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INCIDENCE OF *Listeria monocytogenes* FROM DIFFERENT FOOD SAMPLES IN YOLA

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ABSTRACT

Listeria monocytogenes causes food-borne listeriosis in humans. Vegetables and animal-based foods facilitate transmission and contamination if mishandled. The research aims to isolate and identify *L. monocytogenes* from different food samples in the study area. The Food and Drug Administration, Bacteriology and Analytical Methods (FDA, BAM) were utilized to isolate *L. monocytogenes*. A total of 120 food samples were examined, consisting of 10 samples each of cabbage and fruit salads, as well as 20 samples each of fresh fish, meat, frozen chicken, ice cream, and yoghurt. These samples were purchased at random from various shops and markets in Yola at different times. The identification of isolates was performed using macroscopy, microscopy, biochemical tests, and polymerase chain reaction (PCR) amplification of the 16S rRNA gene. Out of the 120 food samples analyzed, 7 (5.8%) yielded growth of *L. monocytogenes* alongside other Listeria spp., with a high rate of *L. monocytogene* isolation from frozen chicken (3.5%) and cabbage (2.7%). Raw meat and yoghurt samples also yielded 1 (0.8%) growth of *L. monocytogenes* each. Through the examination of various food samples, it was identified that *L. monocytogenes* were present. This can be seen as an opportunity to take proactive measures to ensure the safety of consumers, particularly the elderly, immunocompromised individuals, and pregnant women in the study area**.** Minimizing the potential risk of L. monocytogenes can promote a healthier community.

Keywords: 16S rRNA, Analyzed, Food samples, genomic DNA, *L. monocytogenes***,** Listeria enrichment broth, Listeria Selective agar, Prevalence

INTRODUCTION

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive, motile, non-spore-forming, facultative anaerobic short bacilli about 0.2 × 0.5 to 2μm, widely distributed in nature, and frequently isolated from a variety of food sources, soil, mud, decaying vegetation, contaminated silage, and faecal materials (Buchanan *et al*., 2017). *L. monocytogenes* can thrive and grow in a cold, moist environment and survive in the intestinal tract of at least 37 species of mammals, both domestic and wild. It can grow over a wide range of temperatures from 1°C to 45°C, and it also survives in harsh conditions, such as a wide pH range (4.4 and 9.4), high salinity (40% w/v), low water activity (aw) (≥0.92), and hypoxic conditions (Singh *et al*., 2019).

L. monocytogenes is often present in certain processed foods like dairy products and processed meats. This is due to the possibility of contamination after processing. Foods from animals, including red meat, poultry, dairy products, fish, and seafood, are especially vulnerable and can be easily infected if not properly stored or handled, even if refrigerated. (Desai *et al*., 2019). Contamination of meat and meat products by *L. monocytogenes* was reported in various developed and developing countries (Arsalan and Baytur, 2018). With manufactured ready-to-eat foods being consumed in increasing quantities, *L. monocytogenes* has become recognized as an imperative opportunistic human food-borne pathogen (Mahendra *et al.,* 2017). Although *L. monocytogenes* is infective to all human populations, it has a propensity to cause especially severe problems in pregnant women, neonates, the elderly, and immunosuppressed individuals (Kyoui *et al.*, 2014; Soni *et al.*, 2014).

Listeriosis outbreaks have occurred globally, and certain foods have been linked to this disease. High-risk foods that are associated with listeriosis are those that can be contaminated with *L. monocytogenes*, support its growth to high levels, and do not require further cooking at home. These foods are usually refrigerated, have an extended shelf life, and are contaminated with high levels of the pathogen at the time of consumption (WHO, 2018).

In the initial stages of infection, listeriosis in humans often shows non-specific flu-like symptoms such as chills, fatigue, headache, and muscular and joint pain, as well as gastroenteritis. However, if not treated with appropriate antibiotics, it can progress to septicemia, meningitis, encephalitis, abortion, and, in severe cases, death. (Singh *et al.*, 2019; Vazquez-Boland *et al.*, 2001). According to research studies conducted by Mook *et al.* (2011) and Tolba *et al.* (2012), *Listeria monocytogenes*, a foodborne pathogen, has a hospitalization rate of 91% and an average mortality rate of 30%. These rates are significantly higher than those of other common foodborne pathogens, such as *Salmonella enteritidis* (with a mortality rate of 0.38%), *Campylobacter* species (0.02-0.1%), and *Vibrio* species (0.005-0.01%). This indicates that *L. monocytogenes* is a highly severe disease-causing pathogen. (Altekruse *et al.*, 1997; Mead *et al*., 1999).

MATERIALS AND METHODS

Study Area and Sample Collection

A total of 120 food samples, comprising 10 each of cabbage and fruit salads and 20 each of fresh fish, meat, frozen chicken, ice cream, and yoghurt, were purchased from randomly selected retail shops and markets at different times. These shops and markets are within Yola, the capital of Adamawa State, in the north-eastern part of Nigeria. The samples were transferred to the microbiology laboratory at Modibbo Adama University Yola in sterile polyethene bags. Before examination, the bags were placed on ice and kept in a refrigerator at 4°C, as stated by Oyelami *et al.* (2018).

Sample Processing
Under sterile laboratory conditions, 25g of each of the samples were cut into small sections using sterile blades and processed separately by homogenization using a sterile blender in 50 ml of phosphate-buffered saline (PBS) and sterile distilled water for two cycles (each cycle lasting for 45 seconds with 10 seconds between cycles). 100 ml of melted ice cream and yoghurt were homogenized using the same procedure (Uta *et al.* 2005).

Enrichment of *L. monocytogenes*
The isolation of *Listeria monocytogenes* was done using the Food and Drug Administration (FDA) bacteriological and analytical method procedure, which involves the enrichment of the sample with a Listeria enrichment supplement for 48 hours. Using a sterile syringe, 1 ml of the homogenized sample was added to 9 ml of buffered Listeria enrichment broth (Oxoid, UK) supplemented with Listeria selective enrichment supplement (Oxoid, UK), prepared following the manufacturer's instructions, and incubated for 48 hours at 30 ^{o}C as described by Uta *et al.* (2005).

Isolation *of L. monocytogenes*
Listeria selective agar (Oxford formulation) (Oxoid, UK) plates prepared using the manufacturer's instructions were inoculated by spreading l0µL taken from the enrichment broth and incubated for 24 hours at 37^{0}C.

***L. monocytogenes* differentiation**
Colonies that conform to the morphology of *Listeria monocytogenes* on the Listeria selective media (blackish colonies with sunken centres) were further streaked on nutrient agar plates and incubated aerobically at 37°C for 24 hours to obtain a pure culture for further identification as described by Oyelami *et al.* (2018).

Identification of *L. monocytogenes* isolates
Gram Staining
A smear of the suspected colonies was spread onto a clean, grease-free glass slide and then heat-fixed by passing the slide over a flame. The slide was then stained with crystal violet for a minute and rinsed with clean tap water. The smear was flooded with iodine solution for 1 minute and rinsed with tap water before decolorizing with 75% alcohol for 30 seconds and being rinsed with clean tap water. The slide was counterstained with a secondary stain (Safranin solution) for 30 seconds, rinsed with tap water, and allowed to drain and air-dry, after which it was examined microscopically under the oil immersion objective. An organism that appears purple is gram-positive, while a gram-negative organism appears pink when viewed under an oil immersion objective (Owuama, 2015). Gram-positive rod observations indicated that the organism may be *L. monocytogenes.*

Catalase test
About 2-3 drops of 3% H_2O_2 were placed on a clean, grease-free glass slide. A sterile wire loop was used to pick and transfer a colony of the suspected *L. monocytogenes* isolate from an overnight (24 hours) culture onto the H_2O_2 drops on the slide and observe. Bubble formation within 10 seconds indicates a catalase-positive test (Muhammad *et al.,* 2020).

Oxidase Test
A piece of filter paper was placed in a clean Petri dish, and 2 to 3 drops of freshly prepared oxidase reagent were added. A piece of stick or glass rod was used to remove a colony of the test organism from a 24-hour culture and smeared on the filter paper. The development of a blue-purple colour within 10 seconds indicates an oxidase-positive test, while the absence of a blue-purple colour within 10 seconds indicates a negative oxidase-positive test (Muhammad *et al.,* 2020).

Methyl Red Test
About 5 drops of methyl red indicator were added to the 4-6-hour-old peptone culture of the suspected colony. The appearance of a bright red colour was considered a positive result (Muhammad *et al.,* 2020).

Motility test
A loop full of the suspected colony was inoculated in 5 mL of peptone water in a screw-capped bottle and incubated at 20^{0} °C for 4 hours. Vaseline jelly was rubbed around the cavity of a hanging drop glass slide. A drop of the peptone water culture was placed on a cover slip. The hanging drop slide with a ring of Vaseline at its centre was placed over the drop of peptone slide; it was then quickly inverted and viewed under the microscope using a 40x and 100x objective lens (Owuama 2015). A tumbling motility was observed with *L. monocytogenes*.

Christie, Atkins, and Munch-Petersen (CAMP) Test
The CAMP test was undertaken using *S. aureus* to distinguish *L. monocytogenes* from other Listeria species. A standardized inoculum of *S. aureus* was streaked in a single-part line across a sheep blood agar plate. The standardized inoculum of the suspected *L. monocytogenes* was then streaked at right angles, 2 mm apart, with *S. aureus*. The plates were incubated at 37 °C for 24 h. A zone of enhanced β-haemolysis resembling an arrowhead, circle, or rectangle indicates a CAMP-positive reaction due to the synergistic effect of their hemolysins. The absence of enhanced β-haemolysis indicated a CAMP-negative reaction, as described by Osman *et al.* (2016)**.** *L. monocytogenes* are CAMP-positive for *Staphylococcus aureus*

Carbohydrate Utilization Test
For carbohydrate utilization detection, 1 ml of a standardized inoculum was transferred into test tubes containing 5 ml of xylose and incubated at 37 ^{o}C for 5 days. Positive reactions were indicated by a yellow 5 colour (acid formation) and occurred mostly within 24 to 48 hours (Osman *et al.,* 2016). The same procedure was used to test for glucose, rhamnose, and mannitol.

Molecular confirmation of the *L. monocytogenes* isolates
DNA extraction
Freshly grown *L. monocytogenes* colonies collected from the surfaces of 24-hour nutrient agar culture plates were used for DNA extraction (Maria *et al.,* 2018). Qiagen QIAamp DNA mini kit was used and the DNA extraction procedure was according to the manufacturer's instructions.

DNA Quantification
Nanodrop One (Thermo Scientific) was used to determine the quantity of the extracted *L. monocytogenes* DNA in ng/µL. Its purity was also determined by its absorbance at the A260/A280nm wavelength.

Polymerase Chain Reaction (PCR) Amplification of 16S rRNA
The 16S rRNA gene was amplified for all the *L. monocytogenes* isolates using the universal primers 16SF (AGAGTTTGATCCTGGCTCAG) and 16SR (GGTTACCTTGTTACGACTT) (Mona *et al.,* 2019).

Amplification was performed in a 25µL reaction with 12.5µL master mix (thermos), 10 ng of genomic DNA, and 10 pmol each of forward and reverse primers. The PCR program was performed in a thermo scientific thermo cycler as follows: Initial denaturation at 95 °C for 5 minutes then 95 °C followed by 34 cycles of 95ºC for 1 minute, annealing at 55 °C for 1 minute, extension at 72 °C for 1 minute, and final extension at 72 °C for 10 minutes, as described by Mona *et al.* (2019).

Separation of the 16S rRNA PCR Product

Electrophoresis' separation of the PCR product was performed on 1.5% agarose gel, stained with ethidium bromide. PCR products and a 100-bp DNA marker (5 µL each) were loaded into the gel's separate wells. The process was performed at 110 V and 1.81 A for 1 hour and 15 minutes, and the gel was observed under ultraviolet light. The molecular weight of the PCR product was determined by comparison with a standard 100 bp molecular weight marker as described (Osman *et al.*, 2016); a reaction mixture with no DNA template was incorporated as a negative control.

RESULTS AND DISCUSSION

Microscopic and Biochemical Characteristics of Listeria Species Isolated

The characteristic gram-positive, coccobacilli or short rod-shaped organisms that are catalase positive and oxidase negative, Methyl red-Voges-Proskauer positive, showing typical tumbling motility at 20–25 °C, that do not ferment xylose, that are β-haemolytic on 5% SBA, and are CAMP positive with *S. aureus* were considered *Listeria monocytogenes*. Table 1 shows the microscopic and biochemical characteristics of the Listeria species isolated.

Prevalence of *L. monocytogenes* isolated from different food samples

A total of twenty-one (21) (17.5%) isolates of Listeria species were identified from 120 different commercial food samples. Out of the 21 isolates, eleven 7 (5.8%) were identified as *L. monocytogenes.* Therefore, the percentage prevalence of *L. monocytogenes* from 120 different samples was 5.8%. Table 2 shows the percentage distribution of *L. monocytogenes* obtained from the different food samples.

Molecular Characteristics of *L. monocytogenes*

Extracted DNA Quantification and Purity

Nanodrop spectrophotometric qualification of the extracted *L. monocytogenes*' whole genome DNA revealed the quantity and quality of the DNA which range from 124-131ng/µl and absorbance from 1.85-1.92 at A260/A280nm wavelength. Plate 1 shows the gel picture of the extracted DNA.

PCR Amplified 16S rRNA of L. monocytogenes

Electrophoresis separation of the PCR product revealed the presence of an amplified 1,517bp *L. monocytogenes*16S rRNA in all the isolates as shown in Plate 2.

Table 1: Biochemical Characteristics of Listeria Species Isolated

Biochemical tests				Sugar fermentation				Haemolysis on 5% SBA	CAMP test with *S. aureus*	Species Identified
C	O	MR	VP	GR	L-Rh	D-Xy	αMdm			
+	-	+	+	+	+	-	+	+	+(S)	*L. monocytogenes*
+	-	+	+	+	-	-	-	+	-	*L. seeligeri*
+	-	+	+	+	+	+	+	-	-	*L. welshimeri*
+	-	+	+	+	+	-	+	-	-	*L. innocua*

Key C=Catalase, O=Oxidase, MR=Methyl red, VP=Voges Proskauer, GR=Gram reaction, L-Rh=Rhamnose, D-Xy=Xylose, αMdm=α-Methyl-d-mannoside, SBA=Sheep blood agar, CAMP=Christie Atkins Munch Peterson, *L. monocytogenes=Listeria monocytogenes, L. seeligeri=Listeria seeligeri, L. welshimeri=Listeria welshimeri, L. innocua=Listeria innocua*.

Table 2: Percentage Distribution of *L. monocytogenes* isolates from different food samples

S/n	Food Sample	Number of Samples Examined	Number of samples with Listeria spp	Number of samples with *L. monocytogenes*
1.	Cabbage	10	6(5.0)	2(1.7)
2.	Fruit salad	10	0(0.0)	0 (0.0)
3.	Fresh fish	20	1(0.8)	0 (0.0)
4.	Raw meat	20	4(3.3)	1(0.8)
5.	Frozen Chicken	20	7(5.8)	3 (2.5)
6.	Ice cream	20	0(0.0)	0 (0.0)
7.	Yoghout	20	3(2.5)	1(0.8)
	Total	**120**	**21(17.5)**	**7(5.8)**

Values in parenthesis are percentage

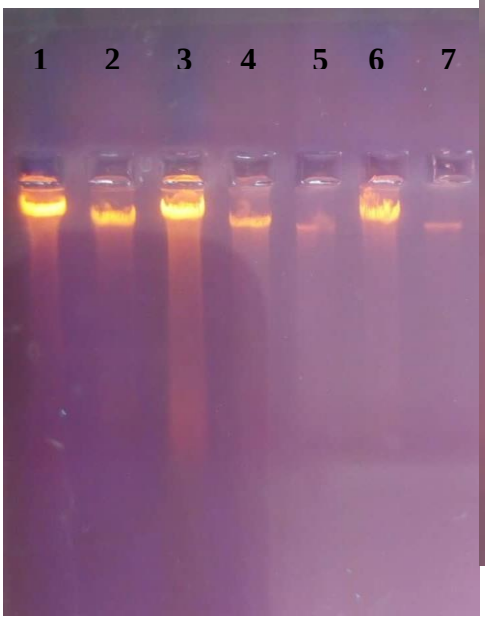

Plate 1

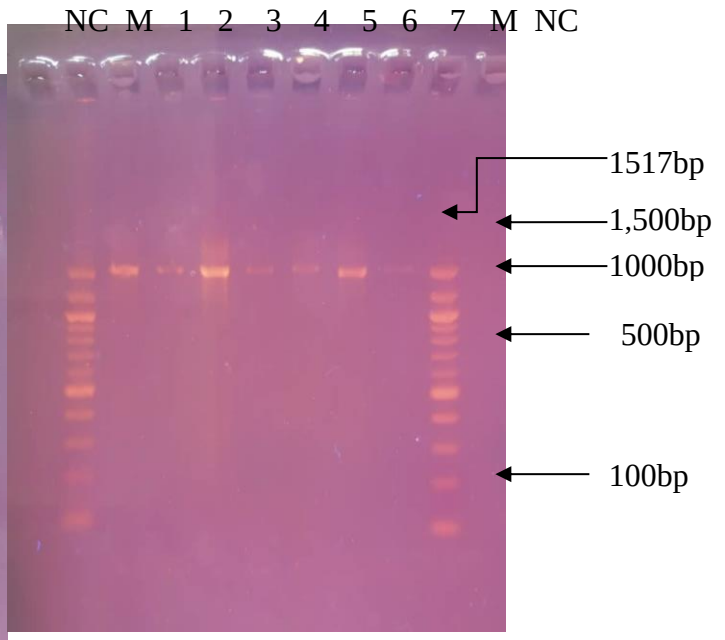

Plate 2

Plate 1: Extracted whole genome DNA of *Listeria monocytogenes* isolates; Lane 1-7 for the isolates Codes Lm2C, Lm3C, Lm4C, Lm8Fc, Lm9Fc, Lm10Fc, and Lm11Y respectively.
Plate 2: Agarose gel of amplified 1,517bp 16S rRNA gene of *L. monocytogenes* NC= Negative control (Reaction mix without template), M= Marker, Lane 1-7 for samples code Lm2C, Lm3C, Lm4C, Lm8Fc, Lm9Fc, Lm10Fc, Lm11Y respectively.
Key; Lm= *L. Monocytogenes* C=Cabbage, Fc=Frozen chicken, Y= Yoghurt

Discussion

Prevalence of L. monocytogenes

The prevalence of *L. monocytogenes* from this study is 5.8% which is relatively low. However, lower prevalence was reported previously from similar food samples Viz.; 0.4% (Luchansky *et al*., 2017), 1.5% (Amajoud *et al.*, 2018), 3% Nayak *et al.*, (2015). But higher prevalence were also reported previously Viz.; 6.5% (Usman *et al.*, 2016), 7.8% (Peter *et al.*, 2016), 12.5% (Akya *et al.*, 2018), 14.2% (Mohamed *et al.*, 2018), 16% (Gallegos *et al.*, 2008), 24.7 (Wang *et al.*, 2021) and 28% by Oyelami *et al*., (2018). The differences may be due to differences in food hygiene practices in the different study areas where the samples were obtained. Also, *L. monocytogenes* prevalence is expected to be lower when food samples are analysed than human samples (Reza and Mehrdad 2018).

In the present study, frozen chicken which is a poultry product, yielded a higher prevalence of 2.5% than any other sample analyzed. The meat sample also yielded the growth of *L. monocytogenes* (0.8%). The findings of this study agree with the statement that meat and poultry products are the leading vehicles of listeriosis among ready-to-eat (RTE) foods (Jay *et al.*, 2005). The higher occurrence of *L. monocytogenes* in frozen chicken and its occurrence in raw meat are expected because *L. monocytogenes* is ubiquitous in the environment (Vitals *et al.*, 2004). Furthermore, the method of slaughter, evisceration of animals and refrigeration (especially of frozen chicken), allows ample opportunity for contamination to occur (Oyelami *et al.*, 2018) because the organism can grow at low temperatures (Singh *et al*., 2019). Also, People handling meat at different levels can be sources of contamination (Oyelami *et al.*, 2018).

The occurrence of *L. monocytogenes* in the meat sample observed in this study is consistent with the result of Oyelami *et al*., (2018). However, this is in contrast with the results of Nayak *et al*., (2015), where no *L. monocytogenes* was detected in meat samples. The detection of *L. monocytogenes* in meat and frozen chicken is of particular concern in terms of consumer safety, as this organism is capable of growing on both raw and cooked meat at refrigeration temperatures (Walker, 2000).

In this study, a high number of *L. monocytogenes* (1.7%) was isolated from cabbage which is a fresh; ready-to-eat vegetable. This is possible because ready-to-eat (RTE) vegetables have been associated with increasing food-borne pathogen outbreaks including *L. monocytogenes* infections resulting in listeriosis (Ntshanka *et al.*, 2022). This also suggests that the cabbage samples may have been contaminated on farms via irrigation water and other farm practices. Therefore, Cabbage as a vegetable sample studied poses a threat to consumers' health and makes them prone to the risk of contracting listeriosis.

Listeria spp other than *L. monocytogenes* was isolated in fresh fish samples analyzed in this study. This finding conforms to that of Nayak *et al*., (2015) who also reported the presence of other Listeria spp but not *L. monocytogenes* in fish. In contrast, Jabir, (2010), reported the presence of *L. monocytogenes* in fish samples.

The analysis of fruit salad and ice cream samples in this study showed no contamination with *L. monocytogenes* and other Listeria spp. This is in contrast to the findings of Nayak *et al*., (2015), who isolated other Listeria spp in ice cream and fruit salad. The absence of *L. monocytogenes* in these food products might be attributed to good environmental and workers' hygiene in the processing factories in the area of study. This is because *L. monocytogenes* is a food-borne pathogen that is widely distributed in the environment and can also be found in the gastrointestinal tract of individuals who

remain as asymptomatic carriers and the risk of its dissemination can be decreased by proper food preparation (Oyelami *et al.*, 2018).

The result of this study showed that a reasonable proportion (5.8%) of the samples failed to meet the U.S. recommended standard (zero tolerance) of *L. monocytogenes* in ready-to-eat food. Such food samples could pose a health threat to the consumer as reported by Kayode *et al*., (2022).

CONCLUSION

L. monocytogenes, a bacterial foodborne pathogen, was found in some of the different food samples that were examined. The isolation and identification of *L. monocytogenes* in these samples suggest a possible risk to consumers, particularly those who are elderly, immunocompromised, or pregnant women. The results of this investigation show that the cabbage, frozen chicken, and yoghurt under investigation contained a significant amount of *L. monocytogenes* alongside other Listeria spp. To reduce the risk of foodborne illnesses caused by pathogens like *L. monocytogenes*, it is essential to continuously monitor food samples, practice safe food handling, and follow the five keys to safer food, which are as follows: 1. Keep clean 2. Separate raw and cooked foods 3. Cook food thoroughly 4. Keep food at safe temperatures 5. Use safe water and raw materials**.** It is crucial to follow these guidelines to prevent contamination and ensure the safety of the food we consume.

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