



ISOLATION OF *LISTERIA MONOCYTOGENES* RECOVERED FROM SOME READY-TO-EAT FOODS SOLD IN KANO, NORTH-WESTERN NIGERIA

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ABSTRACT

Listeria monocytogenes and other *Listeria* species were isolated from some ready-to-eat foods sold in Kano metropolis. The method of United States Food and Drug Administration, Center for Food Safety and Applied Nutrition (FDA/CFSAN) was adopted in the isolation of the organism. A total of 336 food samples were screened. This included 29 beef burger, 30 yoghurt, 32 locally fermented fresh milk (nono), 34 cabbage, 45 grilled meat (tsire), 48 lettuce, 55 meat-pie and 63 roasted meat (balangu) samples. Samples were collected from December 2012 to March 2013. From these samples 38 (11.3%) yielded *Listeria* species. Six isolates (1.8%) were found to be *L. monocytogenes*, 31 (9.2%) were found to be *L. ivanovii* and 1(0.3%) was *L. seeligeri*. Out of the 38 isolates, 7 (18%) were from 'balangu', 9 (24%) cabbage, 14 (37%) lettuce, 5 (13%) meat pie and 3 (8%) 'tsire' samples. *L. monocytogenes* was isolated from 3 lettuce samples (50%), 2 'balangu' samples (33%) and 1 meat-pie sample (17%). On the other hand, *L. monocytogenes* was not isolated from 'tsire', beef burger, 'nono' and yoghurt samples. This study showed the presence of the organism in Kano, which could pose a threat of outbreak or sporadic cases of listeriosis with high morbidity and mortality rates.

Keywords: Isolation, *Listeria monocytogenes*, Ready-to-eat foods, Kano.

INTRODUCTION

Listeria monocytogenes is a ubiquitous bacterium that is responsible for food-borne illnesses in humans. It has been recovered in several countries and from many different foods of animal origin that have been linked to both sporadic cases and outbreaks of listeriosis (Thévenot *et al.*, 2006; Chao *et al.*, 2006). Disease incidence in humans is generally low, but in recent years great attention has been addressed to study the virulence properties of this microorganism due to the severity of the illness and the high mortality rate (20-30%) (Vazquez-Boland *et al.*, 2001; Dussurget *et al.*, 2004).

The organism is wide-spread in the environment. It is found in soil, water, sewage, decaying vegetation and can be readily isolated from humans, domestic animals, raw agricultural products, food processing environment and the home (Ryser and Marth; 1999). The mode of transmission can be by ingestion of contaminated food or transmitted vertically from mother to fetus both in utero and peripartum. Other less frequent possible modes include animal-to-person transmission, resulting for example in cutaneous lesions among veterinarians, or nosocomial transmission, as documented in an outbreak in a newborn nursery through the use of contaminated mineral oil (Schuchat *et al.*, 1991).

Human population responses to *L. monocytogenes* exposures and listeriosis are highly variable. Disease incidence is dependent on a variety of factors, including the presence of virulence factors, dose, general health and immune status of the host (Vazquez-Boland *et al.*, 2001; Dussurget *et al.*, 2004). Listeriosis is a rare disease with high mortality rate causing about 43% of the food poisoning in the

United State (Center for Disease Control (CDC), 2006). It is one of the most virulent food borne pathogens with 20 to 30% of clinical infections resulting in death (Ramaswamy *et al.*, 2007). Listeriosis is responsible for approximately 2500 illness and 500 deaths in the US annually and is the leading cause of death among food borne bacterial pathogens with fatality rates exceeding even *Salmonella* and *Clostridium botulinum* (Dharmarha, 2008). In Nigeria, cases of listeriosis and death caused by the disease were not well documented. Patients might have died before they could obtain medical help because of inaccessibility in most rural areas, poor state of some health facilities and low level of awareness of *L. monocytogenes* among health professionals or absence of the selective laboratory media for the isolation due to the high cost of procurement (Greenwood, 1999). These could lead to wrong diagnosis of listeriosis, although an outbreak of listeriosis was reported in a herd of cattle by Akpavie and Ikheloa in 1992. the research was set up to isolate *Listeria monocytogenes* from some processed foods, Meat, Vegetables, Dairy products and identify the isolates to specie level using biochemical analysis.

MATERIALS AND METHODS

Study Area and Sample Collection

The study area was Kano metropolis. This include; Kabuga, Zoo road, Tarauni, Nassarawa, Bompai road and Municipal. These are places with high number of ready-to-eat and fast food joints. Samples were collected from December 2012 to March 2013. The samples were Meat pies, Beef burgers, Roasted meat (Balangu) and Grilled meat (Tsire), Yoghurt, Locally fermented fresh milk (Nono) Cabbage and Lettuce.

The sample size was 336. This included 29 beef burger, 63 balangu, 45 tsire, 30 yoghurt, 32 nono, 55 meat pie, 34 cabbage and 48 lettuce samples. The work was conducted at the Microbiology/Parasitology of Aminu Kano Teaching Hospital, Kano.

MATERIALS AND METHODS

Isolation of *Listeria monocytogenes*

The United States Food and Drug Administration and Center for Food Safety and Applied Nutrition method of isolation was adopted (Manual, 2004). *Listeria* Enrichment broth (LEB) with *Listeria* selective enrichment supplement was used for enrichment. *Listeria* selective agar base (Oxford formulation) fortified with *Listeria* selective supplement agar plates were used as the selective media for the presumptive isolation of *Listeria* species from the processed food samples.

Sample Preparation

To obtain a true representative of the samples, the composite samples were thoroughly mixed in the following manner; nono and yoghurt were agitated; samples from tsire and balangu were chopped using sterile surgical blades; samples from lettuce and cabbage were also chopped using sterile surgical blades. Aseptic conditions were maintained throughout the period of sample analysis to prevent contamination of samples and media.

Inoculation/Enrichment

- Five grams or milliliters of each sample from the composite mixture was weighed and transferred into a 100ml capacity Erlenmeyer flasks containing 45ml sterilized *Listeria* enrichment broth base with *Listeria* primary enrichment supplement. The samples were properly shaken and incubated at 30°C for 48 hours.
- A loop-full of the broth culture was streaked onto a dried plate of *Listeria* selective agar base (Oxford formulation) + *Listeria* Selective Supplement (Oxoid, Hampshire, UK) and incubated aerobically at 37°C for 24 hours. Colonies that appeared brown black with a depressed center and a surrounding black halo were taken as positive for *Listeria* species.
- Tryptose Soy Agar with 0.6% yeast extract (TSAYE) was sterilized and poured into Petri plates and bijour bottles for slants. Typical colonies from each LSA plate that had blackened were streaked onto TSAYE in order to purify them. The plates were incubated at 30°C for 24 hours. Isolates were transferred to the sterile slants of Tryptose Soy Broth with yeast extract. These were

incubated at 30°C for 24 hours and stored at 4°C for further use (Hitchins, 1998).

Identification of isolates

The isolates were examined with a light microscope for their morphological characteristics, Gram reaction pattern and cellular features. The isolates were further subjected to the following biochemical tests: Catalase, Oxidase and Hemolysis on sheep blood agar in order to confirm the identity of the bacterial species.

Motility

Motility at 25°C was performed using hanging drop procedure: a drop of suspension (a colony suspended in normal saline and incubated at 20-25°C for 2 hours) was placed on a cover glass and inverted over a normal slide supported on a ring of plasticine. It was examined microscopically for motile organisms, using the 10X and 40X objectives. The preparation was brought into focus by focusing first on the edge of the cover glass. True bacterial motility was observed due to the ability of an organism to move itself in different directions in a characteristic tumbling motility. Biochemical screening of the various isolates to specie level were carried out using a standard biochemical tests with Microbact 12L; Plates 9A and 9B (Oxoid, Hampshire, UK) (Hitchins, 2011).

Quality control

Listeria monocytogenes (ATCC 19115) was used as positive controls for checking the efficiency of both culture and media.

RESULTS

In this study, a total of 336 processed foods and vegetable samples in Kano metropolis were examined for the presence of *Listeria monocytogenes*. From the total number of samples collected, *Listeria* species were isolated in 38 samples (11.3%). Out of 38 *Listeria* species, 7 (11%) were from Balangu, 9 (26%) Cabbage, 14 (29%) Lettuce, 3 (7%) Tsire and 5 (9%) Meat pie samples (Table 1). *L. monocytogenes* was isolated from 6 samples (1.8%), 31 isolates (9.2%) were found to be *Listeria ivanovii* and 1 (0.3%) was *Listeria seeligeri*. *L. monocytogenes* was isolated from 3 lettuce samples (50%), 2 (33%) balangu samples and 1 meat pie sample (17%) as shown in Table 1. On the other hand, *Listeria monocytogenes* has not been isolated from cabbage, tsire, beef burger, nono and yoghurt samples. *Listeria ivanovii* has been isolated in cabbage, balangu, lettuce, meat pie and tsire samples, whereas no *Listeria specie* was isolated from any of the beef burger, nono and yoghurt samples (Table 1).

Table 1: Occurrence of *Listeria* species isolated from each food sample.

Sample	No. screened	No. positive for <i>Listeria</i> spp.	<i>L. monocytogenes</i>	<i>L. ivanovii</i>	<i>L. seeligeri</i>	Percentage (%)
Balangu	63	7	2	5	0	11
Beef burger	29	0	0	0	0	0
Cabbage	34	9	0	9	0	26
Lettuce	48	14	3	10	1	29
Meat pie	55	5	1	4	0	9
Nono	32	0	0	0	0	0
Tsire	45	3	0	3	0	7
Yoghurt	30	0	0	0	0	0
Total	336	38	6	31	1	



Plate 9A: Microbact™ *Listeria* 12L, self-contained biochemical based identification system for the definitive identification of *Listeria* species.



Plate 9B: Results are interpreted using the Microbact™ Identification Package (Windows®) MB1244A.

DISCUSSION

A total of 336 samples were screened for *L. monocytogenes* and other *Listeria* species and these were isolated from the examined food samples. *L. monocytogenes* was isolated from lettuce, balangu and meat pie samples. *L. ivanovii* was isolated from balangu, cabbage and lettuce samples. None of the *Listeria* species was isolated from beef burger, yoghurt and nono samples. The absence in both yoghurt and nono samples might be as a result of the production of bacteriocins by the starter culture used for their fermentation which suppresses the growth of *L. monocytogenes*. Shehu and Lamido (1994) reported an average pH of 3.80 for nono sampled in Zaria. Effective fermentation should occur at pH less than 4.4. Consequently, any fermentation at a pH greater than this will be deemed ineffective and will favor the survival of *L. monocytogenes*. This may probably explain why *L. monocytogenes* was not isolated.

In the present study contaminations of food product with *L. monocytogenes* were 3 lettuce samples (6.3%), 2 balangu samples (3.2%) and 1 meat pie sample (1.8%). The non-occurrence of *L. monocytogenes* in Burger, Tsire, Cabbage, Nono and Yoghurt is significant in public health because the products are a ready-to-eat (RTE). Most reports on the occurrence of this food borne pathogen in meats have tended to concentrate on raw meat, meat mixed with salads and poultry (Morobe *et al.*, 2009).

Of public health concern is the occurrence of the microorganism in balangu, meat pie and lettuce. The contamination of lettuce is in agreement with the study of Little *et al.*, (2007), which suggest occurrence of *L. monocytogenes* in prepackaged mixed salads could result from the original contamination of raw material or cross contamination during processing.

Among the food samples examined, the highest *L. monocytogenes* occurrence was encountered in the lettuce samples. Contamination of vegetables with *Listeria* species is thought to occur on the farm mainly

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through soil and animal manure, although, contaminated wash water may also play a role (Ikeh *et al.*, 2010).

The higher occurrence of the organism in lettuce could perhaps be attributed to the fact that lettuce is close to ground, and is usually in contact with farmland, soil, manure etc (Nwachuku *et al.*, 2010). The non-occurrence in cabbage could be due to the interlocked nature of its leaves and the opened lower leaves that are directly in contact with the soil are normally cut off during harvesting. Generally, the presence of the pathogen in lettuce raises enough concern due to the fact that it is consumed raw and by the entire populace, including listeriosis susceptible people. Therefore, creating public awareness by disseminating the information is necessary. Serious precautions regarding the food type, storage system and proper cooking should be considered while handling of food, in order to control *Listerial* food contamination.

This study indicated how these organisms are widely distributed in the society and may infect many people, different foods and also the environment.

Conclusion

L. monocytogenes has been isolated in Kano which pose a threat to human life and can cause outbreak of listeriosis. Many people who have been suffering from unknown causes of meningitis, abortion, still birth, septicemia, endocardities, and so on, might be infected by *L.monocytogenes*. The study represents a database for the isolation of *Listeria* species which can be used especially for further studies.

Recommendations

- More research should be conducted to ascertain the presence of the organism in other food items.
- Great attention has to be given to the decontamination of vegetables that are consumed raw.

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