Listeria monocytogenes and other *Listeria* spp. in Organic Chicken Meat sold in Some Selected Local Markets in Rivers State, Nigeria

Omorodion NJP, Odu NN, Njoku HO

Department of Microbiology, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria.
*Corresponding author. Tel: +2349083622583, E-mail: nnenna.omorodion@uniport.edu.ng, nnennaomorodion@gmail.com

Abstract: *Listeria* is a bacterium that causes the infection listeriosis which is one of the most important food-borne diseases in humans. This study is aimed on the determination of *Listeria* sp. in organic chicken thighs and wings, followed by isolation, identification and confirming the organism through biochemical test. During the research a total of twelve Organic chicken wing and thigh meat samples were collected and twenty five bacteria of the *Listeria* genera were isolated. Of the twenty five isolates obtained, twelve were identified as *L. monocytogenes,* seven were *L. innocua*, three were *L. invanovii* and one was *L. murrayi.* The total viable count of the organic chicken wings and thighs were also carried out, which ranged from $5.6x10^6$cfu/g – $9.25x10^6$cfu/g for the wings and $1.01x10^7$cfu/g – $1.83x10^7$cfu/g for the thighs. *L. monocytogenes* was most prevalent followed by *L. innocua,* then *L. invanovi* and constitute a considerable challenge to food processors and consumers. When ingested, infection starts with sudden onset of fever, headache, nausea and vomiting and may be followed by meningitis, pneumonia, septicaemia and endocarditis and localized abscesses. In pregnancy, abortion, stillbirth or premature labour may occur, According to Food-borne Diseases Active Surveillance Network (FoodNet). The research carried out has shown that chicken meat is conducive for the growth of microorganisms as it is rich in nutrients and possesses optimum growth environments; consequently it should be handled, cooked and stored with extreme care and hygiene consciousness in order to minimise ingestion of pathogen, and suggest that the principles of HACCP for the production and handling of fresh chicken meat as well as establishing legal prosecution for enforcing microbiological standards should be formulated and standardized.

[Omorodion NJP, Odu NN, Njoku HO. ***Listeria monocytogenes* and other *Listeria* spp. in Organic Chicken Meat sold in Some Selected Local Markets in Rivers State, Nigeria.*** *Nat Sci* 2016;14(6):84-88]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 11. doi:10.7537/marsnsj14061611.

Keywords: *Listeria monocytogenes*, *Listeria* spp., Organic Chicken, Meat, Local Markets

1. Introduction

Chicken meat is the commonest poultry meat sold in Nigeria and the most important as it is sold in every market and indispensable in a standard restaurant menu, it provides staple amount of protein with low fat. Because of the increasing demand for chicken meat, Nigerians have taken up chicken rearing in order to make ends meet. Agriculturists and nutritionists are generally agreed that developing the poultry industry of Nigeria is the fastest means of bridging the protein-deficiency gap presently prevailing in the country (Akinwunmi and Ikpi, 1977). In addition, when compared to other livestock, poultry has by far, the quickest and highest rate of turn over. Estimates from consumption and demand surveys in Nigeria indicate that the consumption of poultry is gradually outstripping most other kinds of meat except beef (Akinwunmi and Ikpi 1977). It is therefore not surprising that funds invested in poultry production are recouped faster than any other livestock enterprise. The poultry industry, if properly harnessed can also serve as a source of foreign earnings complementing crude oil which at present constitutes the main source of foreign earnings in Nigeria (The poultry site news 2009).

In poultry production, small scale poultry production represents one of the few opportunities for saving, investment and security against risks. It accounts for approximately 90% of total poultry production (Branckaert, 1999). Despite the acknowledged importance of poultry production, Akanni (2007) opined that it is characterized by low production level due to limited finance for the procurement of basic poultry equipment and materials. The result of this is that many of the small-scale poultry farmers are not encouraged to increase their productivity; thereby moving from small-scale production to a large scale production by small–scale poultry farmers encountered hindrances in the poultry industry which could be detrimental to increase.

The World health Organisation (WHO) defines food borne illnesses as diseases caused through consumption of contaminated food. Contamination of poultry meat occurs during washing, plucking and evisceration. Bacterial number vary considerably on surface of poultry meat, this variation however is greater between birds than birds than it is in different

areas of the same bird (Kabour *et al.*, 2001). Fresh chicken meat is known to undergo deterioration due to microbial action, chemical and physical changes, this deterioration in normal handling are attributed to microbial contamination.

Listeria monocytogenes is a gram positive, non sporing rods, facultative anaerobe. Of the six recognised listeria species, *L. monocytogenes* is the most important as it causes a range of infections in humans. *L.monocytogenes* is a common contaminant of raw poultry meat and most poultry carcasses carry it in low numbers. Ingestion of poultry meat infected with Listeria may lead to Listeriosis. *L. monocytogenes* is a common contaminant of raw poultry meat and may also be found in the further processing environment. About 60 % of chicken carcasses may carry the organism in low number. With ready to eat (RTE) products, there have been concerns over the possible presence and low rates of contamination with *L. monocytogenes* (ICMSF, 1998) determined the *Listeria* spp. in ground chicken meat, chicken meatballs and chicken burgers. Thirty-four of 40 (85%) ground chicken meat, 25 of 30 (83.3%) chicken meatballs and 12 of 30 (40%) burgers were found contaminated with *Listeria* spp. In another study held to determine the contamination of fresh chicken meat parts and edible offal with *Listeria* spp. showed *Listeria* contamination of 90% and 46.6% respectively. *Listeria monocytogenes* was isolated and identified from 23.3% chicken meat parts (leg, breast and wing) and 33.3% edible offals (liver and heart) at the levels of 101-102 and 3.8 MPN/g respectively (Erol *et al.*, 1999). Kalender (2003) isolated *L. monocytogenes* from 4.3% of 206 chicken feaces samples tested. The growth of the organic food industry has increased 20 - 25% in the U.S. over the last seven years. There has also been an increase in foreign markets (Dimitri and Greene, 2002).

Consumers and farmers have been increasingly interested in organic food products because organic foods are considered natural and healthy, although the health benefits as well as the food safety risks have not been clarified and much work still needs to be done. Organic farming emphazies the use of renewable resources to produce foods. Soil and water are also conserved to improve the quality of the environment. Conventional pesticides, fertilizers or other synthetic ingredients are also not allowed. Livestock animals must be given access to outdoor free range and be fed organic feed. In addition, they are not given any antibiotics for either growth promotion or as treatment for any disease. Because of the lack in data and microbiological impact with the way organic farming is done, it is interesting to look at the effect of organisms such as *Listeria* in this new type of environment. Organic livestock production may increase the risk of microbial contamination and thus food-borne illness, due to outdoor production and complete prohibition of antibiotic use. This study is aimed on the determination of sanitary quality and *Listeria* sp. in organic chicken thighs and wings sold in some selected local markets in Rivers State, Nigeria.

2. Methods

2.1. Sample collection

A total of 12 raw chicken meat were purchased (6 thighs and 6 wings) from 2markets in Port Harcourt. The samples collected were transported in a cool pack at +4□C within 1-2 hours of collection to the laboratory for analysis (Woji and Mile-3 market). Samples are categorized as follows:

ROCW1…… Raw Organic Chicken Wings 1
ROCW2…….Raw Organic Chicken Wings 2
ROCT1……...Raw Organic Chicken Thigh 1
ROCT2……...Raw Organic Chicken Thigh 2

2.2. Bacteriological analysis: Using the International Organization for Standardization (ISO, 1996, 2004) produces 25g representative portion from each sample was introduced aseptically into a sterile stomacher bag. Contrary 225ml of Half Fraser Broth (Oxiod, Ltd, Basingst Oke, UK, CM0895) (primary enrichment medium) to obtain a 1:10 sample dilution. The sample is then homogenized for 1-2 min at 260rpm in a stomacher circulator unit followed by incubation for 24hours at 30□C. After incubation period 0.1 ml sub-sample from each Fraser Broth culture is added to 10ml of Fraser Broth (secondary enrichment) and incubated for 48hours at 37□C. A loopful of the Fraser Broth enrichment cultures streaked on the surface of PALCAM Agar, then incubated for up to 48hours at 37□C. Selective agar is observed for suspected colonies at 24 and 48hours of incubation, suspected colonies are those that appeared grayish colours surrounded by black halo and sunken centers on PALCAM Agar. Whenever possible up to 5 suspected colonies showing typical morphology of *Listeria* on the media is streaked on Trypticase soy Agar supplemented with 0,6% of Yeast Extract powder and incubated at 37□C for 24hours. The isolates were identified using various biochemical tests.

2.3. Determination of the total viable count: Twenty-five grams of the chicken sample was homogenized in 225ml 0f peptone water,1ml of the stock solution was transferred into the first test tube containing 9ml of the diluent (peptone water), which gave a 10^{-2} dilution, which was further serially diluted from (10^{-1} -10^{-5}). For each dilution, a new syringe was used to avoid errors and contamination. Aliquots (0.1ml) of each dilution were transferred to the Plate Count Agar (PCA) in duplicates and spread uniformly

using a hockey stick. The plates were incubated aerobically at 37°c for 18-24 hours. Various isolates were identify using the various biochemical tests, colonies were sub-cultured on freshly prepared plate count agar. It was done by streaking a colony in plates containing medium using a sterilized (flamed) wire loop. This was aimed at isolating pure cultures for further identification. The incubation period for subculture was 18-24 hours at 37°C.

3. Results

From the result obtained, the total of 12 Organic chicken Wing and Thigh meat samples were collected and 25 bacteria of the *Listeria genera* were isolated. Of the 25 isolates obtained, 8 were identified as *L. monocytogenes,* 7 were *L. innocua*, 6 were *L. invanovii* and 4 were *L. murrayi*.

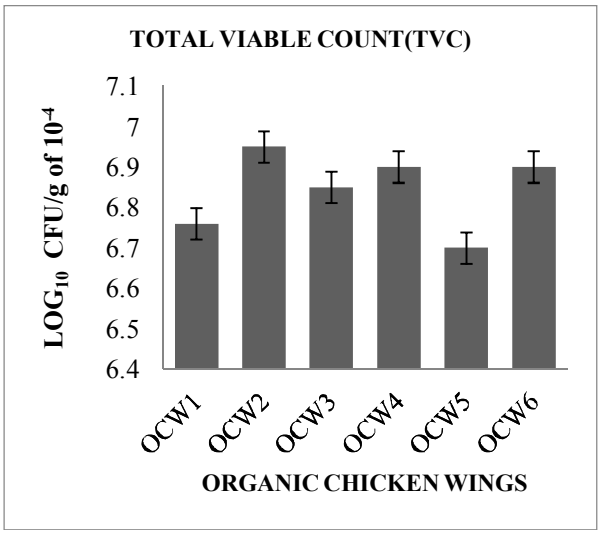

Figure 1: A bar chart showing total viable count of the samples of organic chicken wings from different market.

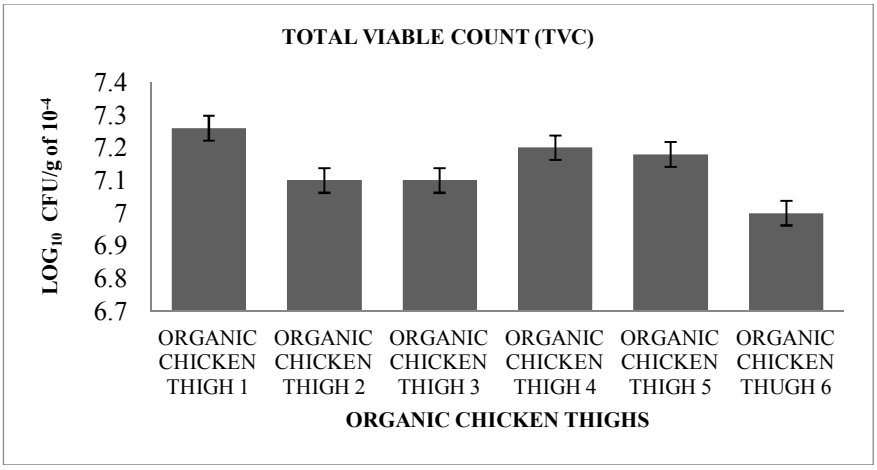

Figure 2: A bar chart showing the total viable count of the samples of organic chicken thighs from different market

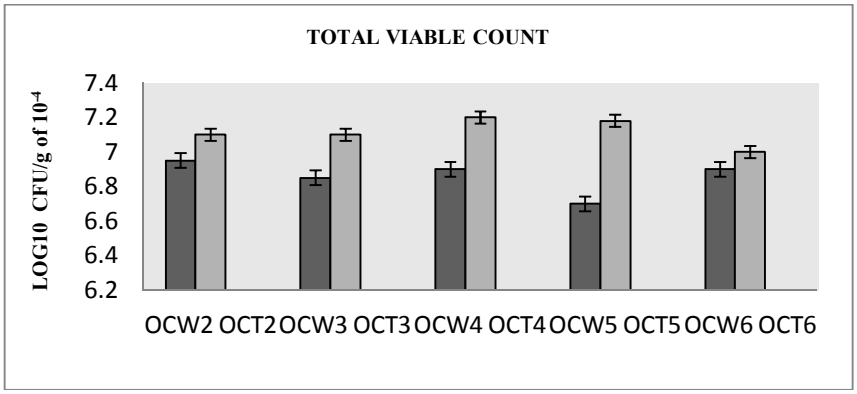

Figure 3: A bar chart showing the comparism of total viable count the organic chicken wings and thighs from different market

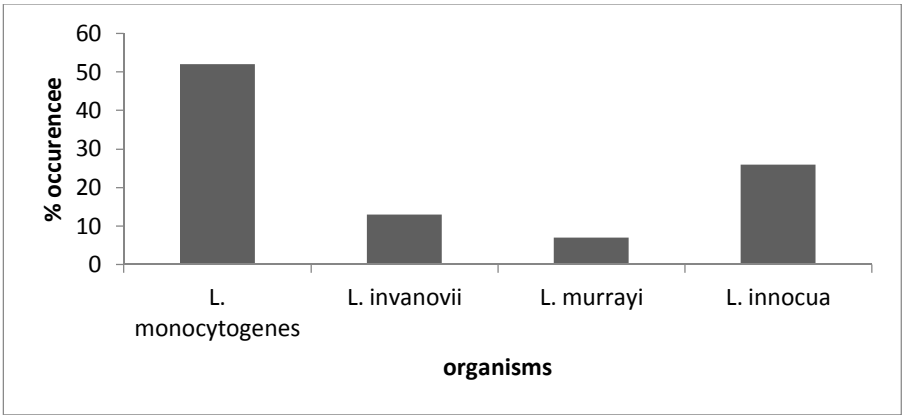

Figure 4: a bar chart showing the occurrence of *listeria species* in the samples.

4. Discussion

Chicken meat is generally accepted worldwide, by various culture and nationalities as it is rich in nutrients and easily digestible. Chicken meat cannot be found naturally sterile as the natural environment of the live birds and their natural flora also influence the microbiological quality of the meat (Mead, 2004). Means of cross contamination include using dirty utensils and equipment, transfer of bacteria from hand, fingers. These contaminations can be avoided if vendors wash their hands after any action that could contaminate hands such as visiting the toilet; as this is a major source of faecal contaminants. The isolated organisms in this study which can constitute public health hazards if ingested. The presence of *Listeria* organism which colonizes the gastrointestinal tract, *Listeria monocytogenes* has the unique ability to cause mammalian cells to absorb the organism into their cytoplasm. Using this ability, the pathogen penetrates the intestinal mucosa and can disseminate by cell-to-cell spread or hematogenously. The bacteria are subsequently found in regional lymph nodes either as free bacteria or within infected macrophages.

During acute infection, many tissues are infected, demonstrating the ability of these bacteria to invade numerous cells in different tissues. The incubation period (time ingestion and the onset of symptoms) for *Listeria* ranges from three to 70 days and averages 21 days (Bryan, 1999). The infection starts with sudden onset of fever, headache, nausea and vomiting and may be followed by meningitis, pneumonia, septicaemia and endocarditis and localized abscesses (Gray and Killinger, 1966). In pregnancy, abortion, stillbirth or premature labour may occur. The infection crosses the placenta and may lead to early-onset or late-onset of neonatal listeriosis in the form of pneumonia, conjunctivitis, meningitis and otitis media (Schuchat et al., 1991).

According to Food-borne Diseases Active Surveillance Network (FoodNet), *Listeria sp* is one of the most reported food-borne bacterial pathogen. The *Listeria* species isolated from the Organic chicken meat were identified as *L. monocytogenes*, *L. innocua*, *L. invanovii* , and *L. murrayi*. *Listeria* were present in all 12 samples examined. The predominant species among the isolated *Listeria* strains was *L. monocytogenes* (52%), followed by *L. innocua (26%),* then *L. invanovii* (13%) and the TVC was high in the organic chicken thighs than wings, this might be due to fact that the thighs is located closer to the anus than the wings which makes more susceptible to contaminants.

According to the World health organization directives on microbial limits, Total bacterial count shall not exceed 5 $x10^5$ colonies per gram of sample. The present study however revealed that the total viable count ranged from $5.6x10^6$cfu/g – $9.25x10^6$cfu/g for the wings and $1.01x10^7$cfu/g – $1.83x10^7$cfu/g for the thighs. Unfortunately, none of the results aligned with the set microbiological limit, this quite similar to the study by Odetunde and Lawal (2011) and different from the study conducted by Sengupta et al. (2011).

In conclusion, the high percentage of the isolation of *Listeria monocytogenes* from organic chicken is a real threat for consumers' health. Therefore, the information obtained from present study may be useful for the consumers and meat products. This study has shown that chicken meat is conducive for the growth of microorganisms (pathogens, non-pathogenic and spoilage organisms) as it is rich in nutrients and possesses optimum growth environments; consequently it should be handled, cooked and stored with extreme care and hygiene consciousness in order to minimise ingestion of pathogens.

References

1. Akanni, I.A., 2007. "Effect of micro-Finance on Small Scale Poultry business in South Western Nigeria" *Emirate Journal of Food and Agriculture*. 9(2): 38-47 http://www.cfa.uaeu.ac.ae. The future of poultry in Nigeria.
2. Akinwunmi .J, Ikpi .A, (1977). "Poultry in Nigeria*" Nigerian Journal of Agriculture*, vol5; Pp 26-30.
3. Bryan F.L. 1980. Poultry and meat products. Edited by Silliker J.H., Elliot R.P., Baird-Parker A.C, Bryan F.L., Christian J.H.B., Clark D.S., Olson J.C. and T.A. Roberts]. Microbial Ecology of Foods, Vol.2: *Food Commodities,* 410 – 458.
4. Erol, I. and U.T. Sireli, 1999. [Listeria manocytogenes presence in frozen broiler carcasses]. Truk. J. Vet. Anim. Sci., 23: 765-770, (In Trukish).
5. Food and Agriculture Organization (F.AO), (1993). Record Poultry Meat Consumption J. Food Prot., 32: 70-72.
6. Dimitri C and Greene C 2002. Recent Growth Patterns in the U.S. Organic Foods Market. Economic Research Service, U.S. Department of Agriculture, Market and Trade Economics Division and Resource Economics Division. Agriculture Information Bulletin Number 777.
7. Gray M. L., Killinger A. H. (1966). "*Listeria monocytogenes* and listeric infection". *Bacteriol. Rev*. 30: 309-382.
8. Jones, D. (1992). Current classification of the genus *Listeria* In: proceedings, *Listeria 1992 pp 7 – 8 Abstr*. The 11th International Symposium on problems of listeriosis, Copenhagen, p2.
9. Kalender H (2003): Detection of Listeria monocytogenes in feces from chickens, sheep and cattle in Elazig province. Turkish Journal Of Veterinary and Animal Science 27, 449-451.
10. Kabour G.A. 2011. Evaluation of Microbial Contamination of Chicken Carcasses during Processing in Khartoum State. M.V. Sc. Thesis Sudan University of Science and Technology, Sudan.
11. Mead, G.C. (2004). Microbiological quality of poultry meat: a Review. *Brazilian Journal of Poultry Science*, 6(3), 135 – 142.
12. Mead, G.C, Hudson, W.R, Hinton M.H (1995). Effect of changes in processing of poultry to improve hygiene control of poultry carcasses with *campylobacter*, epidemiology and infection, *British Poultry science*, 15: 495- 500.
13. Odetude S.K, Lawal, A.K, Akolade, M.A and Bakry S.B. 2011 Microbial flora of frozen chicken part varieties. International Research Journal of Microbiology, 2(11):423-427.
14. Schuchat A, Swaminathan B, Broome CV. 1991. Epidemiology of human listeriosis. *Clinical Microbiology Rev*, 4: 169-1683.
15. Seeliger, H. P. R. (1990). *Listeriosis* – avoidable risk? (Pp 1-4). In: A.J. Miller, J. L. Smith, G.A. and Somkuti, A.G. (eds.). Topics in industrial microbiology: Foodborne listeriosis. New York (NY): Elsevier Science Publications.
16. Sengupta R., Das S. Ganguly and Mukhopadhayay S.K 2011 Survey on microbial quality of chicken meat in Kolkata, India International Journal of Research in Pure and Applied Microbiology 2011; 1 (3): 32-33.
17. Yildirim .Y, Zafer .G, Nurhan .E, (2005), Incidence and antibiotic resistance of *Salmonella sp* on raw chicken carcasses. *Food research Journal*, vol44 (3):725-728.

4/19/2016