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OCCURRENCE OF LISTERIA SPECIES IN SOME FOOD ITEMS SOLD IN PARTS OF KADUNA, NIGERIA

***[1]Hammuel, C., [2]Abdullahi, I. O., [2]Whong, C. M. Z. and [3]Kadima, K. B.**

[1]Department of Microbiology, Faculty of Pure and Applied Sciences, Federal University Wukari
[2]Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria.
[3]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

*Corresponding Author's e-mail: chris2012bansi@gmail.com

ABSTRACT
Listeriosis is a disease of humans and animals cause by pathogenic *Listeria* species and are associated with various types of food items of both animals and plants origin. The aim of this work was to assess the occurrence of *Listeria* species in some foods items sold from four selected markets in two Local Government Areas of Kaduna State. A total number of 400 samples were collected, 100 samples each from the four locations comprising 25 sample each of beef, chicken, lettuce and cabbage were collected from Samaru, Sabon Gari, Kakuri and Ungwan Boro markets. These samples were analysed for *Listeria* species using Listeria selective media and biochemical tests. The isolates of the Listeria species were further confirmed using microgen listeria identification system. The overall prevalence of *Listeria* species in the selected food samples in the four locations was 9.8%. *Listeria* species were isolated from beef (6.0%), Chicken (15.0%), cabbage (6.0%) and lettuce (12.0%). *Listeria ivanovii* (8.0%) was detected in the food samples; other species include *Listeria grayi* (1.5%) and *Listeria welshimeri* (0.25%). Therefore, the presence of *Listeria* species in the selected food samples suggests that the foods might be important source of listeriosis.

Keywords: *Listeria* species, listeriosis, food samples, LGAs

INTRODUCTION

Listeria species are ubiquitous and worldwide in distribution and have been isolated from a variety of sources, including soil, vegetables, meat, silage, faecal material, sewage, water as well as animals and humans (Dimic *et al*., 2010; Greenwood *et al*., 2012 and Jamali *et al*., 2013). These bacteria can grow over a wide temperature range of 1°C to 45°C, with an optimum growth temperature of 37°C. *Listeria* species can grow at pH values between 4.4 and 9.4 and at a water activities ≥0.92 with sodium chloride (NaCl) as the solute (Dimic *et al*., 2010).

There are different species within the genus *Listeria* among which *L. monocytogenes*, *L. seeligeri* and *L. ivanovii*, are associated with pathogenicity. *Listeria ivanovii* is an animal pathogen and is a rare cause of infection in humans, while *L. monocytogenes* causes infection in both human and animal populations (Abdelrazeq *et al*., 2014; Momtaz and Yadollahi, 2013). However, sporadic human infections due to *Listeria seeligeri* and *Listeria innocua* have also been reported (Abay *et al*., 2012). *Listeria monocytogenes* is capable of causing serious invasive illness (listeriosis) including manifestations of septicaemia, meningitis, pneumonia, and encephalitis in both humans and animals (Alzubaidy *et al*., 2013).

Healthy individuals who are infected have few or no symptoms; when symptoms are present, they usually consist of fever, muscle aches, nausea, or diarrhoea. Some may develop more severe symptoms such as meningitis mental changes, brain abscesses, or death (Carrascal-Camacho *et al*., 2014). People with underlying risk factors such as depressed immune response (for example, pregnant woman and their foetus or newborn, cancer patients, AIDS patients) are at higher risk of contracting the disease and some are more likely to have more severe disease (Abdelrazeq *et al*., 2014; Gamboa-Marin *et al*., 2013).

Food contamination by *Listeria* species is one of the leading microbiological causes of food recalls in developed countries, mainly of meat, poultry, sea food and dairy products. Prevention and control measures are based on hazard analysis and critical control point programmes throughout the food industry, and on specific recommendations for high-risk groups (Alsheik *et al*., 2013). Therefore, the aim of this research is determine the occurrence of Listeria species in meat and vegetables because they have been found to be a notable and consistence source of *Listeria* species and are commonly consumed by the populace.

MATERIALS AND METHODS

Study area

The study areas covered were Kakuri and Ungwan Boro markets in Kaduna South local government area. Other areas included in the study were Samaru and Sabon Gari markets in Sabon Gari local government area.

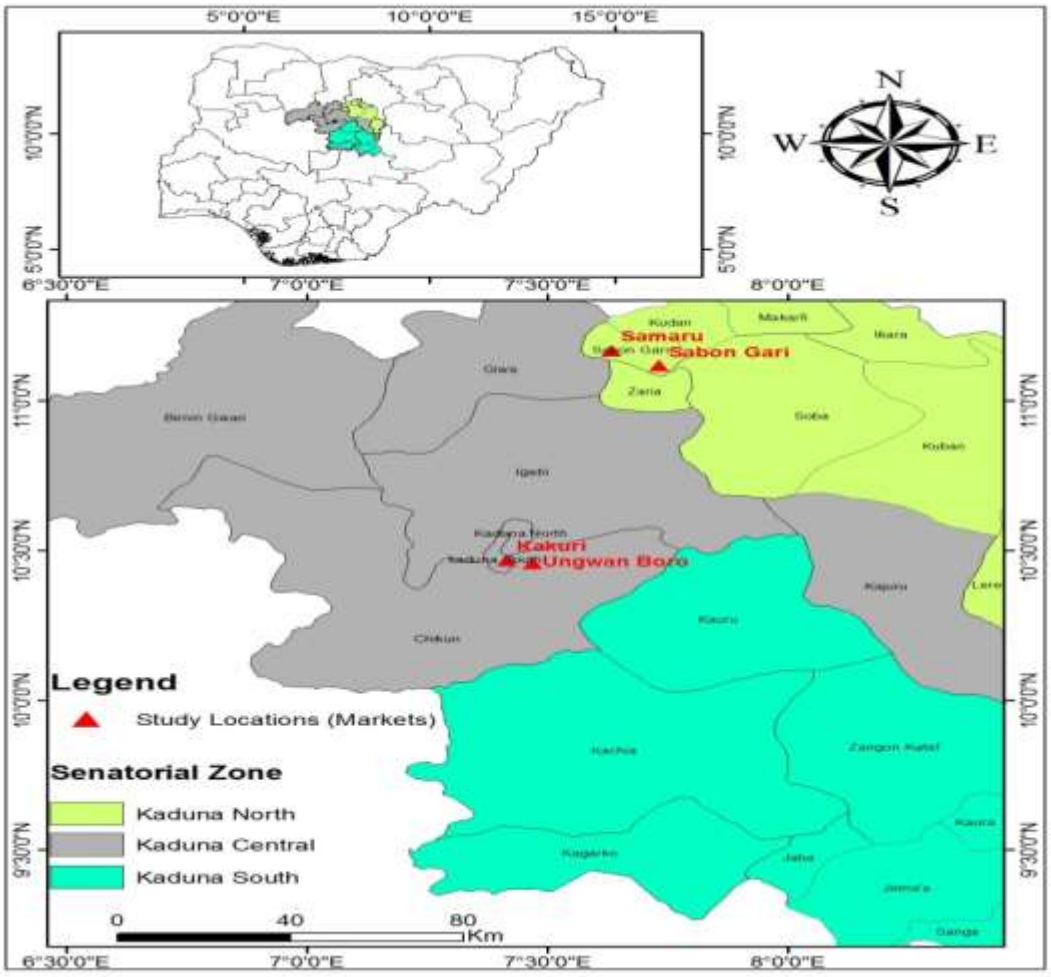

Figure 1: Map of Kaduna showing selected sampling locations (Markets).

Collection of samples sample size and prevalence of *Listeria* species in samples

In this research, a total of 400 samples were collected that include 100 samples of each of fresh beef, chicken lettuce and cabbage from four different locations. The samples were transported in a sampling box containing ice pack to the Department of Microbiology, Ahmadu Bello University Zaria for analysis. The prevalence of Listeria species was calculated thus:

Prevalence of *Listeria* species (%) = Number of positive isolates from the food items ×100

Sample size

Isolation of *Listeria* species

Twenty five grams of each fresh food sample was inoculated into 225 ml of buffered bacteriological peptone water and incubated for 24 hours at 30ºC. Afterwards, twenty five mililitres of each of the incubated buffered bacteriological peptone water with these samples were inoculated into 225 ml of listeria selective enrichment broth medium with listeria enrichment supplement to obtain a ratio of 1:10 and then incubated at 37ºC for 24 hrs. Thereafter, a loop full of each culture from the listeria enrichment broth was sub-culture onto prepared listeria selective agar plates containing listeria selective enrichment supplement and listeria differential supplement and then incubated at 37°C for 24 hrs. Thereafter, *Listeria* species were isolated and characterised using selected biochemical tests.

Biochemical Characterisation

Gram staining: Gram staining was carried out on the suspected colonies on the Listeria selective medium. Isolates which appeared as Gram positive, rod shaped organisms were further charatersied by biochemical tests such as catalase, oxidase, haemolysis, indole, motility, Voges-Poskauer (VP) test (Cheesbrough, 2009) and were confirmed using Microgen identification system.

Catalase test: A drop of 3% hydrogen peroxide (H_2O_2) was placed on a clean dry slide. Thereafter, a freshly prepared colony of the *Listeria* species on nutrient agar medium was placed on the drop of hydrogen peroxide, positive result was indicated by air bubbles as a result of oxygen production, absence of air bubbles indicated negative result (Usman *et al*., 2016).

Oxidase test: A freshly prepared colony was streaked on a piece of filter paper, and a drop of 1% colourless tetramethyl-p-phenylelediamine (TPD) reagent was added. A negative result was indicated by no colour change within 10-30 seconds. Development of a blue-purple colour within ten (10) seconds indicated the positive result

Haemolysis on sheep blood agar: A freshly prepared colony was streaked on sheep blood agar (7%), thereafter, incubated for 24 hrs at 37°C, a positive result was indicated by appearance of narrow zone of β-haemolysis around the colony (Cheesbrough, 2009).

Indole test: The colony of freshly prepared culture of the test organism (after being checked by Gram staining) was inoculated in a bijou bottle containing 3ml of sterile tryptone water. It was incubated at 37°C for up to 48 hrs and then tested for production of indole by dispensing 0.5ml of Kovac's reagent. After it was gently mixed, a negative result showed no red colour in the surface layer within ten (10) mins (den Bakker *et al*., 2013).

Methyl red (MR) test: A 24 h culture of the bacterium was inoculated into a test tube of 3ml of glucose phosphate broth, which contained glucose and a phosphate buffer and was incubated at 37°C for 48 h. The pH of the medium was checked by production of acid as a result of glucose fermentation by adding 5 drops of methyl red solution (0.04%). Development of red colour indicated positive result (MacFaddin, 2000).

Voges-Poskauer (VP) test: A 24 hrs culture of the bacterium was inoculated into a test tube of 3ml of glucose phosphate broth, which contain glucose and phosphate buffer and was incubated at 37°C for 48 h. The culture was treated with 0.6ml of α-naphthol and shaken. Thereafter, 0.2ml of 40% KOH was added to the broth. The tube was allowed to stand for 15 mins, positive result was indicated by colour appearance when allowed to stand for 1 hr; since maximum colour development occurs within one hour after addition of reagents (Suryawanshi, 2014**)**.

Motility test: Small suspension of the pathogen from the 24 hrs culture in *Listeria* enrichment broth was dropped on a slide and covered with a cover glass and then sealed with molten petroleum jelly to avoid drying. The preparation was examined microscopically for motile organisms using 10X and 40X objective lenses (Cheesbrough, 2009).

Identification using microgen listeria identification system

Twenty four hour grown cultured isolates of the *Listeria* spp were suspended or emulsified in a vial containing 2.5ml Listeria suspending medium. The suspension was prepared using MacFarland's standard of 0.5 after mixing it thoroughly to produce homogenous suspension. The microwell test strip containing twelve different substrates was removed from the foil pouch and was placed in the holding frame and the lid was removed. With the aid of sterile Pasteur pipette, 4 drops of the bacterial suspension was transferred to each well of the microwell strip. A drop of haemolysin reagent was added to well 12. The lid was replaced onto the microwell test strip and was incubated at 37ºC for 24 h. After the incubation, the lid was carefully removed from the microwell tests strip and the results were recorded. The tests on the report form have been organised into triplets (sets of 3 reactions). The sum of the positive reactions for each triplet forms a single digit of the octal code. This was used to determine the identity of the *Listeria* spp. isolated. The octal code was entered into the microgen identification system software, which generated a report of the five most likely organisms from the database of *Listeria* species.

RESULTS

The phenotypic characterization of *Listeria* species using microgen kit base on sugar fermentation capacities of the isolates are shown inTable 1 and indicated that 10 of *Listeria ivanovii* had percentage probability identification at 99.03% with octal code of 5751, 9 of the *Listeria ivanovii* had percentage probability identification at 95.31% with octal code of 5643. Five isolates of *L. ivanovii* were identified at 95.91% with octal code of 4657. Two isolates of *L. ivanovii* and another 2 isolates had percentage probability identification at 99.96% (octal code 4454) and 99.87% (octal code 4645) respectively. Other isolates of *L. ivanovii* were identified at 99.79%, 99.79%, 98.23% and 97.15 with octal codes 5757, 4641, 4653 and 5756 respectively. Four isolates of *Listeria grayi* and two isolates of the same species were identified at 96.47% (octal code 4646) and 99.55% (octal code 4643) respectively. *Listeria welshimeri* was identified at percentage probability of 94.32% with octal code of 4561.

Table 1: Phenotypic characterization of *Listeria* spp using microgen kit

Esc	Man	Xyl	Ara	Rib	Rha	Tre	Tag	G-1-P	M-D-Glu	M-D-Man	Hae	Octal code	Probability (%)	Inference organism (No.)
+	-	+	+	+	+	+	-	+	-	+	-	5752	99.03	*L. ivonvii* (10)
+	-	+	+	+	-	+	-	-	-	+	+	5643	95.31	*L. ivonvii* (9)
+	-	-	+	+	-	+	-	+	+	+	+	4657	95.91	*L. ivonvii* (5)
+	-	-	+	-	-	+	-	+	+	-	-	4454	99.96	*L. ivonvii* (2)
+	-	-	+	+	-	+	-	-	+	+	-	4646	96.47	*L. grayi* (4)
+	-	+	+	+	+	+	-	+	+	+	+	5757	99.79	*L. ivonvii* (1)
+	-	-	+	+	-	+	-	-	-	-	+	4641	98.23	*L. ivonvii* (1)
+	-	-	+	+	-	+	-	+	-	+	+	4653	97.21	*L. ivonvii* (1)
+	-	-	+	+	-	+	-	-	+	-	+	4645	99.87	*L. ivonvii* (2)
+	-	+	+	+	+	+	-	+	+	+	-	5756	97.15	*L. ivonvii* (1)
+	-	-	+	+	-	+	-	-	-	+	+	4643	99.55	*L. grayi* (2)
+	-	-	+	-	+	+	+	-	-	-	+	4561	94.31	*L. welshimeri* (1)

Key: Esc = Esculin, Man = Mannitol, Xyl = Xylose, Ara = Arabitol, Rib = Ribose, Rha = Rhamnose, Tre = Trehalose, Tag = Tagatose, G-1-P = Glucose-1-Phosphate, M-D-Glu = Methyl-D-Glucose, M-D-Man = Methyl-D-Mannose, Hae = Haemolysin

A total of 39 *Listeria* species were isolated from this study. The prevalence of *Listeria* species isolated from the food samples are shown in Table 2. Results indicated that of the 400 samples collected, the overall prevalence of all the *Listeria* species in the food samples selected is 9.8% with *Listeria ivanovii* having prevalence of 8.0%, *Listeria grayi* 1.5% and *Listeria welshimeri* 0.25% in all the food samples. The prevalence of *Listeria* species in the food samples was appreciably higher in chicken (15.0%) and lettuce (12.0%). The prevalence of the *Listeria* species in the food items was low in beef meat (6.0%) and cabbage (6.0%). *Listeria ivanovii* was isolated from beef (5.0%), chicken (12.0%), cabbage (6.0%) and lettuce (9.0%). *Listeria grayi* occurred in chicken (3.0%) and lettuce (3.0%). *Listeria welshimeri* was only isolated from beef with occurrence of 1.0%. There was no *Listeria monocytogenes* isolated from any of the food items selected.

Listeria ivanovii had percentage prevalence of 9.0% in food items collected from Sabon Gari market, 8.0% each in food samples collected from Samaru and Ungwan Boro markets while 7.0% of the *Listeria ivanovii* was isolated from food samples from Kakuri market. *Listeria grayi* had percentage prevalence of 2.0% from food samples from Samaru, Sabon Gari and 1.0% from foods from Kakuri and Ungwan Boro markets. The percentage prevalence of *L. welshemeri* from food samples from Sabon Gari market was 1.0%, this organism was not isolated from any of the food samples form Samaru, Kakuri and Ungwan Boro markets. There was no *Listeria monocytogenes* isolated from any of the food samples from any of the markets selected (Table 3).

Table 2: Prevalence of *Listeria* species in different food samples in parts of Kaduna

Food sample type	Total No. of samples	No (%) of sample positive for *Listeria* spp	Listeria species (N = 39)			
			No(%) of *L. ivanovii* in each sample	No(%) of *L. monocytogenes* in each sample	No (%) of *L. grayi* in each sample	No(%) of *L. welshimeri* in each sample
Beef	100	6(6.0)	5(5.0)	0(0.0)	0(0.0)	1(1.0)
Chicken	100	15(15.0)	12(12.0)	0(0.0)	3(3.0)	0(0.0)
Cabbage	100	6(6.0)	6(6.0)	0(0.0)	0(0.0)	0(0.0)
Lettuce	100	12(12.0)	9(9.0)	0(0.0)	3(3.0)	0(0.0)
Total	400	39(9.8)	32(8.0)	0(0.0)	6(1.5)	1(0.25)

Table 3: Prevalence of *Listeria* species isolated from different sampling locations in Kaduna

Isolate	Locations			
	Samaru (n=100) (%)	**S/Gari (n=100) (%)**	**Kakuri (n=100) (%)**	**U/Boro (n=100) (%)**
Listeriaivanovii	8(8.0)	9(9.0)	7(7.0)	8(8.0)
Listeriamonocytogenes	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Listeria grayi	2(2.0)	2(2.0)	1(1.0)	1(1.0)
Listeriawelshimeri	0(0.0)	1(1.0)	0(0.0)	0(0.0)
Total	10(10.0)	12(12.0)	8(8.0)	9(9.0)

S/Gari = Sabon Gari, U/Boro = Ungwan Boro

DISCUSSION

The presence of the *Listeria* species in the fresh beef meat could be as a result of cross contamination in the slaughter house and during distribution or marketing in the study area. Thus, it is generally assumed that meat cannot be free from *Listeria* species because of the unhygienic nature of slaughter house and hence the greater chances of contamination especially in developing countries like Nigeria (Islam *et al*., 2016). Thus, these meat need to be properly cooked to eliminate these pathogens (Alsheik *et al*., 2013). The absence of *Listeria monocytogenes* (0.0%) in the food samples from the four (4) locations in this findings corroborates the report of Okonkwo *et al*. (2014) from Onitsha main market in Anambra state. The authors also could not also isolate *Listeria monocytogenes* from their studies. The absence of the *Listeria monocytogenes* could be because of low numbers of viable cells in the food samples as reported by Beumer and Hazeleger. (2003).

Ndahi *et al*. (2013) also discovered higher prevalence of *Listeria ivanovii* (6.3%) among *Listeria* species isolated from meat and meat products in Zaria. Alsheik *et al*. (2014) reported higher prevalence of *Listeria ivanovii* (19.4%) from chicken in Sudan than the prevalence reported in this research. *Listeria grayi*, *Listeria seeligeri*, *Listeria innocua* and *Listeria welshimeri* were also isolated from meat products, although the latter occurred with the lowest frequency among *Listeria* spp. The occurrence of *Listeria ivanovii*in the food samples can pose a potential health hazards this is because it is an enteric opportunistic pathogen (Dahshan *et al*., 2016).

Another reason for high prevalence of *Listeria ivanovii* could be because it is a zoonotic pathogen that can easily be spread in the environment and might have found it way on the vegetables through application of animal dung and chicken droppings by farmers as well as the use of faecal contaminated water for irrigation (Odjadjare *et al*., 2011). The presence of *Listeria* species in the samples could be attributed to the fact that the organism have been reported to be widely spread in nature, therefore, can contaminate vegetables in the environment (Ieren *et al*., 2013; Dahshan *et al*., 2016; Peter *et al*; 2016). *Listeria ivanovii* has been isolated from various foods including meats, milk and vegetables (Guillet *et al*., 2010).

In this research, the raw chicken harboured most of the *Listeria* species (15.0%); this corroborates the findings of Hussain *et al*. (2016), that poultry is ranked first as one of the major source of listeriosis. The high occurrence of the *Listeria* species in the

chicken meat could be as a result of unhygienic technique used in the removal of the intestinal parts during processing or by contact with contaminated surfaces in the slaughter house. Therefore, the contamination could come within or outside the chicken (Suleiman *et al*., 2017).

The appreciably high level of contamination of lettuce by the *Listeria* species could perhaps be attributed to the fact that lettuce has high percentage of moisture composition and the high level of fermentable sugar such as glucose favourable for the replication of these organisms (Ieren *et al*., 2013). Secondly, it is close to the ground and thus, is usually in contact with the soil and manure (Mohammed and Kawo, 2014).

The occurrence of *Listeria* species in cabbage could also be attributed to the fact that the plant is also close to ground or soil top (which is a reservoir of food borne pathogens) and might have been contaminated through application of organic manures into the farm lands or application of faecal contaminated irrigated water which could be the source these pathogens (Mohammed and Kawo, 2014). Even though the lower leaves of the cabbage which are directly in contact with the soil are normally cut off during harvest but one cannot rule out contamination during the harvest or during transportation and distribution (Mohammed and Kawo, 2014).

The distribution of *Listeria ivanovii* in vegetables could be as result of human activities. These include application of livestock, composed manure and chicken droppings by farmers on farmlands as well as irrigation of the farmlands with untreated or faecal contaminated water as reported by Odjadjare *et al*. (2011); Tiimub *et al*. (2012); Abakpa *et al*. (2013) and Ssemanda *et al*. (2018). However, in the markets, fresh vegetables are exposed to environmental conditions or factors can also introduce all kinds of pathogenic microorganisms.

The occurrence of *Listeria ivanovii* in vegetables could be as result of human activities. These include application of livestock, composed manure and chicken droppings by farmers on farmlands as well as irrigation of the farmlands with untreated or faecal contaminated water as reported by Odjadjare *et al*. (2011); Tiimub *et al*. (2012); Abakpa *et al*. (2013) and Ssemanda *et al*. (2018).The presence of *Listeria ivanovii* in the food samples is in agreement with the report of Guillet *et al*. (2010), that *Listeria ivanovii* is isolated occasionally from animals, vegetables, poultry and environmental sources.

CONCLUSION

The occurrence of *Listeria ivanovii* in the food samples was higher than *Listeria grayi* and *Listeria welshimeri*. *Listeria ivanovii* may pose serious health challenges; this is because it is one of the pathogenic strains which can infect both animal and human population. It is, therefore, recommended that meat and vegetables from these sample locations be thoroughly washed and cooked before consumption.

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