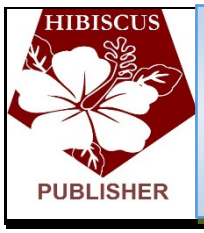
JOBIMB, 2024, Vol 12, No 1, 90-96
https://doi.org/10.54987/jobimb.v12i1.967



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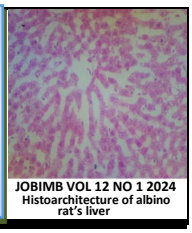

Detection of Haemolysin and Tetracycline Resistance Genes in *Listeria ivanovii* isolated from Foods

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HISTORY

Received: 7th April 2024
Received in revised form: 21st June 2024
Accepted: 30th July 2024

KEYWORDS

Antibiotic
Food
Listeriosis
Resistance Gene
Virulence

ABSTRACT

Listeria ivanovii is an important pathogen that is associated with foodborne diseases. It is responsible for listeriosis in animals, and sporadic cases are present in humans. Drug resistance and virulence among pathogens has been a global health management challenge. This research examined Listeria ivanovii from a few food items for the presence of haemolysin and tetracycline resistance gene. Twenty-five samples each of beef, chicken, lettuce, and cabbage were gathered from four separate locations, for a total of four hundred food samples. The percentage of *Listeria ivanovii* isolates that were resistant to ampicillin, oxacillin, tetracycline, and clindamycin was 25, 53, 22, and 31%, respectively. *Listeria grayi* was 23% resistant to cefoxitin, clindamycin and oxacillin. *Listeria welshimeri* isolate was not resistant to any of the isolates. Two isolates of *Listeria grayi* and sixteen isolates of *Listeria ivanovii* were found to be multidrug resistant. The PCR amplification showed that 14.3% of the isolates of *L. ivanovii* among the selected isolates harboured haemolysin (*hylA*) gene, 28.6% of isolates harboured *tetA* gene and 42.9% isolates had *tetM* gene.

INTRODUCTION

The ingestion of spoiled foods has been a prominent source of pathogenic microorganisms, resulting in a worldwide rise of foodborne illnesses and antibiotic-resistant pathogens, which are severe hazards to public health. as reported by Eruteya *et al*. [1]. *Listeria* species as other food acquired pathogens have also been found to be responsible for foodborne diseases and outbreaks in the globe [2, 3]. The virulence of *Listeria* species in contaminated foods has been a major factor in their pathogenicity and has caused severe infections in individuals with weakened immune systems. Several virulence factors are important in the pathogenicity of *Listeria* species [4]. Some of these virulence factors include: haemolysin protein (*hlyA*), actin polymerization protein (*actA*), invasive associated protein (*iap*), pleiotropic activator protein, phosphatidylinositol phospholipase (*plcA*), exotoxin listeriolysin (LLO) protein, zinc metalloproteinase precursor, glycerealdehydes-3-phosphate protein (*gap*), peptidoglycan N-acetyl glucosamine protein and surface components (proteins) [2, 5]. Studies have provided sufficient evidence that *Listeria* species are resistant to various antibiotics such as rifampin, chloramphenicol, tetracycline, oxacillin, cefotaxime, ampicillin, erythromycin, gentamycin, and vancomycin [6]. *Listeria* species are becoming increasingly resistant to antibiotics, to the point where almost all antibiotics will no longer work due to different strategies these pathogens use to withstand the therapeutic agents. [7].

Since antibiotic resistance may result in the failure of therapeutic treatment attempts, the overuse or indiscriminate use of antibiotics on *Listeria* species is a serious public health concern. The screening for virulence and resistance genes in pathogens from foods is of importance to populace since antibiotic resistance of pathogens is of significant public health concern as reported by Bakshi *et al*. [8] and Duma *et al*. [4]. Therefore, it is essential to conduct this research because it has been discovered that meats and vegetables consumed globally are a significant and reliable source of *Listeria* species.

MATERIALS AND METHODS

Study Area

This study was conducted in Kaduna State, the North central part of Nigeria. Kaduna State is situated at 9° 03' N and 11° 32' N of equator and longitudes 6° 05' E and 80° 38'E of the Greenwich meridian. The Taiwanese-made Etrex® high-sensitivity geographic positioning system (GPS) receiver was used to pinpoint the study areas.

Determination of Sample Size

The sample size was determined using prevalence rate of 37.75% as reported by Eruteya *et al*. [1] in Port Harcourt. To calculate the sample size, the following formula was employed:

$$n = \frac{Z^2P(1-P)}{d^2}$$

n = total number of sample collected
p = prevalence rate of distribution of previous study = 37.75% = 0.3775
Z = standard normal distribution at 95% confident limit = 1.96
d = absolute desired precision of 5% = 0.05

Hence,

$$n = \frac{1.96^2 \times 0.3775\ (1-0.3775)}{0.05^2} = 361$$

The sample size for this research was increased to four hundred (400) samples for uniform distribution and collection of the samples.

Collection of food samples

A total of 400 food samples consisting of 25 samples of beef, chicken, lettuce, and cabbage from Samaru, Sabon Gari, Kakuri and Ungwan Boro markets were collected randomly. To be analysed, each sample was delivered to the ABU Zaria Department of Microbiology in an ice-packed sampling box.

Sample preparation, isolation and identification of *Listeria* species

Twenty-five gram of homogenised food sample was inoculated in 225 mL of buffered bacteriological peptone water and incubated for 24 h at ambient temperature [9]. A 24-hour incubation period was conducted at 37°C after 25 millilitres of the buffered food sample were inoculated in 225 millilitres of Listeria selective enrichment broth medium containing listeria enrichment supplement at a ratio of 1:10. Listeria selective agar plates containing listeria selective enrichment supplement and listeria differential supplement were sub cultured with a loop full of the culture from the listeria enrichment broth and incubated at 37°C for 24 h.

Conventional biochemical tests (Gramme staining, catalase, oxidase, hemolysis, indole, motility, and MR-VP) were used further to identify the colonies from the listeria selective agar. All of the isolates were verified using the listeria microgen identification system and polymerase chain reaction.

Genomic DNA extraction

The genomic DNA was extracted using a Bioneer extraction kit (South Korea). Briefly, a 24 h culture was carefully removed from a medium and suspended into 200 µL of 1 × PBS (phosphate buffered saline) at the concentration of 10^6 and was centrifuged for 5 minutes at 3000×g in a tube. Without disturbing the pellet, the supernatant was cautiously disposed of. After the pellet was re-suspended in 200 µL of 1×PBS, 10 µL of RNase A, 20 µL of proteinase K, and thorough mixing were added.

The mixture was incubated in a tube for 2 minutes at room temperature. Two hundred microlitres of GB buffer was added to the sample and was mixed immediately using vertexing mixer. The mixture was incubated for 10 minutes at 60°C using a multi block heater. Four hundred microliters of absolute ethanol were added and mixed well by pipetting. The lysate was carefully transferred into the upper reservoir of the binding column tube (fitted in a collection tube). The tube was centrifuged at 8000 rpm for 1 minute; the solution from the collection tube was discarded and the collection tube was reused.

Five hundred microlitre of WA1 buffer was added, the tube was centrifuged at 8000 rpm for 1 minute; the solution was discarded from the collection tube, and the collection tube was reused. Five hundred microliter of W2 buffer was added and centrifuged at 8000 rpm for 1 minute; then it was centrifuged once more at 13000 rpm for 1 minute to completely remove ethanol. The 1.5 mL tube used for the binding column was transferred to a new tube for elution. The binding column tube was then filled with 50 microlitres of EA buffer and left to stand at room temperature for one minute. The DNA was extracted by centrifuging it for a minute at 8000 rpm.

Identification using Polymerase chain reaction

The PCR reaction was carried using amplification of *Liv* gene (370bp) which code for putative glycosidase. The procedure outlined by Miladi *et al*. [10] was followed when carrying out the reaction. The primers used for the amplification of genes are listed in **Table 1**. The reaction mixture (20 µl) contained 2.0 µl of both the forward and reverse primers (10 pmol/µl), 0.8 µl dNTP (10 mM), 3.0µl of 10× Taq polymerase (Prome®, USA), 1.8 µl of $MgCl_2$ (Promega®, USA), 0.1 µl of Taq polymerase (5 U/µl) (Prome®, USA), 2.0 µl of genomic DNA and the final volume was adjusted to 20 µl by adding the remaining volume of nuclease-free water.

Amplification proceeded which thermocycler (Gene AMP® PCR System 9700, AB Applied Biosystem, Singapore) with initial denaturation at 94°C for 5 minutes, followed by 25 cycles of denaturation at 95°C for 1 minute annealing temperature of primers was 52°C for 1 minute and extension at 73°C for 1 minute and the last extension for ten minutes at 72°C. 4.0mmol/l, Tris 1mmol/l EDTA, pH 8.0 The 10 µl amplified PCR products were separated using 1.5% agarose gel electrophoresis (Amresco Bioscience, USA) with Tris acetate electrophoresis buffer (TAE, 4.0 mM, Tris 1 mM EDTA, pH 8.0) at 105 V for 35 minute the gel was stained with ethidium bromide and finally visualised under UV transilluminator (BIO-RAD Molecular Imager Gel Doc™ Canada). The image was viewed in a computer for interpretation.

Table 1. Sequence of oligonucleotide primers used in the amplification of *Liv*, *hlyA*, *tetA* and *tetM* genes.

Target gene	Primer sequence	Size(bp)	Reference
Liv gene	5'-GCT GAA GAG ATT GCG AAA GAA G-3' 3'-CAA AGA AAC CTT GGA TTT GCG G-5'	370	[12]
hlyA	5'-CCT AAG ACG CCA ATC GAA-3' 3'-AAG CGC TTG CAA GTC CTC -5'	702	[11]
tetA	5'GGC GGT CTT CTT CTT CAT CAT GC3' 3'CGG CAG GCA GAG CAA GTA GA5'	501	[13]
tetM	5'-GTG GAC AAA GGT AGA ACG AG-3' 3'-CGG TAA AGT TCG TCA CAC AC-5'	406	[14]

Polymerase chain reaction detection of haemolysin A gene
The purpose of the PCR reaction was to detect the presence of hlyA gene (720 bp). The reaction was carried out using the specified method as described by Usman *et al*. [11]. The reaction mixture (20 µl) for multiplex PCR contained 2.0µl of dNTPs (200µm/µl), 2.5 of 10× Taq buffer, 1.5 µl of 25-mM $MgCl_2$, 0.5 µl of each of oligonucleotide primers of *tetA* and *tetM* gene (25pm/µl) (reverse and forward), 0.75 Taq DNA polymerase (3 U/µl) (Promega®, USA), 5.0µl of the template DNA (30 ng/µl) and the volume was make up to 20 µl using nuclease free water.

A thermocycler (Gene AMP® PCR System 9700, AB Applied Biosystems, Singapore) was used to carry out the amplification. The initial denaturation was at 94ºC for 5 minutes, followed by 30 cycles of denaturation at 94ºC for 45 sec, annealing temperature of primers was 55ºC for 1 minute and extension at 72ºC for 1 minute and final extension at 72ºC for 5 minutes. After gel filtration in 1.5% agarose gel containing 0.5µg/mL ethidium bromide at 105V for 35 minutes, the amplified PCR products (10 µl) were finally visualised under a UV transilluminator. (Bio-Red Molecular Imager® Gel Doc™ Canada). The image was viewed in a computer for interpretation.

Standardization of inoculant
The preparation process involved suspending a 24 h isolate colony using a wire loop into a sterile test tube filled with sterile normal saline to achieve a turbidity level that matched the 0.5 scale of McFarland's standard. (1.5×10^8 cells/mL) [15].

Antibiotic susceptibility test
The Kirby-Bauer-NCCLS modified single disc diffusion technique was used to determine the antibiotic susceptibility pattern. [16]. Using a sterile swab stick, the standardised innocula were streaked onto Mueller-Hinton agar that had been prepared. The antibiotic disc was then aseptically placed on the inoculated medium using sterile forceps, and the mixture was incubated at 37°C for a full day.

The zones of inhibition were measured, the outcomes were interpreted in accordance with the CSLI guidelines [17], and all of the data were accurately recorded. Single antibiotic discs such as ampicillin (10 µg), vancomycin (30 µg), tetracycline (30 µg), clindamycin (2 µg), erythromycin (15 µg), ciprofloxacin (5 µg), rifampicin (5 µg), gentamycin (30 µg), amoxicillin (30 µg), chloramphenicol (30 µg), cefoxitin (30 µg) and oxacillin (1 µg) from Oxoid (England) each with expire date of 4th February, 2021 were used.

The Determination of Multiple Antibiotic Resistance (MAR) index
The formula MAR = X/Y, where X is the number of antibiotics to which the test isolates showed resistance, and Y is the total number of antibiotics to which the test organism has been assessed for sensitivity, was used to determine multiple antibiotic resistance [18].

Detection of *tetA* and *tetM* Genes using Multiplex Polymerase Chain Reaction
Multiplex PCR reaction was carried out to detect the presence of *tetA* (501bp) and *tetM* (406 bp) genes in selected isolates of *Listeria ivanovii* that phenotypically exhibited resistance to tetracycline as described by Olowe *et al*. [13] using the forward and reverse primers.

The reaction mixture (25 µl) for multiplex PCR contained 2.0 µl of dNTPs (200 µm/µl), 2.5 of 10× Taq buffer, 1.5 µl of 25-mM $MgCl_2$, 0.5 µl of each of oligonucleotide primers of *tetA* and *tetM* gene (25 pm/µl) (reverse and forward), 0.75 Taq DNA polymerase (3U/µl) (Promega®, USA), 5.0 µl of the template DNA (30 ng/µl) and the volume was make up to 25 µl using nuclease-free water. A thermocycler was used to perform the amplification (Gene AMP® PCR System 9700, AB Applied Biosystems, Singapore) with initial denaturation at 94ºC for 5 minutes followed by 30 cycles of denaturation at 94ºC for 45 sec. Primers were annealed at 55ºC for 1 min and extension at 72oC 1 min, then extended again for five minutes at 72 degrees. The amplified PCR products (10 µl) were resolved by electrophoresis in 1.5% agarose gel (containing 0.5µg/mL ethidium bromide) at 105V for 35 minutes and finally visualized under UV transilluminator (Bio-Red Molecular Imager® Gel Doc™ Canada). The image was viewed in a computer for interpretation.

Detection of Haemolysin A (hlyA) Gene using Polymerase Chain Reaction
The PCR reaction was performed to detect the presence of *hlyA* gene (720 bp). The reaction was carried out using the Usman *et al*. [11] method. The reaction mixture (20 µl) for multiplex PCR contained 2.0µl of dNTPs (200 µm/µl), 2.5 of 10× Taq buffer, 1.5 µl of 25-mM $MgCl_2$, 0.5 µl of each of oligonucleotide primers of *tetA* and *tetM* gene (25pm/µl) (reverse and forward), 0.75 Taq DNA polymerase (3U/µl) (Promega®, USA), 5.0 µl of the template DNA (30 ng/µl) and the volume was make up to 20 µl using nuclease-free water.

Amplification of the gene sample was carried out with a Gene AMP® PCR thermocycler (Gene AMP® PCR System 9700, AB Applied Biosystems, Singapore) with initial denaturation at 94ºC for 5 minutes, followed by 30 cycles of denaturation at 94ºC for 45 sec. The annealing temperature of primers was 55oC for 1 minute, the extension temperature was 72oC for 1 minute, and the final extension was 72ºC for 5 minutes. The amplified PCR products (10µl) were resolved by electrophoresis in 1.5% agarose gel (containing 0.5µg/mL ethidium bromide) at 105V for 35 minutes and finally visualized under UV transilluminator (Bio-Red Molecular Imager® Gel Doc™ Canada). The image was viewed in a computer for interpretation.

RESULTS

Table 2 displays the phenotypic characterisation of Listeria species using a Microgen kit based on the isolates' sugar fermentation capacities. *Listeria ivanovii* were identified as follows: ten had an octal code of 5752 and a percentage probability identification of 99.03%; nine had an octal code of 5643 and a percentage probability identification of 95.31%. At 95.91%, five isolates of *L. ivanovii* with an octal code of 4657 were found. The percentage probability of identification for two *L. ivanovii* isolates and two additional isolates was 99.96% (octal code 4454) and 99.87% (octal code 4645), respectively. Using the octal codes 5757, 4641, 4653, and 5756, respectively, other isolates of *L. ivanovii* were found to be 99.79%, 99.79%, 98.23%, and 97.15. The octal codes for four (4) *Listeria grayi* isolate and two isolates of the same species were determined to be 96.47% and 99.55%, respectively. With an octal code of 4561, a single instance of *Listeria welshimeri* was detected with a 94.32% probability.

Table 2. *Listeria* species phenotypic characterisation with a Microgen kit.

S/No.	Octal code generated from microgen identification system	Percentage identification	Organism inference (No.)
1	5752	99.03	*L. ivonvii* (10)
2	5643	95.31	*L. ivonvii* (9)
3	4657	95.91	*L. ivonvii* (5)
4	4454	99.96	*L. ivonvii* (2)
5	4646	96.47	*L. grayi* (4)
6	5757	99.79	*L. ivonvii* (1)
7	4641	98.23	*L. ivonvii* (1)
8	4653	97.21	*L. ivonvii* (1)
9	4645	99.87	*L. ivonvii* (2)
10	5756	97.15	*L. ivonvii* (1)
11	4643	99.55	*L. grayi* (2)
12	4561	94.31	*L. welshimeri* (1)

KEY: Esc = Esculin, Man = Mannitol, Xyl = Xylose, Ara = Arabitol, Rib = Ribose, Rha = Rhamnose, Tre = Trehalose, Tag = Tagatose, G-1-P = Glucose-1-Phosphate, M-D-Glu = Methyl-D-Glucose, M-D-Man = Methyl-D-Mannose, Hae = Haemolysin.

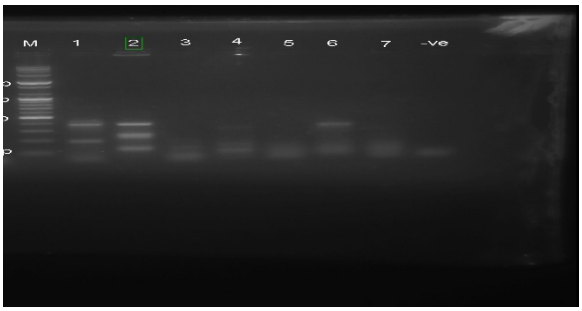

Fig. 1. Agarose gel electrophoresis of amplified *Liv* gene (370 bp) isolates from the food samples. Lane M: DNA lader (100bp), Lane 1, 2, 4 and 6 were the positive isolates, -ve: negative control.

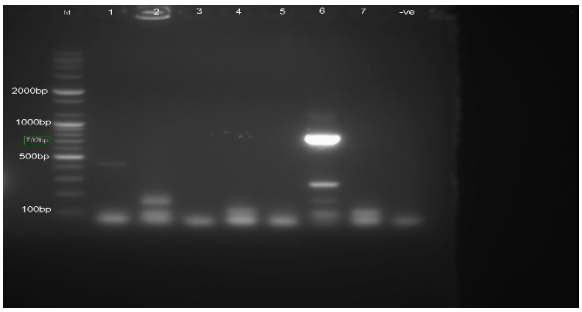

Fig. 2. Agarose gel electrophoresis of amplified *hylA* gene products (720 bp) from some *Listeria ivanovii* isolates. M: DNA lader (100 bp); -ve: negative control; Lane 6 shows the presence of *hylA* gene; Lanes 1, 2, 3, 4, 5 and 7 shows the absence of *hylA* gene.

The agarose gel electropherogram showed the amplification of the 370 bp *Listeria ivanovii* gene. A total of four isolates (57.1%) had amplicon indicated in lanes 1, 2, 4 and 6 as presented in **Fig. 1**. Out of the seven isolates of *Listeria ivanovii* considered for the screening for virulence genes using polymerase chain reaction, only 1(14.3%) isolate from beef at lane 6 had haemolysin gene (*hlyA*) with amplicon size of 720bp as presented in **Fig. 2**. Isolates from chicken (lanes 1-3), lettuce (lanes 4 and 5) and one of the beef samples (lane 7) had no haemolysin (*hlyA*) gene. **Table 3**'s antibacterial susceptibility analysis demonstrated that the isolates of *Listeria ivanovii* and *Listeria grayi* from the chosen food samples were 100% susceptible to ciprofloxacin and rifampicin.

The only isolate of *Listeria welshimeri* was susceptible to ampicillin, tetracycline, amoxicillin/clavulanate, cefoxitin, chloramphenicol, vancomycin, gentamycin, and ciprofloxacin. *Listeria welshimeri* was not resistant to any of the antibiotics selected for this research. *Listeria ivanovii* isolates were resistant to ampicillin (25%), tetracycline (22%), erythromycin (12%), cefoxitin (19%), amoxicillin/clavulanate (16%), clindamycin (31%), gentamycin (6%) and oxacillin (53%). *Listeria grayi* isolates were 17% resistant to ampicillin and chloramphenicol; 33% of the *Listeria grayi* were resistant to cefoxitin, clindamycin and oxacillin.

Table 4. Antibiotic resistance patterns of the *Listeria* species.

	Listeria ivanovii		*Listeria grayi*	
S/N	Pattern	Frequency	Pattern	Frequency
1	DA	3	DA	1
2	TE	3	FOX, DA, OX	1
3	DA, OX	4	AMP, C, FOX, OX	1
4	E, DA,	1		
5	AMP, FOX	1		
6	AMP, AMC	1		
7	OX, FOX, E	1		
8	OX, AMP, DA	1		
9	AMP, AMC, TE	1		
10	OX, FOX, E	1		
11	CN, DA, E, TE	1		
12	OX, TE, CN, AMP	1		
13	AMC, AMP, OX, FOX	2		
14	FOX, OX, AMC, AMP, TE	1		

KEY: AMP = Ampicillin, TE = Tetracycline, E = Erythromycin, AMC = Amoxicillin/Clavulate (Clavulanic acid), FOX = Cefoxitin, C = Chloramphenicol, VA = Vancomycin, DA = Clindamycin, CN = Gentamycin, OX = Oxacillin

The resistance patterns of the *Listeria* species are presented in **Table 4** and the percentage multidrug resistance among the isolates is presented in **Table 5**. This indicated that three isolates of *Listeria ivanovii* were resistant to clindamycin; seven isolates were resistant to two different antibiotics. Four isolates were resistant to three different antibiotics, another four isolates were resistant to four different antibiotics, and one was resistant to five different antibiotics. One of the isolates of *Listeria grayi* was resistant to clindamycin, another isolate was resistant to three antibiotics, and the other isolate was resistant to four antibiotics.

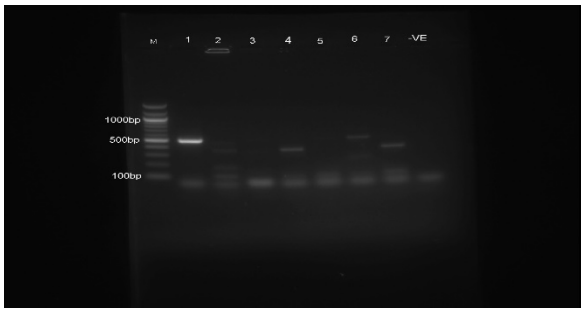

Fig. 3. Agarose gel electrophoresis of amplified *tetA* gene products (501bp) from representative *Listeria ivanovii* isolates. M: DNA marker (100 bp); -ve: negative control; Lane 1 and 6 show the presence of *tetA* gene; Lanes 1, 2, 3, 4, 5 and 7 show the absence of *tetA* gene.

The multiple antibiotic resistant (MAR) indices of the *Listeria* spp as shown in **Table 6** indicate that; six, five, three and two isolates of *Listeria ivanovii* had MAR index of 0.17, 0.25, 0.33 and 0.41 respectively. The MAR index for one of the *L. grayi* isolates was 0.25, while the MAR index for the other isolate was 0.33

Table 3. The antimicrobial susceptibility (antibiogram) of *Listeria* species that were isolated from the foods.

S/N	Antibiotics	*Listeria ivanovii* (n=32)			*Listeria grayi* (n=6)			*Listeria welshemeri* (n=1)		
		R(%)	I(%)	S(%)	R(%)	I(%)	S(%)	R(%)	I(%)	S(%)
1	Ampicillin (10 µg)	8(25)	-	24(75)	1(17)	-	5(83)	0(0)	-	1(100)
2	Tetracycline (30 µg)	7(22)	3(9)	22(69)	0(0)	1(17)	5(83)	0(0)	0(0)	1(100)
3	Erythromycin (15 µg)	4(13)	7(22)	21(66)	0(0)	2(33)	4(67)	0(0)	1(100)	0(0)
4	Amoxicillin/Clavulate (30 µg)	5(16)	-	27(84)	0(0)	-	6(100)	0(0)	-	1(100)
5	Cefoxitin (30 µg)	6(19)	-	26(81)	2(23)	-	4(3)	0(0)	-	1(100)
6	Chloramphenicol (30 µg)	0(0)	5(16)	27(84)	1(17)	0(0)	5(83)	0(0)	0(0)	1(100)
7	Vancomycin (30 µg)	0(0)	2(6)	30(94)	0(0)	0(0)	6(100)	0(0)	0(0)	1(100)
8	Clindamycin (2 µg)	10(31)	11(34)	11(34)	2(33)	4(67)	0(0)	0(0)	1(100)	0(0)
9	Gentamycin (10 µg)	2(6)	0(0)	30(94)	0(0)	0(0)	5(100)	0(0)	0(0)	1(100)
10	Oxacillin (1 µg)	17(53)	-	15(47)	2(33)	-	4(67)	0(0)	-	1(100)
11	Ciprofloxacin (5 µg)	0(0)	0(0)	32(100)	0(0)	0(0)	6(100)	0(0)	0(0)	1(100)
12	Rifampicin (5 µg)	0(0)	0(0)	32(100)	0(0)	0(0)	6(100)	0(0)	1(100)	0(0)

Fig. 3 presents the results of multiplex PCR amplified tetA (501 bp) and tetM (406 bp) gene products electrophoresed on agarose gel in some of the isolates. Of the seven isolates of *Listeria ivanovii* considered for the screening for *tetA* and *tetM* genes, isolates from chicken and beef at lanes 1 and 6, respectively, had *tetA*-resistant genes, and isolates from chicken, lettuce, and beef at lanes 2, 4 and 7, respectively harboured *tetM* gene. The distribution of *tetA*, *tetM* and *hlyA* genes among the selected *Listeria ivanovii* indicate that 2(28.6%) had *tetA* gene, 3(42.86%) had *tetM* gene, and 1(14.3%) had *hlyA* gene (**Table 7**).

Table 7. Distributions of *tetA*, *tetM* and *hlyA* genes in *Listeria ivanovii* isolated from the food samples.

Sample type	No. of Isolates Examined	No. of positive *hlyA* gene (%)	No. of positive *tetA* gene (%)	No. of positive *tetM* gene (%)
Beef	2	1(50)	1(50)	1(50)
Chicken	3	0(0.0)	1(33.3)	1(33.3)
Lettuce	2	0(0.0)	0(0.0)	1(50)
Total	7	1(14.3)	2(28.6)	3(42.9)

DISCUSSION

The antibiotics that showed considerable antibiotic resistance in this research were tetracycline, ampicillin, amoxicillin, oxacillin, erythromycin, clindamycin, cefoxitin and gentamycin, which are extensively used in Nigeria. Excessive exposure of the *Listeria* species to these antibiotics through agriculture and veterinary medicine practices might have led to pathogens' resistance to some of the selected antibiotics. This can eventually lead to the distribution of antibiotic-resistant pathogens in foods and the environment [19]. These antibiotics can provide selective pressure, leading to a higher prevalence of resistant bacteria as reported by Manyi-Loh *et al*. [20] and Muteeb *et al*. [21].

The presence of antibacterial-resistant *Listeria* species in the food samples is of crucial public health implication since there is frequent and uncontrolled use of antibiotics in the study area, as reported by Zeinali *et al*. [12]. Antibiotic resistance poses a serious threat to both humans and animals because it impedes the efficacy of treatment measures and allows antibiotic resistance genes to spread horizontally among different bacteria [22, 23]. Foodborne pathogens including *Listeria* species are showing trends toward resistance to standard available therapies.

According to reports by Aras and Ardic [24] and Tola [25], antibiotic-resistant *Listeria* species have been found in food environments and occasionally occurring human listeriosis cases. Multiple antibiotic resistance (MAR) index value lower than 0.20 indicates that these organisms might have originated from a lower-risk source where the antibiotics are seldom or never used [26]. The results of this study indicate that all isolates of *Listeria* spp. were from higher-risk sources and had frequent exposure to antibiotics, as noted in the MAR index values of more than 0.20 for each isolate [26,15]. Multiple antibiotic resistance (MAR) of *Listeria* species in vegetables could result from the usage of animal waste as fertilizer, which might contain antibiotic residues used to prevent or treat animal diseases and promote animal growth, as reported by Okpo *et al*. [27]. Therefore, antibiotics can be migrated from organic manure to soil and from soil to vegetables and groundwater; bacteria resist these antibiotic residues in the environment. The wide variation of antibiotic resistance patterns between the isolates of *Listeria* species may also be due to their exposure to different antibiotics in the study locations [19].

Listeria species from the meat samples used in this study may have developed antibiotic resistance as a result of overusing antibiotics to treat animal and poultry illnesses. This is because non-registered veterinarians still administer Veterinary drugs, and farmers often purchase and administer the drugs without proper prescription [27]. The presence of *hlyA* gene in multidrug-resistant zoonotic bacteria such as *L. ivnaovii* is of great concern to clinicians in the treatment of patients or animals infected by such an organism [28]. Pathogenic *Listeria* spp in foods harbour virulent genes, which can seriously harm food samples' consumers if not properly cooked or processed to different meat products as ready-to-eat meat products [28]. The low prevalence or absence of *hlyA* gene in some of the isolates in this research could be due to environmental stress and, which include the effect of temperature as earlier reported by Kamp and Higgins [29], Bergholz *et al.* [30], Alessandria *et al*. [31], Ndahi *et al*. [28] and Cordero *et al*. [32] that *hlyA* gene is well express at a very low temperature.

The presence of *hlyA* gene in the *L. ivanovii* implies the organism's pathogenicity. In sheep and cattle, *Listeria ivanovii* can result in severe foetal-placental infections that are linked to outbreaks of neonatal mortality or irregular abortions. *Listeria ivanovii* can attach itself to human amnion-derived cells, penetrate them, lyse the phagosomal membrane, polymerize and recognise the action of the host cell as tails, replicate in the host cell cytosol following phagosomal escape, and spread from one cell to another [33, 34].

In terms of their capacity to infiltrate human epithelial and endothelial cells, *Listeria ivanovii* strains can be compared to *L. monocytogenes*, although they are more effective in doing so. [33]. Even though, the clearance of *L. ivanovii* in human blood cell (septicaemia) is faster than *L. monocytogenes*. Detection of *L. ivanovii* in the faeces and blood of human suggest that the bacterium can cross the intestinal barrier in humans, causing gastroenteritis and disseminating to blood streams. Therefore, *Listeria ivanovii* is foodborne pathogen that causes infections of varying degrees in humans [35]. The detection of tetracycline resistance (*tetA*) and *tetM* genes could be due to frequent exposure of these pathogens to the antibiotic in the environment where they were isolated or frequent abuse of the antibiotic in the treatment of animals or poultry, as

stated earlier [12]. Tetracycline resistance in *Listeria* species might also be acquired through the horizontal gene transfer by plasmid and transposons from the same or different species as reported by Wilson *et al*. [36].

The absence of *tetA* or *tetM* genes in some of the isolates could be because of the loss of the plasmid harbouring these genes since antibiotic-resistance genes are plasmid-acquired genes. In addition, some bacteria do resist antibiotics by efflux pump, which expels drugs from cell at a high rate. Multidrug efflux pumps, which effectively pump various antibiotics, are known to contribute to multidrug resistance. Tetracycline resistance is one of the classic examples of efflux-mediated resistance [37]. The absence of *tetA* and *tetM* genes in some of the isolates could also be associated with modification of the drug target site or prevention of the drug to reach the target site and modification of the antibiotic molecule. Mutations can cause the ability of bacteria to withstand antibiotics; naturally occurring selection will favour the survival of resistant bacteria [13, 36].

CONCLUSION

Of the number of *Listeria ivavovii* screened for the presence of the virulence gene (*hylA*) gene, only one isolate harboured virulence gene. *Listeria* species were resistant to several antibiotics selected for the research. Two isolates of *Listeria ivanovii* had *tetA* and three had *tetM* gene. Therefore, the *Listeria* spp acquired from the selected food of animal origin can pose health hazards to the populace because most people in Nigeria use meats and vegetables as sources of food. The prevalence of these pathogens in lettuce and cabbage can be of great concern due to the fact that they are mostly consumed raw by the populace. It is therefore recommended that butchers seriously consider hygiene to avoid using unclean water for washing, contaminated containers, and contaminated slaughter surfaces and slabs for the sale of meat. Vegetables should be thoroughly washed before consumption since they can be contaminated through wastewater to irrigate farm land; unhygienic means of handling vegetables from the farm and the market can also be sources of contamination. There is a need for handlers of raw foods to be educated on the importance of adherence to hygiene.

ACKNOWLEDGMENT

The researchers acknowledge the support of the National Research Institute for Chemical Technology and the contribution of the technologists in the Department of Microbiology, ABU Zaria, and the staff of DNA LABS, 2A Kinkino Road City Centre, Nasarawa 800283, Kaduna, Nigeria for the successful completion of this research.

COMPETING INTEREST

The authors declared that there is no competing interest.

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