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Research Article

Prevalence and Antibiogram of *Listeria monocytogenes* Isolated from Animals, Humans and Environmental Samples in Jos Metropolis, Plateau State, Nigeria

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Abstract

Listeria monocytogenes is an infectious foodborne pathogen causing the disease listeriosis which is considered a serious public health problem due to severity of symptoms and its high mortality rate specially in high-risk group. The study aimed to determine the prevalence and antibiogram of *L. monocytogenes* isolated from cattle, humans and environment samples at cattle farms in Jos Metropolis, Plateau State, Nigeria. A cross-sectional study was carried out using multistage sampling technique in three selected Local Government Areas where 254 milk, 55 faeces, 227 hand swabs, 95 cloth swabs, 107 water and 104 soil samples totalling 842 samples were collected. The ISO 11290-1 protocol was used aseptically to phenotypically characterize the isolates and the Kirby-Bauer disc diffusion method was used to determine the antibiogram. Data obtained were subjected to descriptive statistics using SPSS 25.0. Results showed out of the 842 samples 58 (6.89%) were positive for *Listeria* species. Distribution of the *Listeria* species showed that 12 (20.69%) were *L. monocytogenes*. The frequency of isolation of *L. monocytogenes* based on location was highest in Jos South LGA (2.08%) and the association was statistically significant (p<0.05). The occurrence of *L. monocytogenes* from the highest to the lowest prevalence was observed in cloth swabs (3.16%), hand swab (2.20%), faecal sample (1.82%) and milk (1.18%) and association was not statistically significant (p>0.05). The isolates tested against 10 antibiotics, showed 100% resistance to ceftazidime, 91.7% to ampicillin, 91.7% ceftriaxone, 83.3% to sulfamethoxazole-trimethoprim while all (100%) were susceptible to ciprofloxacin. The antibiogram showed majority (91.7%) the isolates were resistance to at least four antimicrobial agents in at least three classes of antibiotics. The mean MAR index is 0.58 ± 0.2. These results are of a public health significance because this pathogenic organism have been shown to cross-contaminate meat and other animal products.

Keywords

Listeria Monocytogenes, Prevalence, Antibiogram, Multidrug Resistance, Mar Index, Jos Metropolis

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1. Introduction

Listeria monocytogenes are Gram positive, facultatively anaerobic, non-sporing, short motile bacilli [1, 2]. The bacteria are ubiquitous in nature with high tolerance to vast environmental factors because of their high salt tolerance [3]. *L. monocytogenes* the causative agent of listeriosis, is a serious foodborne zoonotic pathogen affecting mainly the elderly, immunosuppressed individuals, pregnant women and new-borns causing meningitis [4], meningoencephalitis, sepsis [5], febrile gastroenteritis [6], miscarriages [7] and foetal infections [8].

The primary route of infection is through ingestion of contaminated food products such as vegetable salad [9], cheese [10], unpasteurised milk [11] and ready to eat processed meat products [7]. The distribution of *L. monocytogenes* has been documented to occur in various food and environmental sites including milk [12], cheese [13], ready to eat [14, 15], beef and chicken [16, 17], pork, goat meat, cucumbers and carrot [18], liver, spleen, abattoir effluent [19], irrigation water [20] and food production environment [21, 22]. Consequently, *L. monocytogenes* has been reported in foodborne outbreaks due to contamination of food processing plants with virulent strains of this organism [23].

Historically, *L. monocytogenes* has been considered susceptible to a broad range of antibiotics, particularly ampicillin, penicillin, ciprofloxacin, vancomycin and gentamicin. These antibiotics have been used for treating infections, particularly in immunocompromised patients and neonates [8]. However, studies have shown increasing levels of resistance, especially to less commonly used antibiotics, particularly to erythromycin, tetracycline, and other macrolides [19, 20]. Penicillin and ampicillin resistance have occurred due to some strains, particularly those harbouring *bla* (beta-lactamase) genes, have shown resistance or reduced susceptibility [8]. Resistance to macrolides like erythromycin and tetracyclines is of increasing concern. The resistance mechanisms in *L. monocytogenes* are typically due to efflux pumps or modification of the antibiotic target sites [24]. *L. monocytogenes* has also been observed to exhibit multidrug resistance in certain isolates, which complicates treatment regimens. Therefore, understanding the antibiotic susceptibility pattern is critical for clinical treatment of listeriosis and for ensuring food safety. This study investigated the prevalence and antibiogram and of *L. monocytogenes* in animal, humans and environmental samples in some farms in Jos Metropolis, Plateau State, Nigeria.

2. Materials and Methods

2.1. Study Area

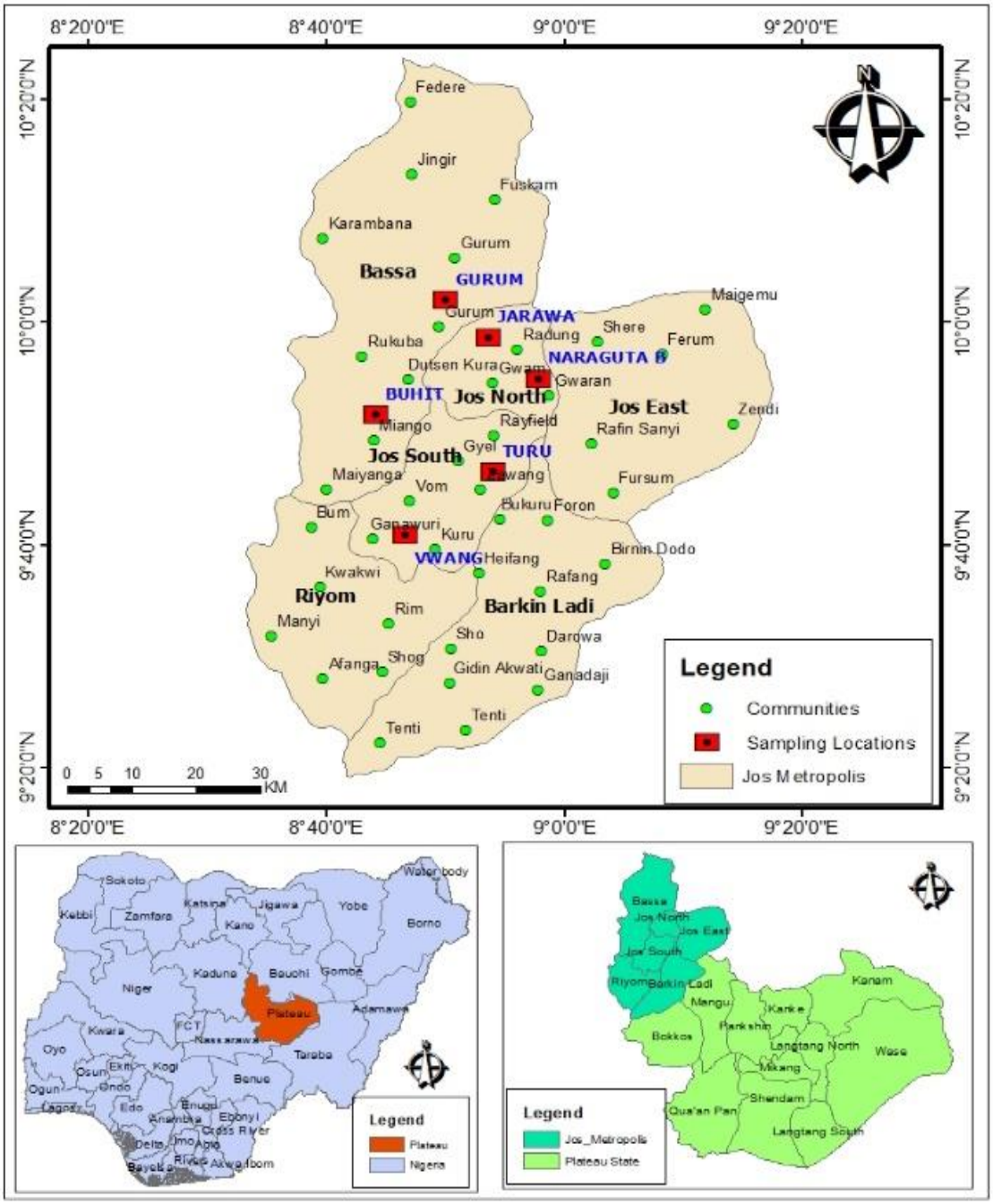
The study was conducted at the Jos Metropolis located between latitudes 9°54 N and 10°10 N and longitudes 8°48' E and 9°30 'E, comprising of Jos North, Jos South and parts of Jos East, Riyom, Bassa, Barkin Ladi and Riyom Local Government Areas (LGAs) of Plateau State, which is also known as Greater Jos Metropolis according to the Plateau State Government (2009) (Figure 1). Plateau State is the 12th largest State in Nigeria with 17 LGAs having an area of 26,899 square kilometres and a population of 3,206,531 million people according to 2006 population Census. The State located in the North-Central geo-political zone of Nigeria shares geographic boundaries with Bauchi, Kaduna, Nasarawa and Taraba States at its North-Eastern, North-Western, South-Western and South-Eastern boundaries respectively. The State has a near temperate climate with an average temperature between 18 ℃ and 22 ℃ and a mean annual rainfall that varies from 131.75 cm (52 in) and 146 cm (57 in). Agricultural activities in the State include crop farming such as maize, guinea corn, cassava, yam, cow peas, rice, fonios which is locally called 'acha', monumental crops, mango, strawberries, flowers, vegetables, tomatoes, onion, cabbage, carrot and cucumber. Plateau State is also an important cattle producing area in Nigeria, with a high concentration of pastoral Fulani and indigenous herders. Plateau State has 1.3million cattle, 1.8 million goats and 1.2million sheep as the population of domestic ruminants as cited by [19].

Ethical Clearance

Ethical approval for the study was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) and the Health Research Ethics Committee (HREC) of the Plateau State Specialist Hospital (PSSH).

2.2. Study Design

A cross-sectional study was conducted to detect the presence of *L. monocytogenes* in animals, humans and the environmental samples in some farms in Jos Metropolis, Plateau State, Nigeria. Multistage sampling method was used to collect data. In the first stage, simple random sampling method was used to select three (3) LGAs from Jos Metropolis, this was done excluding Barkin Ladi LGA from the sampling frame due to logistical volatility and security constraints active within the region at the time of the study while in the second stage, simple random sampling technique was employed to select two wards each in the selected LGAs and in the third stage, simple random sampling method was used to select two farms each based on the owner's consent to participate in the study. The sample size determined was divided proportionately among the subjects sampled.



Source: www.ArcGis.com

Figure 1. *Map of Nigeria showing Plateau State and the sampling locations.*

2.3. Sample Size Determination

Sample size was calculated using the formula by [25] at 95% confidence level and respective prevalence for the different sample types is applied below:

$$n = \frac{z^2pq}{d^2}$$

Where:

n = sample size

Z = z-score at 95% confidence interval (1.96)

P = prevalence

q = 1-p

d = level of significance (0.05)

Sample Size Calculation for Animal samples

Milk sample - Calculated with a prevalence of 18.46% for milk and milk product by [13] = 231.29 samples plus 10% for attrition rate = 254 samples.

Faecal sample - Calculated with a prevalence of 3.4% for cattle farms and beef abattoir by [26] = 50.46 plus 10% for

attrition rate = 55 samples.

Sample Size Calculation for Human Subjects

Hand swab - Calculated with prevalence of 16% from hand swab of farm workers by [27] = 206.52 samples plus 10% for attrition rate = 227 samples.

Animal handlers cloth sample - Calculated with a prevalence of 6.0% from personnel cloths in meat plant by [28] = 86.66 samples plus 10% for attrition rate = 95 samples.

Sample Size Calculation for Environmental Samples

Environmental samples for this study are defined as the cattle farm premises where cattle are housed including the water sources used for the animals.

Water sample - Calculated with a prevalence of 6.8% for irrigation water by [20] = 97.38 samples plus 10% for attrition rate = 107 samples.

Soil sample - Calculated with a prevalence of 6.6% for agricultural soil by [20] = 94.72 samples plus 10% for attrition rate = 104 samples.

Total sample n = 842 samples.

2.4. Sample Collection and Transportation

Fifty millilitres (50ml) of milk and water sample were aseptically collected in a labelled wide-mouthed sample bottles, and fifty grams (50g) of faecal and soil samples were collected into clean labelled polythene bags respectively, while a 5x5 cm dimension of swab from the hands and work cloths of the animal handlers were aseptically collected using sterile swab sticks that were moisten with distilled water and labelled appropriately. All samples collected were packed and transported in a Coleman box containing icepacks to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria for analysis.

2.5. Processing of Samples

Ten grams (10g) of faecal and soil samples were added separately to 90ml of 0.1% peptone (Oxoid® UK CM0009) water in a stomacher bag and homogenized for two minutes in a stomacher at room temperature as described by [12].

2.6. Isolation of *Listeria* Species

The ISO 11290-1 method for the qualitative isolation and identification of *L. monocytogenes* was used with modification as described by [29]. One (1) ml from each milk and water samples, the homogenized soil and faecal samples and swab sticks (cut) from the hand and cloth samples were enriched by adding 9ml of *Listeria* enrichment broth base (Oxoid® UK, CM0862) incorporated with *Listeria* selective enrichment supplement (Oxoid® UK, SR0141E) in labelled plain sample bottles and all the samples were incubated at 37°C for 18-48 hours. A loop-full of the overnight culture were streaked onto the surface of *Listeria* selective agar base (Oxoid® UK, CM0856) plates incorporated with *Listeria* selective supplement (Oxoid®, SR0140E) and were incubated at 37°C for 24-48 h. The colonies were identified by colonial morphology for *Listeria* colonies viz grayish colonies surrounded by black halos with sunken centre. Suspected colonies were inoculated onto Tryptone Soya Agar (Oxoid®, CM0131) supplemented by 0.6% of Yeast Extract Powder (Oxoid®, LP0021) (TSAYE) slants and were incubated at 37°C for 24 hours. The suspected isolates on the TSAYE slants were then stored at 4°C for further identification as described by [30, 31]. The presumptive *Listeria* species were subjected to conventional biochemical tests including catalase, oxidase, β-haemolysis on 7% sheep blood, aesculin hydrolysis, hydrogen sulphide, indole and motility test, Voges-Proskauer, methyl red reaction and carbohydrate fermentation test using mannitol, rhamnose, arabinose, maltose, glucose, sucrose and xylose. Gram's staining was also performed on the suspected isolates.

2.7. Antimicrobial Susceptibility Testing of *Listeria* Species

The antibiotic susceptibility of the isolates was determined by the Kirby-Bauer disc diffusion method on Mueller Hinton agar (MHA, Oxoid, Basingstoke, UK). Standard discs were applied using a disc dispenser following the procedures recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2026 guidelines. Three to five well distinct colonies of the *L. monocytogenes* isolates were transferred into 5ml Brain Heart Infusion Broth (BHIB, Oxoid) and were incubated at 37°C for 24 hours. The overnight broth cultures were diluted using sterile normal saline to a turbidity equivalent to 0.5 McFarland standard (10^8cfu/ml) and were inoculated onto the dried surface of Muller Hinton agar (MHA, Oxoid) plate. Sterile cotton-tipped swabs were used to inoculate the plates using the lawn inoculation technique by streaking back and forth from top of the plate to the bottom of the plate, turning the plate 60 degrees and repeated, then and turn another 60 degrees and repeated. The inoculated MHA plates were allowed to dry at room temperature for approximately 10 minutes and paper discs impregnated with Trimethoprim & sulfamethoxazole (SXT, 25 µg) Streptomycin (S, 10 µg), Gentamycin (GN, 30 µg), Ciprofloxacin (CIP, 5 µg), Ceftazidime (CAZ, 30 µg), Clindamycin (C, 2 µg), Ampicillin (AMP, 30 µg), Ceftriaxone (CRO 30 µg), Amoxicillin (AX, 30 µg) and Vancomycin (VA, 10µ) were placed using a disc dispenser and incubated at 37°C for 24 hours. After incubation, the diameters (mm) of clear zones of inhibition around each disc were measured and interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2026 guidelines, clinical breakpoints for *Staphylococcus, Enterobacterales* and *Streptococcus* were used for the unavailable breakpoints for *L. monocytogenes* [32, 33]. *Staphylococcus aureus* ATCC 25923 was used as quality control strain as described by [12].

2.8. Determination of Multiple Drug Resistance (MDR) and Multiple Antibiotic Resistance Index of *Listeria* Species

The number of classes of antibiotic each isolate was resistant to were noted for the identification of multiple drug resistance (MDR). MDR was performed as described by [34] as the acquired non-susceptibility of an isolate to at least one agent in three or more antimicrobial categories. Multiple resistance and resistance pattern of *Listeria* species isolates were determined and the multi-antibiotic resistance index were calculated using the formula described by [35]:

$$\text{MARI} = \frac{\text{Number of antibiotics an isolate is resistant to}}{\text{Total number of antibiotics used to which the isolates were subjected to}}$$

3. Data Analysis

Data obtained from the study were subjected to descriptive statistical analysis through the use of a statistical software package (SPSS version 25.0). Results were presented in tables, charts, and chi square test were calculated to test for the level of association between positive *L. monocytogenes* and the location from which they were obtained as well as the type of sample. Prevalence was calculated for the isolates using the formula:

$$\text{Prevalence\%} = \frac{\text{Number of positive samples}}{\text{Total number of samples}} \times 100$$

p-value ≤ 0.05 were considered statistically significant.

4. Results

Out of the 842 samples comprising of 254 milk, 55 faeces, 227 hand swabs, 95 cloth swabs, 107 water and 104 soil analysed, 58 (6.9%) were positive for *Listeria* species based on `conventional culture, biochemical and sugar fermentation assays. Species differentiation of the 58 *Listeria* species showed that 12 (20.69%) were *L. monocytogenes* while 2 (3.45%), 14 (24.14%), 2 (3.45), 27 (46.55%) and 1 (1.72%) were *L. grayi, L. innocua*, *L. ivanovii*, *L. welshimeri* and *L. seeligeri* respectively (Table 1).

Table 1. *Relative Species Distribution and Frequency among the Recovered Listeria isolates (n=58).*

***Listeria* Species**	**Frequency of Isolation**	**Prevalence (%)**
L. monocytogenes	12	20.69
L. grayi	2	3.45
L. innocua	14	24.14
L. ivanovii	2	3.45
L. welshimeri	27	46.55
L. seeligeri	1	1.72
Total	58	100

Distribution of the *Listeria* species based on sampling location showed that Jos South LGA (13.19%) had the highest contamination rate, followed by Jos North LGA (5.59%) with the least contamination in by Bassa LGA (5.58%). The frequency of isolation of *L. monocytogenes* was highest in Jos South LGA (2.08%) followed by Bassa LGA (1.40%) with the least isolated in Jos North LGA (1.24%). There was statistically significant association ($p<0.05$) between the prevalence of *L. monocytogenes* and the sampling location in this study (Table 2).

Table 2. *Prevalence of Presumptive Listeria Species Based on Location.*

Location	**No.**	**Listeria**			**Listeria**	**Species**				
(LGA)	**Sampled**	**Species (%)**	**L. grayi (%)**	**L. innocua (%)**	**L. ivanovii (%)**	**L. monocyte-genes (%)**	**L. welshimeri (%)**	**L. seeligeri (%)**	**χ2**	**p-value**
Bassa	215	12 (5.58)	0	4 (1.86)	1 (0.47)	3 (1.40)	4 (1.86)	0		
Jos North	483	27 (5.59)	2 (0.41)	7 (1.45)	0	6 (1.24)	12 (2.48)	0	21.928	0.0383
Jos south	144	19 (13.19)	0	3 (2.08)	1 (0.69)	3 (2.08)	11 (7.64)	1 (0.69)		
Total	842	58 (6.89)	2 (0.24)	14 (1.66)	2 (0.24)	12 (1.43)	27 (3.21)	1 (0.12)		

Distribution of the *Listeria* species showed that the highest prevalence was isolated from cloth swabs (16.84%) which was followed by faecal samples (10.91%), water samples (7.48%), hand swabs (6.61%), soil samples (5.77%) and the least occurring in milk samples (2.76%). The occurrence of *L. monocytogenes* from the highest and lowest was observed in cloth swabs (3.16%), hand swab (2.20%), faecal samples (1.82%) and milk (1.18%). There was however no significant statistically association between the prevalence of *L. monocytogenes* and the sample type (p>0.05) in this study (Table 3).

Table 3. *Prevalence of Presumptive Listeria Species Based on Sample Type.*

Sample type	No. sampled	*Listeria* species			*Listeria* species				χ^2	*p*-value
		Total	*L. grayi* (%)	*L. innocua* (%)	*L. ivanovii* (%)	*L. monocyte-genes* (%)	*L. Welshimeri* (%)	*L. seeligeri* (%)		
Cloth swab	95	16 (16.84)	1 (1.05)	4 (4.21)	1 (1.05)	3 (3.16)	7 (7.37)	0		
Faeces	55	6 (10.91)	0	2 (3.64)	0	1 (1.82)	3 (3.64)	0		
Hand swab	227	15 (6.61)	1 (0.44)	3 (1.32)	1 (0.44)	5 (2.20)	5 (2.20)	0	39.635	0.1121
Milk	254	7 (2.76)	0	0	0	3 (1.18)	3 (1.18)	1 (0.39)		
Soil	104	6 (5.77)	0	2 (1.92)	0	0	4 (3.85)	0		
Water	107	8 (7.48)	0	3 (2.80)	0	0	5 (4.67)	0		
Total	842	58 (6.89%)	2 (0.24)	14 (1.66)	2 (0.24)	12 (1.43)	27 (3.21)	1 (0.12)		

The antibiotic sensitivity testing of the 12 *L. monocytogenes* isolates tested against 10 antibiotics, the isolates showed susceptibility in descending order to the following antimicrobial agents at various percentages: ciprofloxacin 100%, streptomycin 91.7%, vancomycin 58.3%, gentamycin 58.3%, amoxicillin 50%, clindamycin 25%, sulfamethoxazole-trimethoprim 16.7%, ampicillin 8.3% and ceftriaxone 8.3%. In contrast, all 12 isolates of *L. monocytogenes* were resistant (100%) to ceftazidime. Resistance was observed against ampicillin (91.7%), ceftriaxone (91.7%), sulfamethoxazole-trimethoprim (83.3%), clindamycin 75%, and amoxicillin 50%. The least resistance was observed in streptomycin 8.3% followed by gentamycin and vancomycin with 41.7% each. No resistance (0%) was obtained for ciprofloxacin (Figure 2).

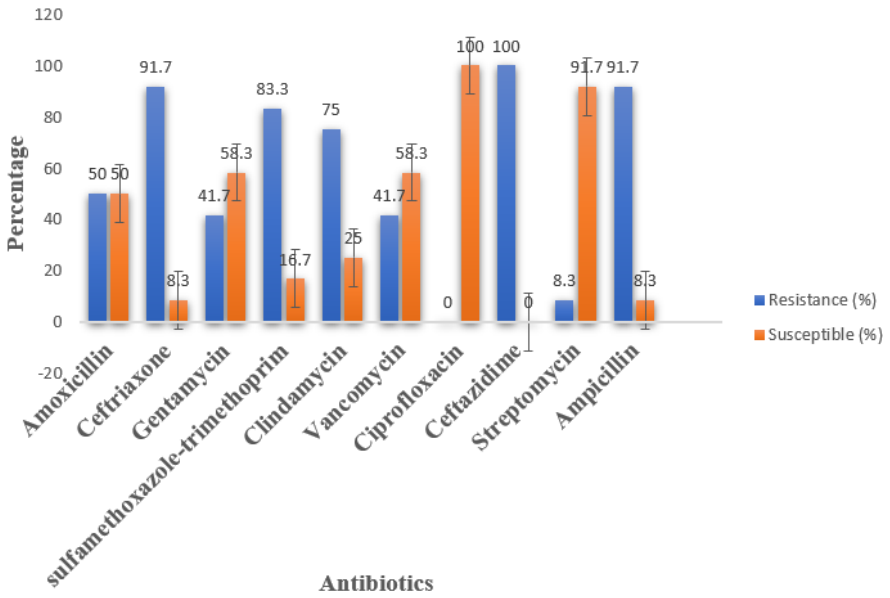


Figure 2. *Percentage Distribution of Antibiotics Susceptibility Testing of Listeria monocytogenes Isolates.*

The cumulative antibiotic resistance profile showed that majority (91.7%) of the *L. monocytogenes* isolates were resistance to at least four antimicrobial agents in at least three classes of antibiotics (Table 4).

Table 4. *Distribution of Listeria monocytogenes isolates based on the cumulative number of resistant antibiotics (n=12).*

Number of Resistant Antibiotics	Frequency	Percent (%)	Cumulative percent (%)
Resistance to less than three antibiotics	1	8.3	8.3
Resistance to four antibiotics	2	16.7	91.7
Resistance to more than four antibiotics	9	75.0	
Total	12	100	100

The isolates showed 9 different antibiograms with ceftazidime occurring in all. Antimicrobial resistance to eight (8) antibiotics: amoxicillin (AX) a penicillin, ceftriaxone (CRO) a cephalosporin, gentamycin (GN) an aminoglycoside, Sulfamethoxazole-Trimethoprim (SXT) a sulphonamide/folate inhibitor, clindamycin (CD) a lincosamide, vancomycin (VA) a glycopeptide, ampicillin (AMP) a β-lactam penicillin and ceftazidime (CAZ) a cephalosporin were the most common patterns occurring in 4 of the 12 *L. monocytogenes* isolates. The mean MAR index is 0.58 ± 0.2 with the minimum being 0.20 and the maximum 0.80 (Table 5).

Table 5. *Antibiogram Pattern and Multiple Antibiotic Resistance (MAR) Index of Listeria monocytogenes Isolates.*

Isolate I. D	Source	Antibiogram	MAR Index
JSC 92	Cloth swab	AX, CRO, CD, AMP, CAZ	0.50
JNF 125	Faeces	AX, CRO, GN, SXT, CD, VA, AMP, CAZ	0.80
JSM 141	Milk	AX, CRO, GN, SXT, CD, VA, AMP, CAZ	0.80
JSM 237	Milk	AX, CRO, GN, SXT, CD, VA, AMP, CAZ	0.80
BM 273	Milk	AX, CRO, GN, SXT, CD, VA, AMP, CAZ	0.80
BH 295	Hand swab	CRO, SXT, CD, AMP, CAZ	0.50
JNC 781	Cloth swab	SXT, CAZ	0.20
BH 801	Hand swab	AX, CRO, GN, SXT, CD, AMP, CAZ	0.70
JNC 813	Cloth swab	CRO, SXT, AMP, CAZ	0.40
JNC 814	Cloth swab	CRO, CD, AMP, CAZ	0.40
JNH 821	Hand swab	CRO, SXT, CD, VA, AMP, CAZ	0.60
JNH 840	Hand swab	CRO, SXT, S, AMP, CAZ	0.50

Amoxicillin AX (penicillin); Ceftriaxone CRO (cephalosporin); Gentamycin GN (aminoglycoside); Sulfamethoxazole-Trimethoprim SXT (sulphonamides/folate inhibitor); Clindamycin CD (Lincosamide); Vancomycin VA (glycopeptide); Ampicillin AMP (β-lactam penicillin); Ceftazidime CAZ (cephalosporin); Streptomycin S (aminoglycoside).

5. Discussion

Listeria monocytogenes has been documented to be the pathogenic species responsible for both human and animal listeriosis [36] and has been responsible for many foodborne listeriosis outbreaks with fatal outcomes [7, 23, 37]. *L. ivanovii* and *L. innocua* have been established as the pathogenic species responsible for animal listeriosis especially in livestock [38, 39].

The study established an overall prevalence of 6.89% of

Listeria species in cattle, humans and environmental samples in some selected cattle farms in three LGA of Jos Metropolis, Plateau State, Nigeria. This result is much lower than the 38.2%, 27.8%, 27.5%, 17.5% and 14.6% reported by [40] isolated from milk and environmental samples in selected areas of Eastern Ethiopia, [41] from raw cow milk (nono) in Ogun and Oyo States, Nigeria, [16] from fresh raw meat retailed in Zaria, Northwestern Nigeria, [42] from raw food and environmental samples in Asaba, Nigeria and [26] from cattle farms and beef abattoirs in Gauteng Province, South Africa respectively. However, the result of this study is slightly higher than the 4.75% prevalence documented by [43] from frozen Atlantic mackerel fish in Nigeria. This is probably due to the fact that *Listeria* species are ubiquitous in nature and are especially found in temperate regions [37].

The results of the different *Listeria* species recorded from the most occurring to the least is, *L. welshimeri*, (46.55%) *L. innocua* (24.14%), *L. monocytogenes* (20.69%), *L. grayi* (3.45%) *L. ivanovii* (3.45%) and *L. seeligeri* (1.72%). All the non-pathogenic *Listeria* species confirmed in this research namely, *L. seeligeri*, *L. ivanovii*, *L. grayi,* and *L, innocua* have been documented in human infections with the exception *L. welshimeri* [44-48]. This result obtained in this study in Jos Metropolis, Plateau State, Nigeria varied with a previous study conducted in Jos North, Jos South and Jos East which are still part of Jos Metropolis where the *Listeria* species isolated were 22.2%, 0.9%, 1.9% and 1.7% for *L. ivanovii*, *L. monocytogenes*, *L. grayi* and *L. seeligeri* respectively in abattoir by-products and effluent [19], however, *L. welshimeri and L. innocua* were not isolated. This could be due to the type of samples analysed in this study that in addition to animal samples, human and environmental samples (soil and water) were included in this study. Probably, this is because *Listeria* is widely distributed in the natural environment, with soil representing a key ecological niche for the persistence of globally distributed isolates where soil runoff may contaminate water sources which then serve as reservoirs that transfer the organism through the environment. Also, studies have documented *L. monocytogenes* circulating in the aquatic environment belonging to clonal complexes that contain the same virulence traits as that frequently isolated from human and animal clinical cases and from globally occurring outbreaks, including hypervirulent clones [49]. The result of this study is higher than the 40.5%, 19.5%, 19.0% and 2.4% prevalence for *L. welshimeri*, *L. monocytogenes*, *L. innocua* and *L. grayi* respectively observation of [50], from broad food sources including ready to eat foods, raw and suya meat, dairy products, fruits and vegetables in Borno State, Nigeria, however, they reported a higher prevalence of 11.9% for *L. ivanovii* but their research did not isolate *L. seeligeri*. [16] observation differed from our report by documenting four (4) *Listeria* species with the most occurring as *L. grayi* (59.1%), *L. innocua* (19.7%), *L. monocytogenes* (12.5%) and the least, *L. ivanovii* (9.1%).

The occurrence of *Listeria* species in the samples analysed was highest in cloth swabs (16.84%) compared with hand swab (6.61%), similarly, *L. monocytogenes* frequency of isolation was highest in cloth swab (3.16%) compared with hand swab (2.20%) among the human samples tested. These findings are lower compared to the 28% and 14% report of [51] from apron of slaughterhouse workers and the 14% and 28% of hand swabs tested in two slaughterhouses in Egypt. This is also lower than the 22.8% report of [27] for *Listeria* species isolated in hand swab and cloth swab of abattoir workers in Turkey and lower than the 5.7% for *L. monocytogenes*. This is probably due to the personal hygiene practice between these countries as poor personal hygiene such as hand-washing during milking, will aid the transmission of the pathogen from animals to humans and human to animals and other objects such as clothing.

The environmental samples i.e. water (7.48%) and soil (5.77%) had the next highest isolation rate of *Listeria* species with only *L. innocua* and *L. welshimeri* isolated in both samples. This differs from positive *L. monocytogenes* isolation in water by researchers including the 20% report of [52] from irrigation water in selected areas of Osun State, Nigeria and the 13% report of [49] isolated from flowing surface waters in Switzerland and also the 6.8% report of [20] isolated from irrigation water and agricultural soil samples in Eastern Cape Province, South Africa. In a research similar to this study, *L. monocytogenes* was not isolated as documented by [53] from soil samples in Iran. This also differs from the works of [20] and [54] who both reported a prevalence of 23% and 1.75% of *L. monocytogenes* in South Africa and India respectively. This difference in isolation of *L. monocytogenes* in these studies is probably due to the differences in sample size. The observed *L. innocua* and *L. welshimeri* prevalence in water samples is indicative that the aquatic environment act as reservoirs for these species. Poor sanitation, runoff contamination from farms, abattoirs and discharge of untreated waste into water bodies which are common challenges in many Nigerian settings are likely contribute to their persistence. The detection of these two species in environmental samples in the study location reflects the environmental distribution and ecological adaptability of non-pathogenic *Listeria* species. These findings are consistent with the understanding that *Listeria* species are ubiquitous in natural environments such as soil, water, and decaying vegetation, where non-pathogenic species often predominate over *L. monocytogenes* [55, 56].

The occurrence of *Listeria* species in the animal samples was highest in faecal samples (10.91%) compared to milk samples (2.76%), likewise, *L. monocytogenes* prevalence was higher in cattle faeces (1.82%) compared to milk samples (1.18%). This is lower than the 27.8% of *L. monocytogenes* findings of [41] in raw cow milk in Ogun and Oyo States, Nigeria. A lower prevalence of 4.7% for *Listeria* species and also a lower prevalence of 1.0% for *L. monocytogenes* isolated from faeces has been reported by [57] in Egypt. In contrast, a much higher prevalence of 37.5% was observed for *Listeria* species by [58] in cattle faeces in Maiduguri, Borno State.

These findings highlight the significant role of both food products and animal reservoirs in the epidemiology of listeriosis. *L. monocytogenes* is a well-recognized zoonotic pathogen capable of persisting in farm environments, contaminating dairy products, and colonizing the gastrointestinal tract of animals. The prevalence milk samples suggests that post-milking contamination, poor milking hygiene, and environmental exposure may amplify bacterial presence in dairy products. The prevalence of *L. monocytogenes* in faecal samples is epidemiologically important, as faecal shedding by livestock is a key source of environmental dissemination coupled with the poor personal hygiene of the animal handlers in this location.

Antibiotics are the most significant class of pharmaceuticals and are one of the most influential medical inventions since their discovery [59]. They are extensively used in animals to prevent, control and treat illnesses, they are also enormously used as growth promoters in animal production [21]. This extensive administration of antibiotics has led to misuse of these drugs in both humans and animals which has invariably contributed to the widespread antibiotic resistance among foodborne pathogens such as *L. monocytogenes* [21]. Antimicrobial resistance (AMR) currently presents a significant challenge to all healthcare systems worldwide [59]. Antimicrobial resistance have been demonstrated to develop in bacteria either through the bacteria exhibiting some intrinsic resistance to certain antibiotics due to their general physiology, through mutations or other kinds of genetic alteration while others by adapting to environmental stresses [60].

The high number of *L. monocytogenes* isolates (100%) resistant to ceftazidime in this study, is expected as the organism is intrinsically resistant to ceftazidime which is often used in selective media to isolate *Listeria* species, leveraging its natural resistance to this antibiotic due to its lack of the specific penicillin-binding proteins (PBPs) that third-generation cephalosporins targets [61]. However, this is in contrast to the observation of [62] that documented a 14% susceptibility to ceftazidime from open-air fish markets in Iran.

Listeria monocytogenes displayed a high resistance (91.7%) to both ampicillin and ceftriaxone, this is similar to a previous finding where 100% of the isolates were resistant to ampicillin in the same study area [19] as well as the 100% report of [16] from fresh raw meat retailed in Zaria, Northwestern, Nigeria but differ from the 100% susceptible report of [63] from food sources in Poland. The high level of resistance to ceftriaxone (91.7%) observed in this study indicates a significant public health concern, particularly in the context of foodborne *L. monocytogenes* infections in Nigeria. Ceftriaxone, a third-generation cephalosporin, is widely used in clinical practice; however, *L. monocytogenes* is intrinsically less susceptible to cephalosporins due to the low affinity of its penicillin-binding proteins for this class of antibiotics. The observed high resistance to β-lactam antibiotic (ampicillin) is highly significant from public health perspective which reflects both intrinsic and acquired resistance mechanisms and appears to be increasingly reported in food and environmental isolates [64]. Resistance of *L. monocytogenes* to ceftriaxone and other cephalosporins has also been reported, a clinical study by [65] reported a lower ceftriaxone resistance of 53.5% and slightly lower ampicillin resistance of 81.4% among *L. monocytogenes* isolates isolated from women with spontaneous abortion in Ugandan tertiary hospitals. Similarly, [57] documented 85.7% resistance to ampicillin from humans, animals and dairy products in Egypt. Increasing resistance to β-lactam antibiotics such as ampicillin is concerning, given that these drugs are considered first-line therapy for listeriosis [66].

The high resistance to clindamycin (75%) observed in this study indicates a high level of antimicrobial resistance among foodborne isolates in Nigeria and raises significant public health concerns. This study agrees with the 71.4% resistance to clindamycin by [67], from locally processed fermented foods in Ethiope West, Delta State, Nigeria. A lower prevalence of 48% was obtained by [68] from diverse food commodities across Sikkim, India. In contrast, a 100% resistance from the two positive *L. monocytogenes* isolates was documented by [69] from different dairy and street food sources in North Karnataka, India. Clindamycin, a lincosamide antibiotic, is not a first-line drug for the treatment of listeriosis, however, it is often used as an alternative in patients allergic to β-lactams. Therefore, increasing resistance to this antibiotic may limit therapeutic options, particularly in resource-limited settings such as Nigeria. A 50% resistance to amoxicillin observed in this study is higher than the 12.5% report of [50] from various ready-to-eat foods in Borno State, Nigeria and differs from the 0% resistance observed by [43] from raw frozen Atlantic mackerel fish in Nigeria. The differences could be due to the type of samples analysed in these studies and also, mackerel fish are found in the Atlantic Ocean, their natural habitat and may not have been exposed to antimicrobials.

The 41.7% resistance to gentamycin and vancomycin each is higher than the 22.2% resistance to gentamycin from a previous study in the same study location [19], this is very concerning that there is growing resistance among the organism in Jos, Plateau State, Nigeria. This this higher than the observation of [70] that documented a 0.72% to 10.2% resistance to gentamycin from clinical, seafood, poultry, raw meat, pork and dairy products in Usa, Iran, Italy, China and Spain, only the Nigerian isolate had 31.7% resistance. This could be due to different geographical region's antimicrobial regulations, handling and stewardship. In Nigeria, non-medical personnel have access to drugs and these antimicrobials are available in the open markets and reconstituted in a cocktail for farmers to buy directly by the drug sellers in the hot sun [71]. Sometimes, the farmers misuse antibiotics by frequently administering it to healthy animals in a bid to avoid infections in those animals [72]. Earlier molecular and surveillance analyses have reported that *L. monocytogenes* isolates are generally susceptible to gentamicin, a key aminoglycoside used in clinical treatment for listeriosis. In the past, resistance genes such as acetyltransferase (*aac*) and phosphotransferase (*aph*) are common in other Gram-positive bacteria which have been rarely

described in *Listeria* species are now being reported with a mechanism of resistance involving active efflux and genetic determinants that affect the uptake of the antibiotic through altered membrane potential [73, 74].

Likewise, vancomycin resistance in *L. monocytogenes* obtained in this study is much lower than the 7% and 14.3% reported by [75] and [67] from foods in Morocco and from locally processed fermented foods in Ethiope West, Delta State, Nigeria respectively. However, a much higher resistance of 83.1% was reported by [76] from fruits and vegetables in selected locations in Nigeria. Vancomycin resistance in *L. monocytogenes* is less commonly reported but is biologically plausible due to the presence of vancomycin-associated resistance genes e.g., *vanC*, *vanR*, *vanS* identified in some environmental isolates. Both gentamicin and vancomycin resistance observed in this study are a significant finding, particularly given the clinical importance of these antibiotics in the treatment of listeriosis. Gentamicin is commonly used in combination with ampicillin as a first-line therapy, while vancomycin is often considered an alternative in severe infections or in cases of β-lactam intolerance [77]. Therefore, resistance to these agents may compromise treatment efficacy and represents a growing public health concern antimicrobial resistance.

The 100% susceptibility of *L. monocytogenes* isolate to ciprofloxacin agrees with the findings of [50, 43] and [41] from some foods in Borno State, from retail raw frozen Atlantic mackerel fish in Nigeria and from raw cow milk (nono) in Ogun and Oyo States, Nigeria respectively. The finding of 100% susceptibility of *L. monocytogenes* to ciprofloxacin in this study indicates a high level of effectiveness of fluoroquinolones against *L. monocytogenes* in the studied area. This result is particularly important given the increasing global concern over antimicrobial resistance in foodborne pathogens. Ciprofloxacin, a fluoroquinolone antibiotic, acts by inhibiting bacterial DNA gyrase and topoisomerase IV, thereby preventing DNA replication and transcription [78]. The complete susceptibility observed suggests that these target mechanisms remain largely conserved and unaffected by resistance mutations in the isolates examined. A lower susceptibility of 73.1% was documented by [79] from ready-to-eat vegetables and fermented milk in Yola, Nigeria. However, this study is not in agreement with the 100% resistant report of [80] from seafood sold in Rivers State, Nigeria. This indicate that while ciprofloxacin remains broadly effective, there is emerging evidence of gradual adaptation and potential resistance development. Studies have demonstrated that *L. monocytogenes* can develop reduced susceptibility to ciprofloxacin under selective pressure through mutations in genes such as *parC* and *fepR*, which affect fluoroquinolone targets and efflux mechanisms [81].

The susceptibility of the isolates to streptomycin (91.7%) is slightly lower than the 100% susceptibility report of [82] from meat products and processing environment in Poland but its slightly higher than the 87.5% report by [50] from some foods in Borno State, Nigeria. The susceptibility of *L. monocytogenes* to streptomycin observed in this and other studies indicates that the majority of isolates remain sensitive to this aminoglycoside, although the presence of resistant isolates (8.3%) suggests the emergence of reduced susceptibility within the population. This might be probably due reduced antibiotic pressure or its non-availability in the markets in the study areas as compared to other drugs such as oxytetracycline, gentamycin, penicillin, ampicillin, amoxicillin to mention a few. The widespread and often uncontrolled use of antibiotics in Nigeria particularly in agriculture and animal husbandry creates strong selective pressure that promotes the emergence of resistant strains. Secondly, environmental reservoirs such as water, soil, and food processing environments serve as hotspots for the accumulation and dissemination of antimicrobial resistance genes. Thirdly, horizontal gene transfer among bacteria in these environments may facilitate the spread of resistance determinants, including those affecting aminoglycosides and glycopeptides.

Multiple antibiotic resistance (MAR) index of the *L. monocytogenes* isolates revealed that the mean MAR index is 0.58 ± 0.20. A slightly lower mean MAR index of 0.343 for cattle and 0.375 for human isolates was documented by [83] from ruminants and humans in New Valley and Beheira Governorates, Egypt. [84] stated that a greater than 0.2 MAR index points to the fact that the isolates originate from high-risk area where the rate of antibiotic usage is much. This study is one of such where 100% the minimum MAR index was ≥0.2. This is similar to the report of [80] who reported 100% of the *L. monocytogenes* isolates have a MAR index ≥0.2 from seafood sold in Rivers state, Nigeria.

The high level of resistance of *L. monocytogenes* to these antibiotics should be of great concern to relevant health agencies, regulators and the general public because this typically suggesting that the isolates originate from high-risk environments where antibiotics are frequently used or misused [35]. Therefore, a mean value of 0.58 strongly implies that the *L. monocytogenes* strains in this study location were exposed to substantial antimicrobial pressure. The implication of a multidrug resistant pathogen as seen in this study is that, it becomes more pathogenic compared to non-multidrug resistant pathogens [85]. This scenario also makes treatment regimen unsuccessful in infected patients [86] and there is additional cost of treatment, as well as prolonged hospital stay and consequent death [87].

The public health implications of these findings are substantial, the presence of non-pathogenic *Listeria* species in environmental samples as recorded in this study may indicate potential contamination with *L. monocytogenes*. Milk serves as a direct vehicle for human infection, particularly when consumed raw or inadequately pasteurized, which is still common in many Nigerian communities as the handlers often drink directly from source. The presence of resistant isolates suggests the potential for emerging resistance and underscores the need for continuous surveillance, antimicrobial stewardship, and prudent antibiotic use by observing withdrawal periods and

using veterinary prescribed drugs, to preserve the effectiveness of antibiotics and prevent future resistance development.

6. Conclusion

The findings in this study demonstrates that *L. monocytogenes* is widely distributed across human, animal and environmental sources in the study area. Multiple drug resistance showed most of the *L. monocytogenes* isolates were resistant to at least four (4) antimicrobial agents in at least three classes of antibiotics. It is therefore recommended that strict hygiene practice among farm workers and high-risk individuals who are susceptible to *L. monocytogenes* infection should observe food safety and food hygiene by cooking food from animal sources thoroughly. Government agencies responsible should enforce regulations on access and use of antimicrobial agents, the use of these agents should be limited to licenced personnel and violators should receive strict penalties in order to reduce the incidence of drug resistance from food animal sources. Licenced personnel should also exhibit good antimicrobial stewardship by performing culture and sensitivity testing to select the most suitable drugs for treatment and ensure the observance of withdrawal periods as well as exploring other sources of therapy like the use probiotics, phage therapies amongst others.

Author Contributions

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Habiba Iliyasu Atta: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing

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Conflicts of Interest

The authors declare no conflict of interest.

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