International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 8, 2025
https://theaspd.com/index.php

Isolation And Molecular Detection Of Virulence Genes Of Listeria Monocytogenes From Ready-To-Eat Foods In The Federal Capital Territory Abuja, Nigeria

Muhammad Asabe Hajara [1,2], Adamu Yusuf Kabiru[3], Jeremiah David Bala[4], Ocheme Boniface Ocheme[5]

[1]Africa Centre of Excellence for Mycotoxin and Food Safety, Federal University of Technology Minna, Niger State

[2]Agriculture and Rural Development Secretariat, Federal Capital Territory Administration, Abuja.

[3]Department of Biochemistry, Federal University of Technology Minna, Niger State

[4]Department of Microbiology, Federal University of Technology Minna, Niger State

[5]Department of Food Science and Technology, Federal University of Technology Minna, Niger State

Abstract

Listeria monocytogenes is a Gram-positive, facultative intracellular food-borne pathogen of significant public health concern, especially due to its presence in ready-to-eat (RTE) food samples. This study determined the prevalence of Listeria monocytogenes in RTE foods across four area councils (Gwagwalada, Bwari, Kuje, and Abaji) in Abuja, Nigeria. A cross-sectional study was conducted, and a total of 225 RTE food samples, comprising tsire (grilled meat), balangu (roasted meat), fermented unpasteurized milk (nono), and soft cheese (wara), were collected proportionately. Samples were processed using standard microbiological methods for isolation, followed by biochemical identification of Listeria monocytogenes and multiplex PCR targeting the virulence genes (iap, inlA, and hylA) was used for molecular identification. The data were subjected to statistical analysis to determine the level of significance between variables. The percentage of positive results was calculated at each stage of detection. The results revealed that out of 225 samples, 59 (26.2%) were positive for Listeria. Spp. by culture, of these, 31 (13.8%) were identified as Listeria monocytogenes through biochemical tests, while 19 (8.4%) were confirmed by multiplex PCR to have the Listeria monocytogenes virulence gene. Abaji area council recorded the highest prevalence rate (48.9%) across all stages. In contrast, Gwagwalada had the lowest prevalence (14.7%). Chi-square tests revealed a statistically significant association ($p < 0.05$) between the area councils and the detection of Listeria monocytogenes using three diagnostic test outcomes. Two-way analysis of variance also showed a statistically significant difference in the level of Listeria contamination amongst the different RTE foods. The prevalence of Listeria across food types in the various area councils revealed that soft cheese had the highest rate (37.3%), followed by unpasteurized milk (30.5%), balangu (20.3%), and tsire (11.9%). Amongst the Listeria spp. isolated, Listeria monocytogenes was the most isolated with 13.3%, followed by Listeria ivanovii (8.0%), and Listeria seeligeri (4.4%). Only 8.4% of the Listeria monocytogenes isolates were confirmed by multiplex PCR, from which the virulence genes, iap and inlA, with characteristic band sizes of 131bp and 255bp, respectively, were detected. This study confirms that Listeria contamination of RTE foods in Abuja remains a serious public health concern and a threat to food safety. RTE food should be properly processed and handled to minimise the risk of microbial contamination and foodborne illness. Regular microbiological testing and monitoring should be conducted to ensure product safety and quality.

Keywords: *Abuja, Listeria monocytogenes, Public Health, Ready-To-Eat Foods, Virulence gene,*

1.0 INTRODUCTION

Listeria monocytogenes is an opportunistic foodborne pathogen belonging to the genus Listeria, a Gram-positive, non-spore-forming, facultatively anaerobic, rod-shaped, ubiquitous, and persistent bacterium in food processing plants (Manyi-Loh et al., 2025). Due to inadequate hygiene and manufacturing practices, this pathogen contaminates foods such as fresh or frozen fruits and vegetables, unpasteurized dairy products, sausages, and fish (Abebe et al., 2020).

Ready-to-eat (RTE) foods are defined as any food in a raw state or one that is handled, processed, mixed, cooked, or prepared and is consumed without any further processing (Bovo et al., 2015). Ready-to-eat foods are the most important source of both sporadic cases and outbreaks of listeriosis in humans (Kaptchouang-Tchatchouang et al., 2020). The disease can develop after consumption of a wide range

of foods, such as meat products (Godfray et al., 2018), dairy products, especially in ripened cheeses (Jackson et al., 2019). Animals are also susceptible to listeriosis but can carry *Listeria monocytogenes* asymptomatically (Schoder et al., 2022). Listeriosis caused by *Listeria* species is an emerging zoonotic disease requiring continuous surveillance in order to prevent outbreaks in humans (Dhama et al., 2015). The invasive form of listeriosis is observed primarily in high-risk population groups such as the elderly individuals with lowered immunity, pregnant women and newborns (Gezali et al., 2016).

According to the World Health Organisation (WHO, 2018), the rapid increase in cases of food-related diseases has been associated with poor hygiene practices, inadequate cooking, improper holding temperatures, and the use of contaminated equipment by the food outlets. In Nigeria, there have been limited reports documenting outbreaks of listeriosis in the country (Kaptchouang-Tchatchouang et al., 2020). Globally, Food Borne Diseases pose a significant public health challenge (Omotayo and Denloye, 2013). The first confirmed human case of listeriosis in Nigeria was reported in 1982 by Onyemelukwe et al. (1983). Since then, foodborne listeriosis has remained underreported and poorly documented in Nigeria. *Listeria monocytogenes* has been isolated from abroad range of raw and processed foods, including milk, cheese, ice cream, pâtés, cold cuts, smoked fish and other RTE products (Swaminathan and Gerner-Smidt, 2007; WHO, 2023). The persistence of *L. monocytogenes* in food processing environments and its ability to grow at refrigeration temperatures make it a particularly challenging pathogen in food safety management, requiring strict hygiene practices and a robust monitoring system (European Food Safety Authority and European Centre for Disease Prevention and Control, 2022). The Government of Nigeria launched its National Policy on Food Hygiene and Safety in 2000, as part of the Nigerian National Health Policy, which aimed to achieve high standards of food hygiene and safety to promote public health, reduce foodborne diseases, and ultimately eliminate the hazards associated with poor food handling practices (Bamaiyi, 2011).

Recognising these challenges, our study aimed to identify and isolate *Listeria monocytogenes* in ready-to-eat foods within Nigeria's federal capital territory (Abuja) and to assess the antibiotic susceptibility of these isolates.

2,0MATERIALS AND METHODS

2.1 Study Area

This study was conducted in the Federal Capital Territory (FCT), Abuja, Nigeria. Abuja, the capital city of Nigeria, was officially designated in 1976 as the new capital to replace Lagos, primarily due to its central location, accessibility, and potential for expansion. The territory was carved out from parts of the former Nasarawa, Niger, and Kogi States, and it serves as the seat of the Federal government. The FCT spans an area of approximately 7,315 km2, with the Abuja city proper covering 2,753 km2 and lies between latitude 8°25'N and 9°25'N of the Equator and longitude 6°45'E and 7°45'E of the Greenwich Meridian. The topography is characterized by undulating plateaus and scattered hills. It falls within the Guinea Savannah ecological zone, which features moderate climatic conditions, marked by a distinct dry season (November–March) and wet season (April–October), with average annual rainfall between 1,100 and 1,600 mm and temperatures ranging between 25°C and 35°C.

Administratively, the FCT is divided into six area councils: Abaji, located in the southern axis and bordering Kogi and Niger States; Kwali, in the southwest, known for its rural landscape and traditional crafts; Kuje, found in the central-southern belt, acting as a peri-urban transition zone; Gwagwalada, located in the west and serving as a major gateway into the FCT; Bwari, in the northern corridor, which hosts several academic institutions; and the Abuja Municipal Area Council (AMAC), which encompasses the city center and serves as the hub of administrative, diplomatic, and commercial activities. (Figure 3.1)

According to the most recent population estimate by the National Bureau of Statistics (NBS, 2022), the Federal Capital Territory has a projected population of 3,067,500 residents. The population continues to grow rapidly due to migration, urbanization, and economic opportunities in the capital city.

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 8, 2025
https://theaspd.com/index.php

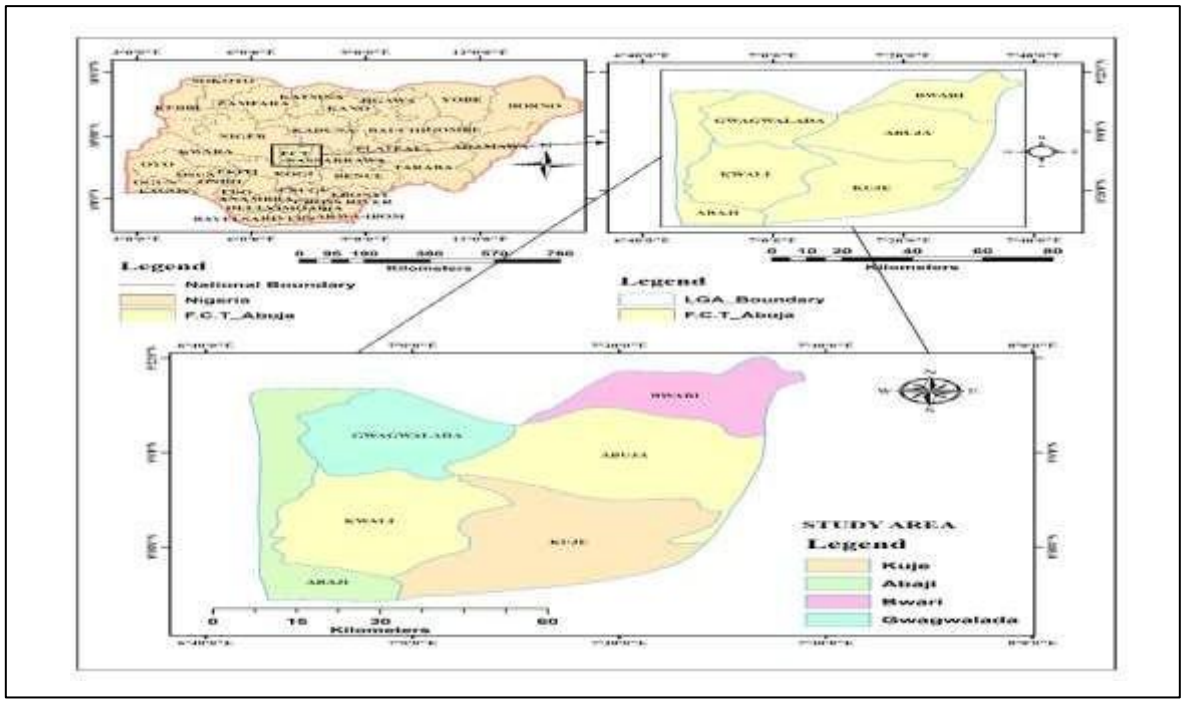

Figure 3.1: Map of Abuja showing the various area councils and the study area.
Source: Geography Department FUT Minna, 2024

2.2 Study Design

The study is a cross-sectional study.

2.3 Sample Size Determination

The sample size was calculated using the formula of Thrusfield (2007):

$$n = \frac{z^2pq}{d^2} \quad \text{Eqn (1)}$$

Where; n =sample size
p= expected prevalence
d=desired absolute precision
z=standard normal deviate for 95% confidence level (1.96)
q=1-p

- Calculation of sample size for cattle using 10.2% prevalence by Musa et al. (2017) and absolute precision of 0.04%

$$N = \frac{1.96^2 \times 10.2 \times 1\text{-}10.2}{0.04^2}$$

$$= \frac{3.842 \times 10.2 \times 9.2}{0.0016}$$

$$= 225 \text{ samples}$$

Therefore, a total of 225 milk and meat sample was collected.

2.4 Sample Collection and Transportation

Abuja, the federal capital territory of Nigeria, was used as the study area for this research. A survey was conducted in the four randomly selected area councils of the city (AMAC, Gwagwalada, Bwari, and Abaji). A total of 225 ready-to-eat (RTE) food samples comprising meat and milk products were collected from four Area Councils of the Federal Capital Territory (FCT), Abuja, Gwagwalada, Bwari, Kuje, and Abaji, based on their food market activity and population density. The samples were distributed proportionally across the selected area council based on estimated vendor density and market capacity (Arbitrary research-based proportion): therefore, 68 from Gwagwalada (30%), 56 each

from Bwari and Kuje (25% each), and 45 from Abaji (20%). The meat samples included balangu (roasted meat) and tsire (grilled meat), while the milk samples comprised unpasteurized milk (nono) and locally made soft cheese (wara). In each Area Council, meat and milk samples were collected in equal numbers where possible. Specifically, Gwagwalada contributed 17 samples each of balangu, tsire, unpasteurized milk, and soft cheese; Bwari and Kuje each contributed 14 samples of each type; and Abaji contributed 11 samples of balangu, tsire, each, and 11 samples of unpasteurized milk and 12 samples of soft cheese. Samples were collected during peak vendor hours (12:00–4:00 pm) using random and systematic walk-through sampling techniques. Approximately 30g of solid sample and 20 ml liquid sample were aseptically collected using sterile gloves and tools, placed in pre-labelled sterile containers, and immediately transferred into cool boxes containing ice packs. All samples were transported to the microbiology laboratory within 2–4 hours and refrigerated at 4°C until processing, which was carried out within 24 hours.

2.5 Isolation and Identification of Listeria

Enrichment protocol and *Listeria* isolation and identification were done using the United States Food and Drug Administration and Centre for Food Safety and Applied Nutrition method of isolation (Hitchins & Whiting, 2001). A standard enrichment protocol for microbiology food testing was used (1:10). 10g of each meat sample and 10ml of milk sample were weighed and aseptically enriched in 90ml of *Listeria* enrichment broth (Oxiod, UK) containing selective *Listeria* enrichment supplement (Oxoid, UK). The mixture was homogenized in a stomacher for 2 minutes and incubated for 24hours at 37°C.

A loopful of the enriched sample was streaked on a *Listeria* agar plate containing Listeria selective supplement (Oxoid, UK) and incubated at 37°C for 24 hours and observed for growth of colonies. Thereafter, plates were examined for brownish-black colonies with a raised convex halo, which were considered presumptive *Listeria* species. Representative presumptive colonies were subcultured on tryptic soy agar (TSA) to get pure colonies, which were stored on TSA slant. Thereafter, selected representative colonies were further identified to species level based on biochemical analysis using Gram stain, catalase, oxidase, motility and sugar fermentation tests, which included Rhamnose, Mannitol, Maltose and Sucrose. Typical colonies of different isolates were transferred to TSA plates and incubated for 24hours. Colonies from the TSA plates were preserved on TSA agar slant, stored at -4 ℃ for further tests (MacFaddin, 2000; American Society for Microbiology, 2023).

2.6 DNA Extraction using Qiagen kit Method

Following the overnight culture of each isolate, DNA extraction was done using AccuPrep® Genomic DNA Extraction Kit from Bioneer (K-3032) according to the manufacturer's instructions.

2.7 PCR Amplification and Detection of Virulence Genes

Multiplex PCR amplification of the targeted fragments of the virulence-specific genes (hlyA, inlA and iap) was done using forward and reverse primers as presented in Table 3.1 (Furrer et al., 1991). PCR set-up was done using 10µl PCR primer, 6µl Nuclease water, 2µl Primer, and 2µl DNA sample to make up a 20µl PCR reaction set-up. The reaction mixture was then heated in the thermocycler using the following amplification procedure: 95°C for 5 min for initial denaturation, followed by 35 cycles at 94°C for 1 minute for denaturation, 55-68°C for 30 seconds for annealing, 72°C for 1 minute for extension, and 72°C for 5 minutes for final extension. During this experiment, the control strain of *L. monocytogenes* ATCC 19117 was used as a positive control and nuclease (Dnase) free water as a negative control. PCR amplification products were fractionated by electrophoresis in 2.0% agarose gel (Promega, USA) in 1xTBE pH 8•3 at 6V/cm for 15-20 minutes, and visualized by staining with ethidium bromide. The Listeria monocytogenes isolates were screened for the hlyA, inlA and iap genes by multiplex polymerase chain reaction (m-PCR) using the primers and previously described conditions, as presented in Table 1 below.

Table 1: *Listeria monocytogenes* isolates specific-primers for the hlyA, inlA and iap genes and Conditions for the Multiplex PCR.

Parameters	Oligonucleotide sequences (5'-3')	Length	Genes	Reference

Forward primer (F)	CCTAAGACGCCAATCGAA' 702bp	hlyA	Furrer *et al*., 1991
Reverse primer (R)	AAGCGCTTGCAAGTCCTC		
Forward primer (F)	ACAAGCTGCACCTGTTGCAG 131bp	Iap	Furrer *et al.*, 1991
Reverse primer (R)	TGACAGCGTTGTTAGTAGCA		
Forward primer (F)	AGATCTAGACCAAGTTACAACGCTTCAG 255bp	inlA	Usman *et al.*, 2016
Reverse primer (R)	TAATATCATTTGCTGTTTTATCTGTC		
Protocol:			
Initial denaturation	95°C for 5 minutes	35 cycles	Usman *et al*., 2016
Denaturation	94°C for 1 minute		
Annealing	55-68°C for 30 seconds		
Extension	72°C for 1minute		
Final **e pair** extension	72°C for 5 minutes		

Key: bp = base pair

3.0 RESULTS

3.1 Distribution and Detection rate of *Listeria monocytogenes* in ready RTE Foods across the four Area Councils of Abuja, Nigeria.

The prevalence of *Listeria monocytogenes* recorded for the various area councils of the total number of samples collected (225), using the Isolation, Biochemical and Multiplex PCR detection methods show a prevalence of 14.7%, 7.4%, and 2.9% respectively for Gwagwalada; 25.0%, 14.3%, and 8.9% respectively for Bwari; 23,2%, 12.5%, and 7.1% respectively for Kuje; 48.9, 24.4%, and 17.8% respectively for Abaji. The overall prevalence for isolation as 26.2**%** for isolation, 13.8% for biochemical, and 8.4% for multiplex PCR. As shown in Table 2.

Table 2: Distribution and Detection Rate of *Listeria monocytogenes* in Ready-to-Eat Foods Across Four Area Councils in Abuja, Nigeria

Area Council s	Sample Collected	Isolation				Biochemical				Multiplex PCR			
		-Ve	+ve (%)	X^2	p-value	-ve	+ve (%)	X^2	p- value	-ve	+ve (%)	X^2	p- value
Gwagwa Lada	68	58	10(14.7)	13.24	0.0041	63	5(7.4)	10.29	0.0162	66	2(2.9)	11.51	0.0093
Bwari	56	42	14(25.0)			48	8(14.3)			51	5(8.9)		
Kuje	56	43	13(23.2)			49	7(12.5)			52	4(7.1)		
Abaji	45	23	22(48.9)			34	11(24.4)			37	8(17.8)		

Total	**225**	**166**	**59(26.2)**	**194**	**31(13.8)**	**206**	**19(8.4)**	

Key: X^2 = Chi-square, +Ve = Positive and -Ve = Negative

3.2 Occurrence and distribution of *Listeria* species isolated from the selected ready-to-eat food samples by area councils in Abuja, Nigeria

The presence of *Listeria* species in RTE foods from the four area councils (Gwagwalada, Bwari, Kuje, and Abaji). The overall occurrence shows that out of 225 total sample collected during the study, 59 (26.2%) were positive for *Listeria* specie, of which 11.9% were from Tsire (grilled meat), 20.3% from balangu (roasted meat), and 30.5% were from fermented unpasteurized milk (nono), and 37.3% were from soft cheese (wara). For each area council, Gwagwalada showed a 14.7% prevalence, out of which 10.0% was for tsire; 20.0% were from balangu; 30.0% were from fermented unpasteurized milk, and 40.0% were from soft cheese. Bwari had an occurrence of 25.0%, of which 14.3% were from tsire and balangu each and 28.6% were from fermented unpasteurized milk, and 42.9% were from soft cheese. While Kuje area councils showed an occurrence of 23.2%, out of which 7.7% were from tsire ;23.1 were from balangu;30.8% were from fermented unpasteurized milk, and 31.8% were from soft cheese; Abaji showed an occurrence of 48.9%, out of which 13.6% were from tsire;22.7% were from balangu, and 31.8% were from fermented unpasteurized milk and soft cheese. This occurrence and distribution of *Listeria* spp. From RTE food samples (Table 3).

Table 3: Occurrence and Distribution of *Listeria* species Isolated from Selected Ready-to-Eat Food Samples by Area Councils and Food Types in Abuja, Nigeria

Area Councils	Number of Samples Collected	Number of +ve Sample (%)	Tsire (Grilled Meat) (%)	Balangu (Roasted Meat) (%)	Unpasteurized Milk (Nono) (%)	Soft Cheese (Wara) (%)
Gwagwalada	68	10(14.7)	1(10.0)	2(20.0)	3(30.0)	4(40.0)
Bwari	56	14(25.0)	2(14.3)	2(14.3)	4(28.6)	6(42.9)
Kuje	56	13(23.2)	1(7.7)	3(23.1)	4(30.8)	5(38.5)
Abaji	45	22(48.9)	3(13.6)	5(22.7)	7(31.8)	7(31.8)
Total	**225**	**59(26.2)**	**7(11.9)**	**12(20.3)**	**18(30.5)**	**22(37.3)**

ANOVA; f = 5.75, P-value = 0.0113

3.3 Distribution of *Listeria* species isolated from the selected ready-to-eat food samples

Based on the total number of food samples collected, 225, 59 were positive for *Listeria* species, out of which 12.5% were from Tsire (grilled meat), 21.4% were from balangu (roasted meat), while 32.1% were from unpasteurized milk (nono), and 38.6% were from soft cheese (wara). *Listeria monocytogenes* isolated from 12.9%, 19.4%, 29.0%, 38.7 of each food sample respectively with an overall prevalence of 13.8% while *Listeria ivanovii* were isolated from 11.1%, 22.2%, 27.8%, 38.8%, respectively of each food sample with overall prevalence of 8.0% and *Listeria Seeligeri* were isolated from 10%, 20%, 40%, 30% respectively of each food sample with overall prevalence of 4.4% (Table 4).

Table 4: Distribution and Percentage of *Listeria* species Isolated from the Selected Ready-To-Eat Food Samples by Food Types in Abuja, Nigeria

RTE Food Sample	No Screened	No Positive for *Listeria* species (%)	*Listeria monocytogenes* (%)	*Listeria ivanovii* (%)	*Listeria seeligeri* (%)
Tsire (Grilled Meat)	56	7(12.5)	4(12.9)	2(11.1)	1(10.0)

Balangu (Roasted Meat)	56	12(21.4)	6(19.4)	4(22.2)	2(20.0)
Unpasteurized Milk (Nono)	56	18(32.1)	9(29.0)	5(27.8)	4(40.0)
Soft Cheese (Wara)	57	22(38.6)	12(38.7)	7(38.8)	3(30.0)
Total	**225**	**59(26.2)**	**31(13.8)**	**18(8.0)**	**10(4.4)**

3.4 Multiplex PCR Detection of Virulence Genes in *Listeria monocytogenes.*

The gel electrophoretic profile of PCR products targeting *Listeria monocytogenes* virulence Genes (plate I - III) represents the results of a multiplex PCR amplification of virulence genes from *Listeria monocytogenes* isolates recovered from ready-to-eat (RTE) food samples. Selected samples show single or dual bands, indicating the presence of either or both targeted genes. Isolates demonstrating bands at both 131 bp and 255 bp confirm the simultaneous presence of iap and inlA genes, which further supports their identification as virulent strains of *L. monocytogenes*. Nineteen (8.4%) out of the 31(26.2%) biochemical tested positive samples were positive for multiplex PCR to detect the virulence gene of *Listeria monocytogenes* (Plates I, II, III). The virulence gene iap gene and inlA gene were among the virulence genes detected based on a characteristic band size of 131bp and 255bp, respectively, shown on different lanes.

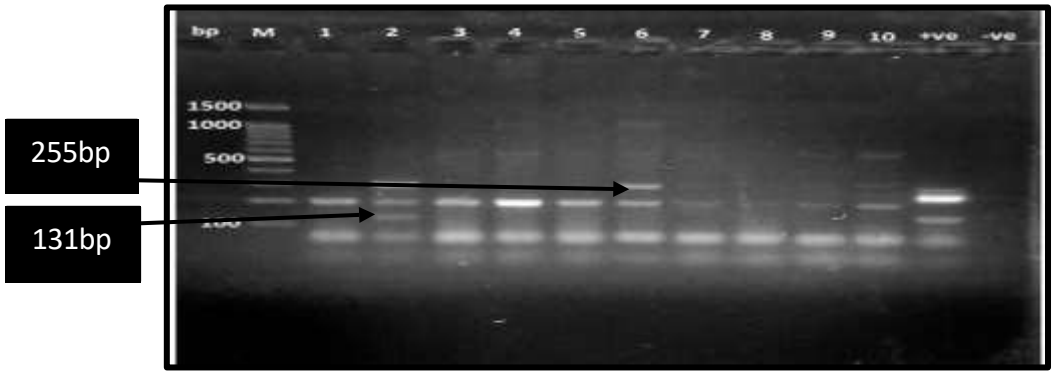

Plate I: Gel Electrophoretic Profile of PCR Products Targeting Listeria Monocytogenes Virulence Gene (Sample1-10)

Well loaded with 100bp plus DNA ladder is labelled as M; Positive and Negative control are in well +ve and -ve respectively. Lane 2 was identified as positive for iap (131) and inlA (255) gene.

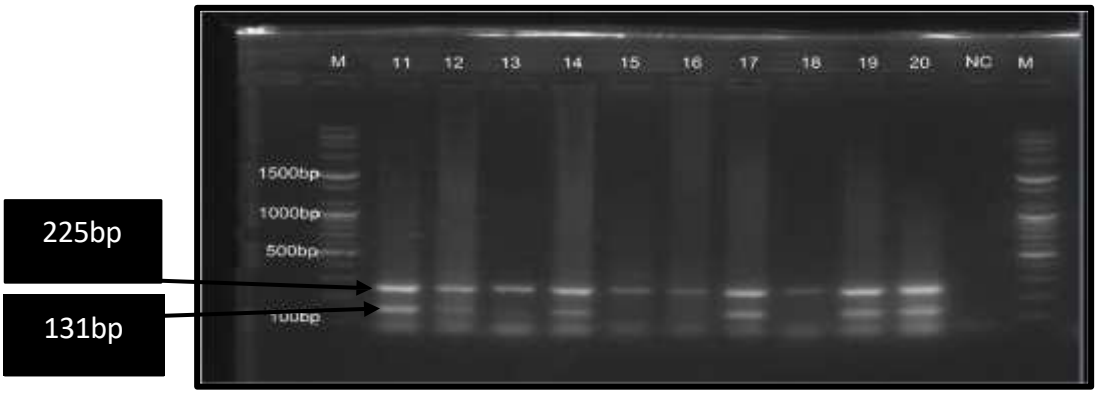

Plate II: Gel Electrophoretic Profile of PCR Products Targeting *Listeria Monocytogenes* Virulence Gene (Sample11-20)

Well loaded with 100bp plus DNA ladder is labelled as M; Negative control is in well NC. Lane 11 to 20 was identified as positive for iap (131) and inlA (255) gene.

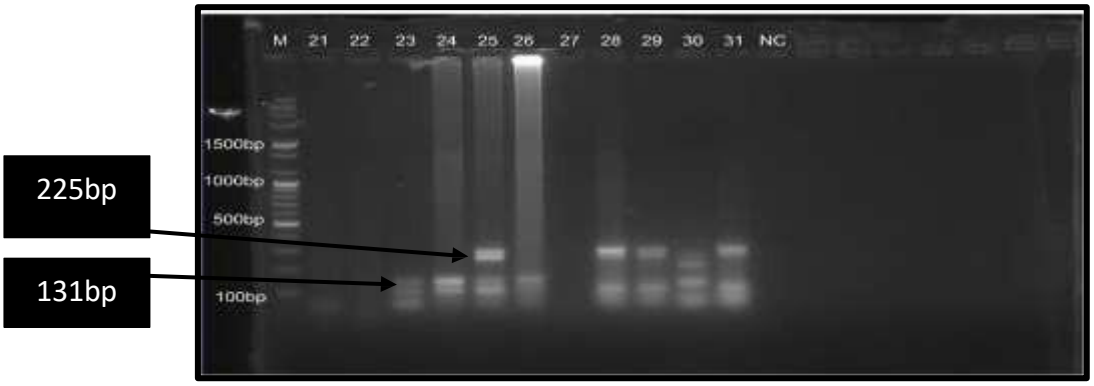

Plate III: Gel Electrophoretic Profile of PCR Products Targeting *Listeria Monocytogenes* Virulence Gene (Sample 21-31)

Well loaded with 100bp plus DNA ladder is labelled as M; Negative control is in well NC. Lane 23,24.25,26,28,29,30,31 was identified as positive for iap (131) and inlA (255) gene.

4.0 DISCUSSION

The study showed that the occurrence of *Listeria monocytogenes* was higher in Abaji, recording the highest detection rates across all methods: isolation, biochemical and multiplex PCR. In contrast, Gwagwalada showed the lowest occurrence. Chi-square test revealed statistically significant association between the area councils and the detection of *Listeria monocytogenes* at all three diagnostic test outcomes (isolation; p= 0.0041, biochemical; p= 0.0162, multiplex PCR; p= 0.0093), indicating that *L. monocytogenes* detection was not randomly distributed but influenced by geographic or environmental factors and location where the RTE foods was collected matters, and the significantly higher detection rates in Abaji could be attributed to the fact that Abaji is semi-rural area with a large agrarian population, and many residents process and sell food in informal or unregulated settings, especially traditional ready to eat foods like balangu, tsire, nono and wara which often lack standardized hygienic practices compared to other urban areas like Gwagwalada. Moreso, open defecation and improper refuse disposal are still common, and these increase the microbial loads in the environment. *Listeria* can survive in soil, water, and decaying vegetables, so food processed or sold in such environments has a higher risk of contamination. This finding implies that the RTE foods sold in the Abaji area councils are more likely to be exposed to *Listeria* contamination; therefore, residents are likely at higher risk of contracting listeriosis.

In comparison to other studies, the overall prevalence of 26.2% for *Listeria* species was lower than 33.8% reported by Eruteya *et al.* (2014) in Port Harcourt and 49.1% by Okorie et al. (2020) in Enugu State, but slightly higher than 21.5% prevalence reported by Chukwu *et al.* (2019). But similar to 28% by Ndahi *et al.,* 2014 in Zaria town and 27.5% by Olaniyan *et al.,* 2022 in North West Zaria. While the prevalence recorded using the biochemical detection method in this study was 13.8%, which was similar to 12.3% by Adetunji and Isola (2011) in Oyo State. and also, similar to 14.7% when compared to a study conducted in South Africa by Matle *et al.,* 2019.

For PCR-based detection in our study, a prevalence of 8.4% for *Listeria monocytogenes* recorded was similar to 7.8% prevalence by Peter *et al.,* 2016 in Makurdi, but much lower than 20% reported Ebakota *et al*., 2018 in southern Nigeria. This difference in prevalence could be attributed to the number and type of ready-to-eat food processed or environmental factors.

In other African countries, *Listeria monocytogenes* in RTE Foods also varies. In South Africa, a prevalence of 15.8% in RTE meat was reported by Mokoena *et al*. (2020). Notably, during the major listeriosis outbreak between 2017 and 2018, which was linked to processed meat. This finding is comparable to or slightly higher than the values obtained in this study. On a global scale, developed countries typically report lower prevalence rates due to stringent regulatory controls and improved Food safety practices. The European Food Safety Authority (2022) reported 1.4% of *Listeria monocytogenes* detection in RTE Food across the European Union, which is much lower than that

obtained in this study. In the USA, routine surveillance data from the USDA and CDC show *Listeria monocytogenes* prevalence ranging from 0.5% - 2% in packed or industrially processed RTE Foods. In Asia higher prevalence rate has been observed in the informal food sector, such as 6.6% chicken meat and dairy products in China by Li *et al.,* 2018 and 7.2% in India by Kumar *et al.,* 2017. Which was still lower than that obtained in this study. This could be attributed to geographic distribution and stringent hygienic practices. The presence of *Listeria* species among the ready-to-eat foods sampled showed that soft cheese (wara) had the highest contamination rate, followed by fermented unpasteurized milk (nono), balangu (roasted meat), and tsire (grilled meat). This finding aligns with previous studies, such as Ifeanyi et al. (2013); Kelly *et al.* (2018), which have consistently identified soft cheese and fermented unpasteurized milk as high-risk foods for *Listeria* contamination due to their high moisture content, neutral pH, and poor handling or refrigeration conditions, especially in informal markets. The highest prevalence of *Listeria* species-positive food samples was observed in Abaji, followed by Bwari, Kuje, and Gwagwalada. This trend reflects the relatively poorer sanitary practices and regulatory oversight in more rural and peri-urban areas like Abaji, where open markets and inadequate storage practices may predispose food to microbial contamination. Analysis of Variance (ANOVA) revealed a statistically significant difference (0.0113) in the level of *Listeria* contamination among the different ready-to-eat foods sampled, meaning not all food types have the same level of contamination. This suggests some ready-to-eat foods, particularly soft cheese and fermented unpasteurized milk, have significantly higher contamination levels. This difference could be attributed to factors such as poor hygienic handling/post-processing contamination, storage temperature abuse, especially for dairy products, cross-contamination from equipment or handlers, and lack of pasteurization in dairy- based foods like milk and cheese.

In comparison to other studies, the occurrence recorded in this study for soft cheese, which was 37.3%, was higher than 30% reported by Kelly *et al*. (2018) in the USA. The prevalence in raw milk of 30.5% found in this study was higher than 8% in dairy products reported by Musa *et al.* (2022) in Kaduna State. While Ifeanyi *et al.* (2013) found 17.8 % in raw milk in Enugu state, which is lower than the present study but understandable given that their sample came from more regulated urban retail points. Bello *et al.* 2014 reported a prevalence of 18% in roasted meat (balangu) and 8% in Tsire in Kano, Nigeria, which is also lower than 20.3% prevalence found in this study. This study recorded a prevalence of 13.8% *Listeria monocytogenes* from a variety of RTE Food samples (tsire, balangu, unpasteurized milk, and soft cheese). The dominance of this species is expected, as it is the most pathogenic and frequently isolated *Listeria* species in foodborne outbreaks. However, the presence of *L. ivanovii* and *L. seeligeri*, though less common, still poses public health concerns, particularly for immunocompromised individuals. The prevalence is higher 11.3% Bello *et al*. (2014) in Kano state, and also higher than 8% and 10.2% reported by Musa *et al.* (2022) in Kano State and Eze *et al.* (2020) in Nsukka, respectively. But similar to 12.5% by Olaniyan *et al.* (2022) in Zaria, Kaduna state and lower than 17.5% reported by Ifeanyi *et al*. (2013). While the prevalence recorded for *L. ivanovii* aligns with Salihu et al. (2008), who found *L. ivanovii* in 7.1% of RTE meat in Sokoto State. But lower than 9.2% by Kawo and Bello, 2016, in Kano state. Many Nigerian studies do not focus on *L. ivanovii*, making this finding important. And for *L. seeligeri* is also notable because this species is rarely reported in Nigerian food studies, possibly due to a lack of molecular speciation in some older works. It is generally considered less pathogenic but still relevant for food hygiene assessments.

Globally, *L. ivanovii* and *L. seeligeri* are rarely found in food due to advanced hygiene and screening systems. In Europe, *L. monocytogenes* prevalence is typically <2 %, due to strict food safety protocols (European Food Safety Authority, 2022). While in South America, *L. monocytogenes* was found in 10-25% of cheese and raw milk in Brazil, aligning with this study.

In Asia, a study by Jamali et al. (2013) in India found *L. monocytogenes* in 20-30% in dairy and meat products, which is higher than recorded in this study. And also, in a study in China by Li *et al.* (2018), a prevalence of 15.2%, which closely matches this study.

In this study,19 (8.4%) out of 31 biochemically positive *Listeria* isolates were confirmed to be *L. monocytogenes* by multiplex PCR targeting virulence genes. The two virulence genes detected were iap

and inlA, with amplicon sizes of 131bp and 255bp, respectively. This molecular confirmation demonstrates the enhanced specificity of PCR over conventional biochemical methods for detecting pathogenic *L. monocytogenes* strains. The relatively low PCR confirmation rate (8.4%) compared to biochemical positive suggest that some biochemically identified isolates may belong to non-pathogenic *Listeria* species or that biochemical tests can yield false positives. This underscores the importance of molecular techniques in accurately identifying pathogenic strains, especially those posing significant food safety risks. In comparison with other studies, this is much lower than 17% reported by Ifeanyi *et al*. (2013) in raw milk from urban retail outlets using conventional culture and PCR methods, and Aditunji and Isola (2011) reported a prevalence of 10% in cheese samples in southwest Nigeria. Compared to this prevalence, these studies showed higher detection rates, possibly due to sample type, sampling area, or detection genes used. In conclusion, the prevalence recorded revealed that there is a considerable level of contamination in RTE foods circulating in Abuja, posing a serious threat to public health, especially among vulnerable populations. The area council's distribution showed that Abaji had the highest prevalence at each detection stage, whereas Gwagwalada had the lowest. This variation likely reflect difference in food handling practices, sanitary conditions, market structures, and regulatory enforcement across the area councils. The statistically significant association between the area councils and diagnostic outcomes emphasize that geography and local practices influence food safety outcomes. The decline in detection rates from isolation to PCR highlights the potential for false positives when relying solely on culture and biochemical methods. While culture methods are useful for screening, PCR offers greater specificity and accuracy, affirming the importance of molecular tools in foodborne pathogen surveillance. Two key virulence genes of *Listeria monocytogenes*, iap and inlA, were detected among the confirmed isolates, with respective band sizes of 131bp and 255bp. The detection of these genes highlights the pathogenic potential of the isolate, posing a significant food safety risk, especially to vulnerable populations such as the elderly, immunocompromised individuals, and pregnant women. The study confirms the presence of virulence genes of *Listeria monocytogenes* in commonly consumed RTE foods in Abuja, linking human health, food safety, and environmental sanitation to prevent the transmission and escalation of drug- resistant *Listeria* strains through the food chain.

Acknowledgement

The entire academic staff of ACEMFS Federal University of Technology, Minna, and the Faculty of Veterinary Medicine, University of Abuja. We appreciate the contribution you all made towards the success of this work.

Author contributions

Hajara Asabe Muhammad designed the experiment. Adamu Yusuf Kabiru, Jeremiah David Bala, and Ocheme Boniface Ocheme supervised the whole research work and provided the necessary materials. We were all involved in the discussion, as well as read and approved the final manuscript.

Conflict of interest

There is no conflicting interest.

REFERENCES

1) Manyi-Loh, C.E., & Lues, R. (2025). Listeria monocytogenes and Listeriosis: The Global Enigma. Foods, 14(7), 1266.
2) Abebe, E., Gugsa, G., & Ahmed, M. (2020). Review of major food-borne zoonotic bacterial pathogens. Journal of Tropical Medicine, (1), 4674235.
3) Bovo, F. (2015). Definition of Food Safety Criteria for Bacteria Food-Borne Pathogens in Ready-to-Eat Products.
4) Kaptchouang-Tchatchouang, C.D., Fri, J., De Santi, M., Brandi, G., Schiavano, G.F., Amagliani, G., & Ateba, C. N. (2020). Listeriosis outbreak in South Africa: comparative analysis with previously reported cases worldwide. Microorganisms, 8(1), 135.
5) Godfray, H. C. J., Aveyard, P., Garnett, T., Hall, J. W., Key, T. J., Lorimer, J., & Jebb, S. A. (2018). Meat consumption, health, and the environment. Science, 361(6399), eaam5324.
6) Jackson, K.A., Gould, L.H., Hunter, J.C., Kucerova, Z., & Jackson, B. (2019). Listeriosis outbreaks associated with soft cheeses, United States, 1998–2014. Emerging infectious diseases. 24(6), 1116.
7) Schoder, D., Guldimann, C., & Märtlbauer, E. (2022). Asymptomatic carriage of Listeria monocytogenes by animals and humans and its impact on the food chain. Foods, 11(21), 3472.
8) Dhama, K., Karthik, K., Tiwari, R., Shabbir, M. Z., Barbuddhe, S., Malik, S. V. S., & Singh, R. K. (2015). Listeriosis in animals, its public health significance (food-borne zoonosis) and advances in diagnosis and control: a comprehensive

review. Veterinary Quarterly, 35(4), 211-235.
9) Gezali, A., Feyissa, B., & Kula, J. (2016). Listeriosis and its public health importance: a review. Glob Vet, 17, 52-62.
10) World Health Organisation (2018). Joint FAO/WHO Microbial Risk Assessment Series: Risk assessment of Listeria monocytogenes in ready-to-eat foods: Technical report. (Microbiological risk assessment series; no. 5), Listeriosis (WHO international). (Accessed October 20th, 2023).
11) Omotayo, R., & Denloye, S. (2013). The Nigerian Experience on Food Safety Regulations. FAO/WHO Global Forum of Food Safety Regulations; Marakesh, Morocco. pp. 28–30.
12) Onyemelukwe, G.C, Lawande, R.V., Egler, L.J., & Mohammed, I. (1983). Listeria monocytogenes in Northern Nigeria. Journal of Infectious Diseases. 6, 141-145.
13) Swaminathan, B., & Gerner-Smidt, P. (2007). The epidemiology of human listeriosis. Microbes and Infection, 9(10), 1236–1243.
14) World Health Organisation. (2023). Listeriosis – Key facts. https://www.who.int/news-room/fact-sheets/detail/listeriosis
15) European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC). (2022). The European Union One Health 2021 Zoonoses Report. EFSA Journal, 20(12), e07666. https://doi.org/10.2903/j.efsa.2022.7666
16) Bamaiyi, P. (2011). 'Emerging food pathogens: A case study of E. coli 0104:H4'. Continental Journal of Animal Veterinary Research. 3, 21–27.
17) Nigeria National Bureau of Statistics (2022). Abuja population. r/depopulation review.com, accessed 13/03/2023.
18) Hitchins, A.D., & Whiting, R.C. (2001). Food-borne Listeria monocytogenes risk assessment: Additional Contamination. 18(12), 1108-17.
19) MacFaddin, J.F. (2000). Biochemical Tests for Identification of Medical Bacteria, 3rd ed. Lippincott Williams & Wilkins, 2000. (Catalase, oxidase, motility media, detailed methods).
20) American Society for Microbiology. (2023). Carbohydrate fermentation test protocol. ASM Science. https://asm.org/protocols/carbohydrate-fermentation-test-protocol.
21) Furrer, B., Candrian, V., Hofelein, C., & Luthy, J. (1991). Detection and identification of Listeria monocytogenes in cooked sausage products and in milk by in vitro amplification of haemolysin gene fragments. Journal of Applied Bacteriology. 70, 372-379.
22) Usman, U.B., Kwaga, J.K.P., Kabir, J., Olonitola, O.S., Radu, S., & Bande, F. (2016). Molecular characterisation and phylogenetic analysis of Listeria monocytogenes isolated from milk and milk products in Kaduna, Nigeria. Canadian Journal of Infectious Diseases and Medical Microbiology. 14, 1- 7.
23) Eruteya, O. C., Odunfa, S. A., & Lahor, J. (2014). Listeria spp. in raw cow and goat meat in Port Harcourt, Nigeria. Br Biotechnology Journal, 4(2), 204-214.
24) Okorie-Kanu, O. J., Anyanwu, M. U., Ezenduka, E. V., Mgbeahuruike, A. C., Okorie-Kanu, C. O., Ugwuijem, E. E., & Vidal, M. (2020). Occurrence and antibiogram of Listeria species in raw pork, beef, and chicken meats marketed in Enugu State, Southeast Nigeria. Veterinary World, 13(2), 317.
25) Chukwu, O. O., Nwaiwu, O., & Okoli, I. C. (2019). Prevalence of Listeria monocytogenes and other Listeria species in RTE meat products in Enugu State, Nigeria. African Journal of Microbiology Research, 13(8), 147–154. https://doi.org/10.5897/AJMR2018.9017
26) Ndahi, M.D., Kwaga, J.K.P., Bello, M., Kabir, J., Umoh, V.J., Yakubu, S.E., & Nok, A.J. (2014). Prevalence and antimicrobial susceptibility of Listeria monocytogenes and methicillin-resistant Staphylococcus aureus strains from raw meat and meat products in Zaria, Nigeria. Lett Applied Microbiology. 2014;58(3):262–269. doi: 10.1111/lam.12183.
27) Olaniyan, S. E., Kwaga, J., Saidu, A. S., & Usman, U. B. (2022). Multiple Anti-microbial Resistance Profile and Molecular Detection of Some Virulence Genes of Listeria monocytogenes Isolated from Fresh Raw Meat Retailed in Zaria, Northwestern Nigeria. Afro-Egyptian Journal of Infectious and Endemic Diseases, 12(1), 3-15.
28) Adetunji, V. O., & Isola, T. O. (2011). Antibiotic resistance of Escherichia coli, Listeria, and Salmonella isolates from retail meat tables in Ibadan municipal abattoir, Nigeria. African Journal of Biotechnology, 10(30), 5810–5814. https://doi.org/10.5897/AJB10.2226
29) Matle, I., Mbatha, K.R., Lentsoane, O., Magwedere, K., Morey, L., & Madoroba, E. (2019). Occurrence, serotypes, and characteristics of Listeria monocytogenes in meat and meat products in South Africa between 2014 and 2016. J Food Saf. 2019;39(4):1–14.
30) Peter, A., Umeh, E.U, Azua, E.T, & Obande, G.A. (2016). Prevalence and Antimicrobial Susceptibility of Listeria monocytogenes Isolated from Beef, Pork and Chicken Sold in Makurdi Metropolis. MRJI. 2016;14(11):1–7.
31) Ebakota, D.O., Abiodun, O. A., & Nosa, O. O. (2018). Prevalence of antibiotic-resistant Listeria monocytogenes strains in Nigerian ready-to-eat foods. Food Safety, 6(3), 118-125.
32) Mokoena, M.P., Magwedere, K., Zewotir, T., & Hemberger, M.Y. (2020). Prevalence of Listeria monocytogenes in RTE meat during the 2017–2018 listeriosis outbreak in South Africa. International Journal of Environmental Research and Public Health, 17(11), 3892. https://doi.org/10.3390/ijerph17113892

33) Li, W., Bai, L., Ma, X., Guo, Y., Fu, P., Yao, X., & Li, X. (2018). Characterisation of Listeria monocytogenes isolated from retail ready-to-eat food in China. Food Control, 92, 316–321. https://doi.org/10.1016/j.foodcont.2018.04.051
34) Kumar, S., Nehra, K., & Yadav, S. (2017). Occurrence of Listeria monocytogenes in street-vended foods and its antibiotic resistance in India. Journal of Food Science and Technology, 54(5), 1464–1471.https://doi.org/10.1007/s13197-017-2606-1
35) Ifeanyi, C. I. C., Bassey, B. E., & Ikeneche, N. F. (2013). Prevalence and antibiotic susceptibility pattern of Listeria monocytogenes among pregnant women in Nsukka, Nigeria. African. Journal of Microbiology Research, 7(11), 948–952.
36) Kelly, A. J., Gould, L. H., Hunter, J. C., Kucerova, Z., & Jackson, B. R. (2018). Listeriosis outbreaks associated with soft cheese, United States, 1998–2014. Emerging Infectious Diseases, 24(6), 1116–1118.
37) Musa, T. L., Abubakar, M. B., & Sani, R. A. (2022). Prevalence and antibiotic resistance of *Listeria monocytogenes* in unpasteurized milk in Kano State, Nigeria. Nigerian Journal of Animal Production, 49(1), 61–67.
38) Bello, A. M., & Kawo, A. H. (2014). Isolation of Listeria monocytogenes recovered from some ready-to-eat foods sold in Kano, North-Western Nigeria. Bayero Journal of Pure and Applied Sciences, 7(2), 8-12.
39) Eze, E. A., Nwaiwu, O., and Eze, V. C. (2020). Prevalence and antibiotic susceptibility profile of Listeria monocytogenes isolated from ready-to-eat foods in Nsukka, Nigeria. African Journal of Clinical and Experimental Microbiology, 21(1), 32–40. https://doi.org/10.4314/ajcem.v21i1.6
40) Salihu, M.D., Junaidu, A.U., Magaji, A.A., & Ahmed, A. (2008). Isolation and prevalence of *Listeria monocytogenes* in milk and milk products in Sokoto State, Nigeria. Journal of Animal and Veterinary Advances, 7(6), 678–681.
41)Kawo, A. H., & Bello, A.M. (2016). Antimicrobial susceptibility profile of Listeria species isolated from some ready-to-eat foods sold in Kano, North-Western Nigeria. Bayero Journal of Pure and Applied Sciences, 9(1), 217-222.gy (pp. 195-222).
42) Jamali, H., Radmehr, B., & Thong, K.L. (2013). Prevalence, characterisation, and antimicrobial resistance of Listeria species and Listeria monocytogenes isolates from raw milk in farm bulk tanks. Food Control, 34(1), 121–125.