria comprises ten species: *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. grayi*, *L. ivanovii*, *L. seeligeri*, *L. marthii*, *L. rocourtiae*, *L. fleischmannii* and *L. weihenstephanensis (*Bubert *et al.,* 1999). Two of these species, *L. monocytogenes*and *L. ivanovii*, are potentially pathogens (Filiousis *et al*., 2009). The other eight *Listeria* species are essentially saprophytes that have adapted for survival in soil and decaying vegetation *(*Bubert *et al*., 1999). *Listeria monocytogenes*, a facultative intracellular pathogen, repeatedly found in meat and meat products, chicken, raw milk, soft cheese, pasteurized dairy products, fish and fish products, and vegetables (Hassan *et al.*, 2006). This strain is responsible for severe foodborne infections in humans of all ages but especially pregnant women, infants less than four weeks old, the elderly and immune compromised individuals. It also cause invasive disease in many ruminants including, cattle, sheep, and goats (Indrawattana *et al.*, 2011). The bacterium possesses properties that favor it as a foodborne pathogen. At variance with most other pathogens it is relatively resistant to acid and high salt concentrations, grows at low temperature, down to freezing point, which means it may grow in refrigerated foods, measures commonly used to control the growth of pathogens in foods (Jay, 1996). Before the development of *Listeria* selective media, this property was used for selective enrichment of the bacterium from complex matrices, with *Listeria* outnumbering the competing flora after incubation of the enrichment culture at refrigeration temperature for weeks or months (Johnson *et al.,* 1990). Although the occurrence of *Listeria* in various foods has been investigated in several countries (Adzitey and Huda, 2010; Awaisheh, 2010; Montero *et al*., 2015) there is limited information regarding its prevalence in frozen raw beef and chicken in Nigeria. So the present study has conducted to determine the occurrence of *Listeria* species in frozen beef and chicken sold in Benin City, Southern Nigeria.

2. Materials and Methods

2.1 Sample Collection

A total of two hundred and forty samples which consist of one hundred and twenty frozen beef and one hundred and twenty frozen chickens were randomly purchased from different markets (Oba, New Benin, Uselu and Aduwawa) in Benin metropolis. The meat samples were collected in sterile plastic bags, placed in ice packs, and then transported to the laboratory for further analysis.

2.2 Isolation of *Listeria* species

Aseptically, 10 g of each sample was added to 90 mL *Listeria* enrichment broth (LEB) (Oxoid, UK) containing selective *Listeria* enrichment supplement in a bottle. It was homogenized for 2 min at room temperature (28 ± 2 °C) and incubated at 37 °C for 24 to 48 h. After incubation, a loopful of growth from LEB was streaked on the *Listeria* selective agar (LSA) plate (Oxoid, UK) and incubated at 37 °C for 24 to 48 h. Presumptive colonies of *Listeria* species on both plates having a black color on LSA plates were re-streaked on nutrient agar slants incubated at 37 °C for 24 h, and stored at 4 °C before subjection to Gram's staining and further biochemical characterization.

2.3 Confirmation and Identification

Colonies suspected to be *Listeria* were transferred onto trypticase soy agar (Becton, Dickinson and company, France) with 0.6% yeast extract (Lab M, UK) and incubated at 37 ºC for 18 to 24 h, before being subjected to the standard biochemical tests: Gram's staining, catalase reaction, oxidase reaction, β - hemolysis on sheep blood agar and acid production from mannitol, rhamnose and xylose. The confirmed isolates on the basis of criteria suggested by Seeliger and Jones (2009) were further identified using the polymerase chain reaction (PCR).

2.4 Isolation of plasmid DNA using zymo research plasmid miniprep™ classic

The cells were grown on tryptone soya broth at 37 °C for 24 h and transferred into the micro tubes and centrifuged at 5000 × g, 28 ± 2 °C to obtain the pellets. Plasmid DNA was isolated using Zymo research plasmid miniprep™ classic according to the manufacturer instructions (Zymo Research, Irvine, USA).

2.4.1 Agarose gel preparation

Electrophoresis of the plasmid DNA was carried out on a 0.8% agarose gel in a 2 mM Tris-Borate-EDTA (TBE) buffer. The gel was then electrophoresed in a horizontal tank at a constant voltage of 60 °C for 90 min. After electrophoresis, plasmid DNA bands were viewed by fluorescence under a short wave ultraviolet light transilluminator and the photograph were taken using a photo documentation system. The molecular weights of the plasmid were extrapolated and calculated using the standard molecular weight marker preloaded in the wells.

2.4.2 Molecular identification

DNA Extraction

DNA was extracted using the QIAamp DNA Mini Kit (250) cat no 51306. A 16S primer was used for eight isolates. Buffer AE placed into 70 °C water bath. Then, 180 µL of ATL buffer was added to the isolates, 20 µL of Proteinase K was added and the samples were then

incubated at 56 °C until completely lysed. The tubes were centrifuged at 5000 × g, 28 ± 2 °C for 10 min to collect condensation, 200 µL of Buffer AL was added and vortexed for 15 sec. Then, tubes were incubated at 70 °C for 10 min. Tubes were centrifuged to collect condensation. Two hundred and thirty (230 µL) of ethanol (96–100%) was added and mixed by vortex for 30 sec. Then, the samples were carefully applied to QIAamp spin column and centrifuged at 8000 rpm per minute. The spin column was placed in a clean 2 mL collection tube and the filtrates were discarded. 500 µL Buffer AW1 was added, spin at 8000 rpm per minute and placed in the collection tube and filtrate discarded. Following was the addition of 500 µL Buffer AW2, spin at 8000 rpm per minute and placed into the collection tube and filtrate discarded again. Centrifugation was done at a full speed for 3 min, column was placed in labeled 1.5 mL tube and 200 µL of the preheated (70 °C) Buffer AE was added, the tubes were incubated at (70 °C) for 5 min and then centrifuged at 8000 rpm per minute. The filtrate solution (200 µL) was placed back into the spin column. Then, 200 µL of the preheated (70 °C) Buffer AE and the tubes were incubated at (70 °C) for 5 min and then centrifuged at 8000 rpm per minute. Afterwards, the spin columns were discarded and ran on agarose to visualize the DNA of isolates.

2.4.3 Polymerase chain reaction (PCR)

PCR Product Purification

Absolute ethanol (20 µL) was added to PCR product and incubated at 37 °C after which it was spun at 10000 rpm for 15 min. The supernatant was decanted and spun again at 10000 rpm for 15 min then 40 µL of 70% ethanol was added and the supernatant decanted again. It was then allowed to air dry and 10 µL of ultrapure water and the product was checked in 1.5% agarose. The purified PCR product was used for another PCR reaction which then became the amplicon used for sequencing reaction.

2.4.4 16S rRNA Amplification

Extracted DNA templates were subjected to PCR using set (Forward and Reverse) of primers targeting 16S rRNA of isolates. PCR was performed with 27F-AGAGTTTGATCMTGGCTCAG and 1492R-AAGGAGGTGWTCCARCCGCA. The primers allowed amplification of the 16S rRNA genes of the isolates.The thermocycling conditions for initial denaturation was rapid thermal ramp to 96 °C, 94 °C for 5 min followed by denaturation at 94 °C for 30 sec. Anneallation temperature was at 56 °C for 30 sec, extension temperature at 72 °C for 45 sec. The final extension temperature was at 72 °C for 7 min, holding temperature was at 10 °C then 36 circles. Each of rapid thermal ramp to 96 °C, 96 °C for 10 sec, rapid thermal ramp to 50 °C, 50 °C for 5 sec, and rapid thermal ramp to 60 °C, 60 °C

for 4 min and finally rapid thermal ramp to 4 °C until ready for purification. Rapid thermal ramp used was 1 °C/sec.

2.4.5 Purifying Extension Products

The 48-well reaction plate was removed from the thermal cycler and briefly centrifuged. A 5 µL of 125 mM EDTA was added to each well. Then 60 µL of 100% ethanol to was also added to each well. The plate were sealed with aluminum tape and mixed by inverting 4 times and incubated at 37 °C for 15 min, then centrifuged at 1650 ×g for 45 min at 4 °C. The plates were inverted and centrifuged at 185×g then 60 µL of 70% ethanol was added to each well, centrifuged at 1650xg for 15 min at 4 °C. The plates were then inverted and centrifuged at 185×g for 1min and the samples were re-suspended in injection buffer. The product from the purification process was loaded on the 3130×l genetic analyzer (Applied Biosystems) to give the sequences. MEGA6 was used to view and analyze the obtained data.

Generating Consensus Sequence

The base were edited with MEGA6 software after which the reverse sequence in each case was made to complement the forward sequence by reverse complement. Then a pairwise alignment was carried out on both forward and reverse sequence and the consensus sequence was obtained from the aligned sequence. The consensus sequence was pasted on Blast at NCBI to obtain closely related strains.

Determination of antibiotic susceptibility

The organisms were tested for antibiotic susceptibility using the disk diuffusion method (Balouiri *et al*., 2016). The test organisms were subcultured in 5% blood agar for 24 h at 37 °C. The colonies were then inoculated in normal saline solution. The turbidity was then adjusted to equal 0.5 McFarland standard giving a final inoculum of 1.5×10^8 CFU/mL. About 100 mL of inoculum of test organism was spread on Mueller Hinton agar plate (Remel™, Thermo Fisher Scientific, USA). The standard antibiotic disks were then laid carefully on the agar followed by incubation at 37 °C for 24 h. Antibacterial activities were evaluated by measuring the diameters of zones of inhibition in mm against the test organism.

2.5 Statistical Analysis

The distribution of the *Listeria* species in the various meat types and parts were subjected toanalysis of variance (ANOVA) and Duncan multiple range test to determine means that differed (Ogbeibu, 2005).

3. Results and Discussion

The total viable counts of the *Listeria* isolates from all samples of beef and chicken is shown in Table 1. The viable counts of *Listeria* isolates in the beef samples ranged between 3.11×10^3 CFU/g to 1.36×10^4 CFU/g while for chicken samples it ranged between 1.56×10^3 CFU/g to 1.84×10^4 CFU/g. It was observed that the *Listeria* counts in chicken samples was higher than that of the beef.

Table 1 Mean viable counts of the *Listeria* isolates from frozen beef and chicken samples obtained from open markets in Benin City

Source Code	Total Viable Counts (CFU /g)	
	Beef samples	**Chicken samples**
MO_1	$4.56 \pm 0.85 \times 10^{3a}$	$1.84 \pm 0.45 \times 10^{4a}$
MN_2	$1.36 \pm 0.39 \times 10^{4b}$	$1.09 \pm 0.16 \times 10^{4b}$
MU_3	$1.22 \pm 0.31 \times 10^{4b}$	$3.00 \pm 0.75 \times 10^{3c}$
MA_4	$3.11 \pm 0.69 \times 10^{3a}$	$1.56 \pm 0.24 \times 10^{3c}$

Note: MO_1, MN_2, MU_3 and MA_4 are Oba market, New Benin market, Uselu market and Aduwawa market respectively.

Mean with different alphabets along vertical array indicates significant difference ($p < 0.05$)

Table 2 shows the phenotypic characteristics used for the identification of *Listeria* isolates in beef and chicken samples. The biochemical tests were: Gram's staining, motility test, catalase, oxidase, H_2S production and sugar fermentation. *Listeria*spp identified were: *L. monocytogenes, L. ivanovii, L. grayi and L. innocua.*

The frequency of occurrence of *Listeria* isolates in beef and chicken sample is shown in Table 3**.** It was observed that *L. monocytogenes* had the highest prevalence (34.78%) followed by *L. ivanovii* (30.43%), *L. grayi* (26.09%) and *L. innocua* (8.70%) in that order for beef. For chicken samples, *L. innocua* had the highest prevalence (31.82%), followed by *L. monocytogenes* (27.27%), *L. ivanovii* (22.73%) and *L. grayi* (18.18%) in that order. It was observed that *L. innocua* had the lowest prevalence of (8.70%) in beef samples while *L. grayi had the* lowest prevalence of (18.18%) in chicken samples as shown in Table 3.

Table 2 Cultural, morphological and biochemical characteristics of *Listeria* isolates in beef and chicken samples

Characteristics	LS1	LS2	LS3	LS4	LS5	LS6	LS7	LS8
Cultiral								
Margin	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth
Colour	greyish-black	greyish-black	greyish-black	bluish-green	bluish-green	greyish-green	greyish-green	cream
Shape	round	round	round	irregular	irregular	regular	regular	regular
Size	small	small	small	small	small	small	small	small
Morphological								
Gram's stain	+	+	+	+	+	+	+	+
Cell type	cocci	cocci	cocci	rods	rods	rods	rods	rods
Cell arrangement	Short chains	Short chains	Short chains	Short chains	Short chains	Short chains	Short chains	Short chains
Biochemical								
Motility	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+
Coagulase	-	-	-	-	-	-	-	-
H2S production	-	-	-	-	-	-	-	-
Sugar fermentation								
Glucose	+	+	+	+	+	+	+	+
Xylose	-	-	-	+	+	-	-	-
Mannitol	-	-	-	-	-	+	+	-
Rhamnose	+	+	+	-	-	+	+	-
OBIS	-	-	-	+	+	+	+	+
Beta haemolysis	+	+	+	+	+	-	-	-
Possible organism	*Listeria monocyte genes* 1	*Listeria monocyte genes* 2	*Listeria monocyte genes* 3	*Listeria ivanovii* 1	*Listeria ivanovii 2*	Listeria grayi 1	Listeria grayi 2	Listeria innocua

Note: KEY: + is positive, - is negative, LS1- LS8 are *Listeria* species

Table 3 Frequency of occurrence of Listeria isolates in frozen beef and chicken

Isolates	BEEF		CHICKEN	
	No. of isolates	Frequency (%)	No. of isolates	Frequency (%)
L. monocytogenes	8	34.78	6	27.27
L. ivanovii	7	30.43	5	22.73
L. grayi	6	26.09	4	18.18
L. innocua	2	8.70	7	31.82
	23	**100**	**22**	**100**

The antibiotic susceptibility pattern of eight (8) *Listeria* isolates screened for susceptibility to ten (10) antibiotics before curing is shown in Table 4 The results showed that 7 (87.5%) were resistant to ampiclox, 5 (62.5%) were resistant to amoxacillin, none (0%) were resistant to pefloxacin, 7 (87.5%) were resistant to septrin, none (0%) were resistant to gentamycin, 1 (12.5%) was resistant to zinnacef, 1 (12.5%) was resistant to rocephin, none (0%) were resistant to ciprofloxacin, 2 (25.0%) were resistant to streptomycin and 1 (12.5%) was resistant to erythromycin as shown in Table 4.

Table 4 Antibiotic sensitivity pattern of the *Listeria* isolates from frozen beef and chicken samples before curing

***Listeria* isolates**	**APX**	**AM**	**PEF**	**SXT**	**CN**	**Z**	**R**	**CPX**	**S**	**E**
L. ivanovii S1	R	R	I	R	S	S	S	S	I	S
L. grayi G1	R	I	S	R	S	S	S	S	S	S
L. monocytogenes M1	R	R	I	R	S	R	I	S	R	I
L. monocytogenes M2	R	R	I	R	S	I	I	S	S	S
L. grayi G2	R	I	I	R	S	I	S	S	I	S
L. monocytogenes M3	R	R	I	R	S	I	R	S	R	S
L. ivanovii S2	R	R	S	R	S	I	S	S	S	R
L. innocua	I	I	S	I	S	S	S	S	S	S

Note: 15 mm and above are regarded as sensitive (S), 11–14 mm are regarded as intermediate (I), 10 mm and below are regarded as resistant (R).

Key: R – Resistant, I – Intermediate, (S) – Sensitive, APX – Ampiclox 30 µg, Z – Zinnacef 20 µg, AM – Amoxacillin 30 µg, R – Rocephin 25 µg, CPX – Ciprofloxacin 10 µg, S – Streptomycin 30 µg, SXT – Septrin 10 µg, E – Erythromycin 10 µg, PEF – Pefloxacin 10 µg, CN – Gentamycin 10 µg.

Table 5 shows the antibiotic susceptibility pattern of eight (8) isolates screened for susceptibility to ten (10) antibiotics after curing. It was observed that plasmid curing increased *Listeria* sensitivity to test antibiotics. The number of isolates previously resistant to ampiclox was reduced from 7 to 3, amoxicillin was reduced from 5 to 1, septrin was reduced from 7 to 1 and streptomycin was reduced from 2 to 1. The only isolate resistance to erythromycin became sensitive after plasmid curing.

Table 5 Antibiotic sensitivity pattern of the *Listeria* isolates from frozen beef and chicken samples after curing

***Listeria* isolates**	**APX**	**AM**	**PEF**	**SXT**	**CN**	**Z**	**R**	**CPX**	**S**	**E**
L. ivanovii S1	S	S	S	I	S	S	S	S	I	S
L. grayi G1	R	S	S	I	S	S	S	S	S	S
L. monocytogenes M1	R	I	S	R	S	R	I	S	R	S
L. monocytogenes M2	I	I	I	I	S	S	I	S	S	S
L. grayi G2	S	S	S	S	S	I	S	S	I	S
L. monocytogenes M3	R	R	S	I	S	S	R	S	I	S
L. ivanovii S2	I	I	S	I	S	S	S	S	S	S
L. innocua	I	S	S	S	S	S	S	S	S	S

Note: 15 mm and above are regarded as sensitive (S), 11–14 mm are regarded as intermediate (I), 10 mm and below are regarded as resistant (R).

Key: R – Resistant, I – Intermediate, (S) – Sensitive, APX – Ampiclox 30 µg, Z – Zinnacef 20 µg, AM – Amoxacillin 30 µg, R – Rocephin 25 µg, CPX – Ciprofloxacin 10 µg, S – Streptomycin 30 µg, SXT – Septrin 10 µg, E – Erythromycin 10 µg, PEF – Pefloxacin 10 µg, CN – Gentamycin 10 µg.

Table 6 shows the multiple antibiotic resistance (MAR) and plasmid profile. The multiple antibiotic resistance (MAR) index of the isolates ranged from 0.2–0.5 with the highest being *L.*monocytogenes M1, *L.*monocytogenes M3, *L. grayi G1* and the lowest *L. innocua*. Two (2) of these isolates harbored plasmids with molecular weight ranging from 2500–5100 bp.

Table 6 Multiple antibiotic resistance **(**MAR**)** and plasmid profile

***Listeria* isolates**	**MAR**	**Number of plasmids**	**Plasmid size (bp)**
Listeria ivanovii S1	0.3	0	-
Listeria grayi G1	0.2	0	-
Listeria monocytogenes M1	0.5	1	5000
Listeria monocytogenes M2	0.3	0	-
Listeria grayi G2	0.2	0	-
Listeria monocytogenes M3	0.5	1	5000
Listeria ivanovii S2	0.4	0	-
Listeria innocua	0.0	0	-

Figure 1 shows the Plasmid profile of six (6) isolates on agarose gel electrophoresis stained with ethidium bromide. It was observed that *Listeria monocytogenes* Ci189 and *L. monocytogenes* Lm N1546, of different strains carried plasmid band with a molecular sizes of 5000 bp. The other isolates were plasmid negative. In plate 2 is shown the PCR products of 16S rRNA gene of *Listeria* isolates on agarose gel. Isolates (strains) that were sequenced include: *Listeria monocytogenes* Lm N1546, *L. monocytogenes* Ci189, *L. monocytogenes* LM 1296, *L. ivanovii subsp. Londoniensis* WSLC 30167, *L. ivanovii subsp. Ivanovii* WSLC 3010, *L. grayi* ATCC 25401, *L. grayi* H3506 and *L. innocua* ERULIS-61 and they had molecular weights of 1500bp.

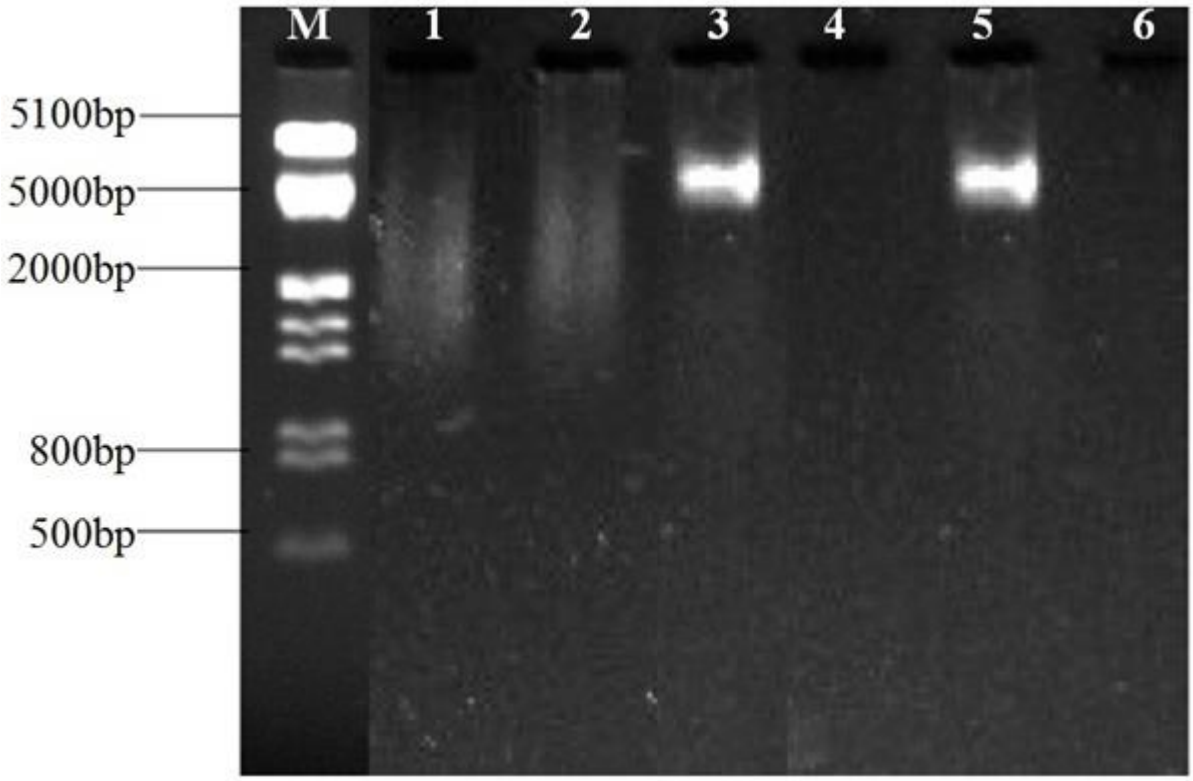

Figure 1 Plasmid Profiling of *Listeria* species stained with ethidium bromide

Lane 1 = *Listeria ivanovii subsp. Ivanovii* strain WSLC 3010, Lane 2 = *Listeria grayi* strain ATCC 25401, Lane 3 = *Listeria monocytogenes* strain Ci189, Lane 4 = *Listeria monocytogenes* strain LM 1296, Lane 5 = *Listeria monocytogenes* strain Lm N1546, Lane 6 = *Listeria grayi* strain H3506.

Table 7 shows the blast sequence of 16SrRNA gene of *Listeria* isolates with identity and accession numbers. The organisms had 87–94% identity with the blast sequence.

Table 7 Blast Sequence of I6s rRNA Gene

S/No.	Description	Query Length	Max. Score	Extension Total Score	Query Cover	E-Value	Percent homology	Accession No.
1.	*Listeria ivanovii subsp. ivanovii* strain WSLC 3010, complete genome	855	1192	7153	95%	0.0	93%	CP009577.1
2.	*Listeria grayi* strain ATCC 25401 16S ribosomal RNA gene, partial sequence	855	1210	1210	95%	0.0	94%	JN852815.1
3.	*Listeria monocytogenes* strain Ci189 16S ribosomal RNA gene, partial sequence	853	750	750	79%	0.0	87%	KM010020.1
4.	*Listeria monocytogenes* strain LM 1296 16S ribosomal RNA gene, partial sequence	846	754	754	58%	0.0	94%	KF588563.1
5.	*Listeria grayi* strain H3506 16S ribosomal RNA gene, partial sequence	851	1227	1227	97%	0.0	93%	NR_11 6351.1
6.	*Listeria monocytogenes* strain Lm N1546, complete genome	850	1214	7252	96%	0.0	93%	CP013724.1
7.	*Listeria ivanovii subsp. londoniensis* strain WSLC 30167, complete genome	847	1229	7363	95%	0.0	94 %	CP009575.1
8.	*Listeria innocua* strain ERULIS61 16S ribosomal RNA gene, partial sequence	851	1208	1208	95%	0.0	93%	KU208087.1

4. Conclusions

Listeriosis has been recognized to be one of the emerging zoonotic diseases during the last two decades and is contracted mainly from the consumption of contaminated food and meat products (Uyttendaele *et al.,* 1999). Beef and chicken meat have been frequently contaminated with *Listeria*speciesand may serve as vehicle of other pathogenic organisms. The frequent occurrence of *Listeria* sppin beef and chicken may pose a potential risk for consumers. Molla *et al*. (2004) reported that raw beef products showed a high level of contamination with *Listeria* species (50.6%). It is generally assumed that beef and chicken meats cannot be free from *Listeria* because of slaughter methods and food processing that allows greater chance for contamination.

Human infections primarily result from eating contaminated food and meat products which may lead to serious and potentially life-threatening listeriosis (El-Malek *et al.,* 2010). Increasing evidence suggests that substantial cases of human listeriosis are attributable to the food borne transmission of *L. monocytogenes (*Vitals *et al.,* 2004*).* It has been reported that *L. monocytogenes*was isolated from 12.2% of ground beef and 37% from minced chicken meat in Japan (Inou *et al.,* 2000). Furthermore Baek *et al. (*2000) observed a frequency of 30.2% in chicken meat in Korea and in Spain 32% (Capita *et al.,* 2001). Prevalence of Listeria in beef accounts for 2.6% in Ethiopia (Gebretsadik e*t al.,* 2011), 19% in Jordan (Awaisheh, 2010) and 52% in Canada (Bohaychuk *et al.,* 2006) respectively.

In this study, beef sold at New Benin market had the highest counts (1.36×10^4 CFU/g) while the lowest was Aduwawa market (3.11×10^3 CFU/g). Chicken sold at Oba Market had the highest total counts (1.84×10^4 CFU/g) while the lowest was Aduwawa market (1.56×10^3 CFU/g). The high counts found in beef and chicken could be as a result of high level of unhygienic practices by the abattoir workers, meat and chicken vendors (Akano *et al.,* 2013). The high total viable counts from markets such as Oba and New Benin markets could be attributed to improper cleaning and sanitizing of storage facilities and poor hygiene within the store and more importantly due to erratic power supply in this area. Microbial proliferation are known to occur with temperature abuse **(**Daniel *et al.,* 2015**).**

Contamination rates with *Listeria monocytogenes* were higher for beef than chicken. The acceptable level of *Listeria* species is 2.0×10^3 CFU/g in food and environmental samples (Ohue *et al.,* 2013). The concentration of *Listeria* species less than 100 CFU/g can be considered to be low risk to consumers, although the possibility of infection from low numbers, especially among the most susceptible population group (neonates, the elderly, pregnant women and the immunocompromised cannot be underestimated (Uyttendaele *et al.,* 2009; Ohue *et al.,* 2013). This result is in agreement with the results of other authors (Dhanashee

et al., 2003; Pesavento *et al.,* 2010) and suggests the presence of a significant public health hazard linked to the consumption of beef and chicken contaminated with *Listeria monocytogenes.*

The high occurrence of *L. monocytogenes*in raw meat is expected, because *Listeria monocytogenes*is ubiquitous in the natural environment (Vitals *et al*., 2004). Therefore, higher incidence of *Listeria* in chicken could be attributed to contamination caused by mincing machine, knives, chopping board, cleaning cloth, other working surfaces and human contact. Many of these pathogenic bacteria which are contaminants of frozen chicken may get in during production and processing through sources such as the air, contaminated water, soil, processing surfaces, processing equipment, and during distribution (Ikeh *et al.,* 2010).

Moreover, *L. Monocytogenes* has been strongly implicated particularly in the contamination of beef and chicken meat stored at low temperatures. Storage of meat products at low temperature conditions may allow the growth of significant numbers of *Listeria*spp leading to food-borne illnesses among consumers. People handling beef and chicken at different levels can also be sources of contamination. In this study, a total of four (4) *Listeria* species were isolated and characterized from frozen beef as *Listeria monocytogenes (*34.78%), *L. grayi (*30.43%*), L. ivanovii (*26.09%*) and L. innocua (*8.70%*)*. Four (4) *Listeria* species were also isolated and characterized from frozen chicken samples as *L. monocytogenes (*27.27% *), L. grayi (*22.73%*), L. ivanovii (*18.18%*) and L. innocua (*31.82%*)*. This finding is similar to the reports of other studies which reported a 30–70% prevalence of *Listeria monocytogenes* in meat products (Dhanashee *et al.*, 2003; Vitals *et al.*, 2004). However, other studies have shown a lower incidence of the pathogen in meats (5% and 17%) (Rorvik *et al.*, 2001; De Simon *et al.*, 2000). It is necessary to improve hygiene and provide adequate storage conditions from slaughter houses through the beef and chicken vendors to avoid growth of the pathogen to high levels, because cross contamination represents the major factor in the introduction of *Listeriamonocytogenes* to meats products **(**Daniel *et al.,* 2015**).** The importance of frozen beef and chicken as a vehicle for the transmission of various diseases, especially in countries where hygienic standards are not strictly enforced has been well documented (Liu *et al*., 2007).

*L. monocytogenes (*34.78%*)* was the predominant isolate from the beef samples and *L. innocua (*31.82%*)* from chicken samples while other *Listeria* species were less common. The result agrees with (Okutani *et al.,* 2004). These authors stated that *L. monocytogenes*and *L. innocua*are most often reported with most investigators reporting one or the other to be most predominant followed by other species among five related species. The incidence rate of 27.27% of *L. monocytogenes* from chicken samples observed in this study is high compared to

prevalence rate of 13% observed from poultry products by Lawan *et al.* (2013). However, prevalence rate as low as 13.6% were recorded by Alsheikh *et al*. (2013) in retail broiler chickens.

The results of this study reveals that the *Listeria* spp were highly resistant to amoxicillin, ampiclox and septrin but susceptible to gentamycin and ciprofloxacin. This finding corroborates the reports of Ennaji *et al.* (2008) who observed high susceptibility of *Listeria* isolates to gentamycin and ciprofloxacin. Safdara and Armstrong (2003) and Chukwu *et al.* (2006) had observed a continuing pattern of emergence of strains of *Listeria* spp isolated from food and clinical cases of listeriosis which are resistant to one or more antibiotics. The complete resistance shown by amoxicillin and ampicloxindicates that, these widely used drugs are ineffective in the treatment of listeriosis. This is similar to reports from a study where environmental isolates were shown to be resistant to amoxacillin and ampiclox (Daniel *et al*., 2015). The resistance to antibiotics can be due to selective antibiotic pressure (Hanchung *et al.,* 2004).

The choiced drug for treating listeriosis is a β-lactam (ampicillin or penicillin) alone or combined with an aminoglycoside (gentamicin) (Ramaswamy *et al*., 2007). However, all confirmed *Listeria* isolates found in this study were sensitive to ciprofloxacin, erythromycin, gentamicin and pefloxacin.

The emergence of antibiotic resistant isolates from poultry products is of medical and public health importance because of the ability of the organisms to interact with man, either through contact or consumption (Hanchun *et al*., 2004), thus initiating listeriosis, which may defy almost all the antibiotic chemotherapy. Although the incidence of antibiotic resistance is currently low, the range of antibiotics to which resistance has been acquired is wide. It is of concern that this expanding range now includes a number of first choice antibiotics such as penicillin, ampicillin, tetracycline and gentamycin used in the treatment of listeriosis. Results from this study showed that *Listeria monocytogenes*strains are susceptible to the first choice antibiotics used in the treatment of listeriosis which are erythromycin and gentamycin. Also *Listeria* spp were susceptible to septrin, pefloxacin used as second choice antibiotics in the treatment of listeriosis especially in patients allergic to penicillin. All *Listeria* strains were resistant to amoxicillin and ampiclox. The widespread use of ampiclox in human and veterinary therapy, alongside the length of time over which it has been available in Nigeria and other countries of the world could account for this trend. It is known that *L. monocytogenes* can either acquire or transfer antibiotic resistances' genes from plasmid and transposons of other bacterial species including *Enterococcus* spp either *in vivo* or *in vitro* in the intestinal tract (Pourshaban *et al.,* 2002). This is very important because bacteria that acquire new resistance

are not disrupted by antibiotics during a therapy. The multiple antibiotic resistance (MAR) index of the isolates whichranged from 0.2–0.5 shows that plasmids carrying antibiotic resistant genes can lead to the possible emergence of antibiotic resistant strains. This may also affect the choice of optimal initial therapy for severe listeric infection especially in compromised individuals (Safdar and Armstrong, 2003; Akano *et al*. 2013).

Molecular characterization of the *Listeria* isolates (strains) sequenced include: *Listeria monocytogenes* Lm N1546, *Listeria monocytogenes* Ci189, *Listeria monocytogenes* LM 1296, *Listeria ivanovii subsp. londoniensis* WSLC 30167, *Listeria ivanovii subsp. ivanovii*WSLC 3010, *Listeria grayi* ATCC 25401, *Listeria grayi* H3506 and *Listeria innocua* ERULIS-61. In 2005 and 2011, Switzerland experienced two listeriosis outbreaks linked to the consumption of soft cheese and imported cooked ham products, respectively, which were contaminated with *Listeria monocytogenes* LM 1296 and *L. ivanovii*strains (Taurai *et al.,* 2016). *Listeria innocua* strains was isolated from a 62 years old woman in a hospital in France (Monique (2003). It was reported that cases of *Listeria monocytogenes* strain Ci189 was found in blood sample of neonates (Montero, 2015). *Listeria grayi* (ATCC 25401) was isolated from food products of animal, plant, and fish origin (Rapose, 2008). The majority of the listeriosis cases occur in elderly, immunosuppressed patients, pregnant women and neonates. Although the symptoms of listeriosis in pregnant women are often mild, there is a high risk of trans-placental transmission causing miscarriage, congenital defects, preterm birth and even stillbirth (Montero, 2015).

This study has confirmed a high prevalence of *Listeria* species in frozen beef and chicken sold in Benin City, Nigeria and resistance to amoxicillin, ampliclox and septrin. Thus the isolation of *Listeria* species in poultry and beef pose a health risk for high-risk individuals. Beef and chicken must be thoroughly cooked or grilled before consumption to eliminate *Listeria* spp. The study underscores the need for implementation of proper hygienic and sanitary measures during slaughtering, skinning and evisceration operations to prevent contamination of beef and chicken with *Listeria* species.

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