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. monocytogenes 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 x 0.5 to 2 μm, 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 (Kyoi 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 °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 0µL taken from the enrichment broth and incubated for 24 hours at 37°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% H2O2 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 H2O2 drops on the slide and observe. Bubbling 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°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 test, 1 ml of a standardized inoculum was transferred into test tubes containing 5 ml of xylose and incubated at 37°C for 5 days. Positive reactions occurred mostly within 24 to 48 hours (Osman et al., 2016). The same procedure was used to test for glucose, thannose, 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 16S F (AGAGTTTGATCCTGGCTCAG) and 16S R (GGTTACCTTGTAGCAGCTT) (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 95°C 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. monocytogenes16S rRNA in all the isolates as shown in Plate 2.

Table 1: Biochemical Characteristics of Listeria Species Isolated

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th>Sugar fermentation</th>
<th>Haemolysis on 5% SBA</th>
<th>CAMP test with S. aureus</th>
<th>Species Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>O</td>
<td>MR</td>
<td>VP</td>
<td>GR</td>
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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

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

Values in parenthesis are percentage
The occurrence of contamination (Oyelami, 2018) at different levels can be sources of contamination (Oyelami, 2018). The higher occurrence of contamination to occur (Oyelami, 2016), allows ample opportunity for the environment (Vitals, 2022) to be a threat to consumers’ health and makes them prone to the risk of contracting listeriosis.

L. monocytogenes, which is ubiquous in the gastrointestinal tract of individuals who also be found in the gastrointestinal tract of individuals who practices. Therefore, Cabbage as a vegetable sample studied 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.

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 are not infected with the pathogen.

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.
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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REFERENCE


