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Research Article

Detection of *Listeria monocytogenes* In Raw Food and Environmental Samples In Asaba Nigeria

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Abstract

Listeria monocytogenes is a bacterium that is pathogenic for man and for most animal species. *Listeria monocytogenes* causes listeriosis which is an infection that starts after ingestion of the causative agent L. *monocytogenes*. Factors that contribute to the spread of this infection are food that is not properly heat-processed, long storage of such food, food that is produced in non-hygienic food plants, as well as cooled food. In this research, 126 raw food samples were collected from Ogbogonogo market, Mile 5 market, anwai market, yanga market and food stores in asaba. Microbiological testing was done according to internationally prescribed standards ISO 11290-1 (1996). The isolation of *Listeria monocytogenes* was carried out using a single enrichment broth and plating method. Phenotypic characterization such as Gram's staining, Catalase and motility tests were carried out according to the methods of Hitchins and Jinneman, 2011. Among the 126 samples from the food products, Listeria species was presence in 22 samples (17.5%) and from the positive samples, *Listeria monocytogenes* was found in 14 (63%). Serious foodborne illnesses are linked to *Listeria monocytogenes*, therefore the presence of L. *monocytogenes* in these food materials and the food processing environment highlights the dangers of consuming inadequately prepared food items.

1. Introduction

Listeria monocytogenes is a bacterial pathogen that is widely distributed in nature. *Listeria monocytogenes* is psychotropic and can tolerate high salt as well as a wide pH range. *Listeria monocytogenes* has been associated with a number of foodborne outbreaks in a variety of refrigerated food products, such as ready-to-eat (RTE) meat, dairy products, processed vegetables, meats, fish and seafood [1, 2] as well as in environmental sources such as soil and water. *Listeria monocytogenes* is a pathogenic bacterium responsible for listeriosis, a serious infection usually caused by eating food contaminated with the bacterium. Listeriosis can lead to severe health issues such as septicemia, meningitis, encephalitis, and, in pregnant women, it can cause miscarriages, stillbirths, and preterm labor. The elderly, immunocompromised individuals, and newborns are also particularly susceptible to listeriosis. The Centers for Disease Control and Prevention (CDC) estimates that approximately 1,600 people get listeriosis each year in the United States, with about 260 resulting in death .

The incubation period is typically 30 days, but can vary from 1-90 days. Initial flu-like symptoms are often followed by vomiting and diarrhoea and in a few cases potentially fatal meningitis and septicemia may develop. Infection in pregnant women can lead to miscarriage and overall mortality rates of 30% are typical in outbreaks. Transmission of infection is now thought to be mainly foodborne and person-to-person transmission is rare, although pregnant women may pass infection to the foetus [2].

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L. *monocytogenes* is ubiquitous in the environment and can be isolated from soil, plant material, animals and even marine fish and seafood. It has also been found in a very wide variety of food products, but its presence is particularly hazardous in chilled processed foods with a long shelf life, such as smoked fish, pâtés, soft cheeses and ready-to-eat cooked meats [3]. This is because it is able to grow slowly at temperatures as low as 0oC and may therefore multiply to dangerous levels in refrigerated foods unless controlled. Listeria species are also common colonizers of food factories where they may form biofilms that are difficult to remove. These biofilms can act as reservoirs of persistent L. *monocytogenes* contamination for processed foods if not controlled. The presence of Listeria species in the food factory environment is often used as an indicator for L. *monocytogenes* contamination. It can enter food-processing settings via incoming raw materials and the movement of personnel and equipment. L. *monocytogenes* can colonize in the form of biofilms on food- processing equipment and (non) food-contact surfaces. Inadequate cleaning and disinfection procedures may lead to persistence of the bacterium for prolonged periods in food-processing environments. L. *monocytogenes* is present in food processing environments (FPEs) due to a saprophytic lifestyle. Temporal breakdowns in hygiene barrier efficiency, poor hygiene practices and unhygienic design of equipment may trigger L. *monocytogenes* food plant contamination, [3, 4]. *L. monocytogenes* can spread throughout the food processing facility due to contaminated contact materials, inappropriate personnel movements and food workflows [5]. Such contamination can be an intermediate step in transmission from their original habitat in the environment (in biofilms, water and organic soil residues) to food contact surfaces (FCS) and food under processing [6].

L. *monocytogenes* infection can result in two types of human illness: the non- invasive form of listeriosis affects the digestive system and results in symptoms including fever, muscle aches and sometimes gastrointestinal symptoms (nausea or diarrhoea), whereas the more serious invasive listeriosis is associated with clinical presentations of central nervous system infection, sepsis, and bacteremia. Because of the invasiveness of L. *monocytogenes*, listeriosis fatalities are particularly associated with high-risk populations, e.g. individuals with compromised immune systems such as persons with hematological malignancies (e.g. leukemia), persons suffering from liver cancer, older adults ($>$ 74 years of age), pregnant women, and new born babies [4, 7]. *Listeriosis* is a serious foodborne illness caused by this pathogen, especially in susceptible populations, including children, pregnant women, the elderly, and individuals with compromised immune systems [8]. The symptoms of *listeriosis* include mild flu-like infection to severe cases of invasive infection, in which the bacteria spread from intestines to the blood, causing bloodstream infection, or central nervous system infection, causing meningitis and encephalitis [8]. In pregnant women, the infection may get transmitted from mother to neonate, causing spontaneous abortion or the birth of a premature infant with meningitis. A long shelf life makes ready to eat (RTE) foods susceptible to bacterial growth, especially foods with intrinsic factors that support the growth of L. *monocytogenes*, e.g., foods with pH between 4.5 and 9 [9] and water activity $\geq$ 0.92 [10]. Studies have reported the presence of L. monocytogenes in RTE foods during storage at a refrigerated temperature [9].

Listeria *monocytogenes* is ubiquitous in the environment and can enter the food chain through primary production, that is, contamination can occur at the farm level, particularly in raw materials like milk, vegetables, and meat [11]. The prevalence of L. *monocytogenes* in food production environments has been identified as a cause of many *listeriosis* outbreaks. For example, a *listeriosis* outbreak in 2011 that was linked to cantaloupe was originated from the food production environment [12]. Similarly, in 2015, the *listeriosis* outbreak linked to ice cream was also found to have originated in the food production environment L. *monocytogenes* may enter the food production environments through different routes, such as incoming raw material, equipment, employee activity, air flow, traffic flow, soil, water, and vegetation [13]. The prevalence of L. *monocytogenes* in a food production environment depends on several factors, including the type of food, processing method, incoming raw material, the effectiveness of cleaning and sanitation protocols, the sanitary design of equipment and facilities, and employee training [14]. The bacterium can persist in food processing facilities, where it can form biofilms on equipment and surfaces, leading to cross-contamination. Contaminated food products can spread Listeria to other foods and environments during transport and storage.

Effective detection of Listeria *monocytogenes* is vital for ensuring food safety and public health. Early and accurate detection and vaccination helps prevent the distribution of contaminated food products [15], thereby reducing the incidence of *listeriosis* outbreaks. Given the significant public health implications, the detection and control of Listeria *monocytogenes* in food and environmental samples are critical. Hence, this study was aimed at detecting Listeria *monocytogenes* in food and environmental samples in Asaba. Nigeria.

2. Materials and methods

Sample collection

In this study, a total of 126 raw food samples were purchased from Ogbogonogo market, Oko market along Ezenei road, Abraka market along Benin - Asaba expressway, Mile 5 market along Infant jesus road, Anwai market by Infant jesus, Yanga market Asaba - Ibusa road and from food stores within Asaba due to a reported current outbreak of listeria foodborne disease in Asaba, Nigeria between March 2024 and May 2024. The food samples were collected using the Ziploc bags and stored 4°C. The foods samples were grouped into 5 groups: 23 were dairy-based, 25 meat-based, 26 poultry-based, 29 vegetable-based, and 23 fish-based. The samples except for vegetable were subjected to heat treatment. The samples were taken to the laboratory and processed.

The isolation of Listeria *monocytogenes* was carried out using a single enrichment broth and plating method. For each sample, 25 g was added to 225 mL of one-broth Listeria and stomached for 30 seconds. It was incubated at 30 oC for 24 hours. After 24 hours enrichment, the broth cultures were agitated and a loopful was inoculated onto Brilliance Listeria agar. This was incubated appropriately (37 oC for 24 – 48 hours). The plates were examined for blue-green colonies with white halos, the suspected colonies were subcultured on Tryptone Soya Agar (TSA, Oxoid) and incubated at 37 o C for 24 hours. Phenotypic characterization such as Gram's staining, Catalase and motility tests were carried out according to the methods of Hitchins and Jinneman, 2011. Oxoid Listeria Latex Agglutination Test, Oxoid Biochemical Identification System (O.B.I.S.) and MICROBACT Listeria 12L system were also used for identification.

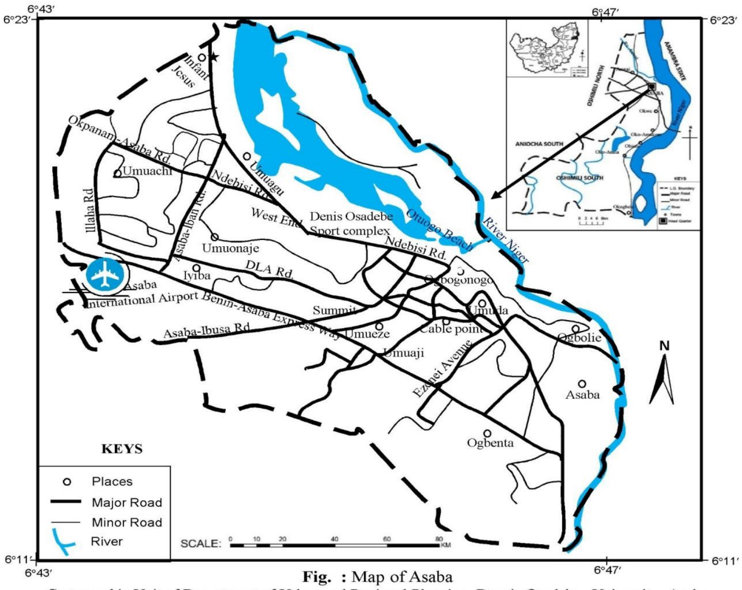

Fig. : Map of Asaba
Cartographic Unit of Department of Urban and Regional Planning, Dennis Osadebay University, Asaba

Figure 1: Map showing the location of study.

3. Results

One hundred and twenty six (126) raw food sample that was used in this research with meat products 25 with 7 (28.0%) positive for listeria spp, fish products 23 with 8 (34.8%) positive for listeria spp, vegetable food 29 with all sample negative for listeria spp, poultry foods 26 with 5 (19.2%) positive for listeria spp and dairy products 23 with 2 (8.7%) for for listeria spp.

Table 1: Presence of Listeria spp in raw food.

Food Products	Listeria spp Number of sample	Positive	Percentage
Meat products	25	7	28.0
Fish products	23	8	34.8
Vegetable food	29	-	-
Poultry foods	26	5	19.2
Dairy products	23	2	8.7
Total	**126**	**22**	**90.7**

Fourteen (14) of the twenty two (22) raw food that was positive for Listeria spp was also positive for Listeria *monocytogenes* with fish products 6 (26.1%), *poultry*4(15.4%), meat 3 (12%), *dairyfood*1(4.3%).

Table 2: Presence of Listeria monocytogenes in food samples.

Food Products	Number of positive SamplesofL.monocytogenes	Percentage	
Meat products	3	12	
Fish products	6	26.1	
Vegetable food	-	-	
Poultry foods	4	15.4	
Dairy products	1	4.3	
Total	**14**	**57.8**	

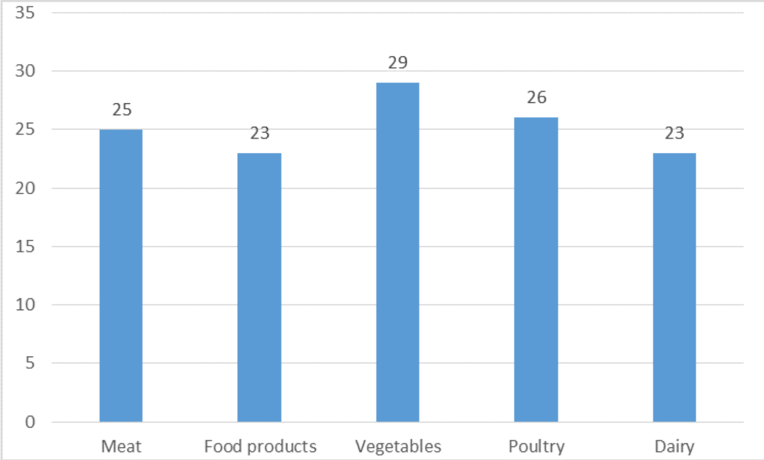

Figure 2: Chart showing the number of food products that was sampling.

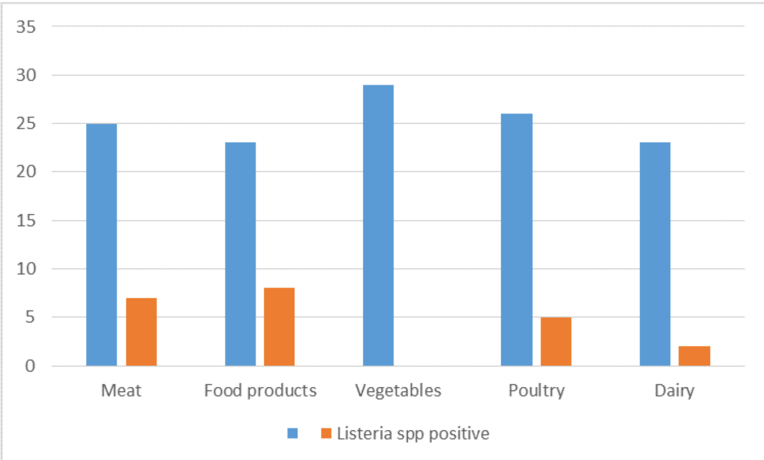

Figure 3: Chart showing the number of samples positive for *Listeria spp*

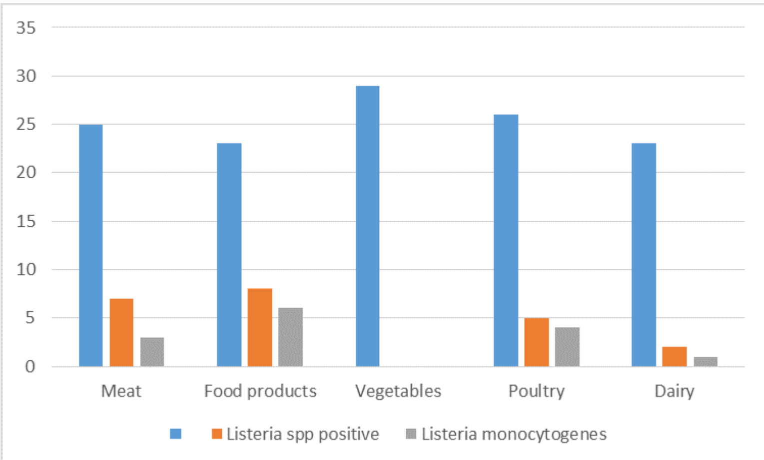

Figure 4: Chart displaying the number of positive samples for *Listeria monocytogenes*

4. Discussion

Serious foodborne illnesses are linked to *Listeria monocytogenes*. The presence of L. *monocytogenes* in food goods highlights the dangers of consuming inadequately prepared food items. From locally processed food, Listeria *monocytogenes* was found (meat, fish product, poultry food and dairy products). According to reports, L. *monocytogenes* might withstand a number of food processing and microbiological control procedures in the food business, such as routine cleaning and meticulousness [16]. One of the harmful organisms found in traditional fermented foods from Africa is L. *monocytogenes* [17]. They are a serious risk to food safety because they can withstand extreme temperatures, low moisture contents, high acidity, and high salinity during food processing and storage [18]. Listeria *monocytogenes* recovered from these food and environmental products suggest that there may be a health risk for users. While this study focused on raw food products, other research has highlighted the risk of pathogen contamination of plant products when irrigated with contaminated water sources [19]. Microbial contamination can be influenced by the use of contaminated containers, the use of untreated water in food processing, and the storage of food crops in contaminated environments [20].

Food safety is threatened by contaminated foods that include germs [21]. Inadequate hygiene standards and incorrect application of the hazard analysis critical control point (HACCP) principles during processing may have contributed to the detection [19]. Cross-contamination from the environment, utensils, processing water, and packaging materials might result in the occurrence of L. *monocytogenes* in these food items [22]. Contaminated food products can spread Listeria to other foods and environments during transport and storage, therefore it is crucial to test food products and lab environments for possible contamination by Listeria *monocytogenes*.

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