Occurrence of *Listeria monocytogenes* and other *Listeria* spp in Conventional Chicken Meat purchased from some retailer outlets in Rivers State, Nigeria

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Abstract: The aim of the study was to determine the occurrence of *Listeria monocytogenes* and other *Listeria* spp. in conventional chicken meats a total of 16 samples comprising of 8 wings and 8 thighs obtained from retailer outlets in four different locations (Rumuola, Rumuosi, Aluu and Choba). The total viable counts ranges from $5.6x10^6$-$5.6x10^7$ cfu/g for the wings, while the counts from the thighs ranges from $7.0x10^6$ to $8.8x10^8$ cfu./g. Counts were higher from samples obtained from Rumuola retailer store and least counts were obtained from Aluu and Choba retailer stores. High occurrence of Listeria spp were found more on the chicken thighs. The percentage occurrence of *Listeria monocytogenes* (53%), *Listreia innocua* (17%), *Listeria invanovii* (15%), *Listeria murrayi* (9%) and *Listeria welshimeri* (6%). Due to the high microbial contamination, it is very essential that the microbial level of any processed food be kept as low as possible, this because the microbial load is a reflection of the food quality. It very important that regulatory agencies be set up to ensure the enforcement of microbiological safety of poultry meats, by providing documents containing microbiological standards clearly specified to be used for assessment of safety and monitoring the nature and quality of poultry meats.
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1. Introduction

Listeria monocytogenes is a widely distributed food pathogen that can cause listeriosis which is a serious invasive human illness with mortality rates on average approaching 30% (Vazquez-Boland et al. 2001). Seeliger (1990) reported that listeriosis may have occurred before the advent of Murray et al. (1926) report and were discussed under names like pseudotuberculosis and argentophilic septicaemia which were completely unknown in laboratory animals.

The genus *Listeria* could best be characterized into seven species. The genus is closely related to the genera Brochothrix and Bacillus (Collin *et al.,* 1991). The seven species are considered to represent two closely related, but distinct lines of descent, with *L. monocytogenes, L. ivanovii, L. innocua, L. seeligeri and L. welshimeri* forming one group known as true Listeria organisms, with *L. grayi and L. murrayi* representing the other line (Collins et al., 1991; Jones, 1992). However, only *L. monocytogenes and L. ivanovii* are pathogenic for man and animals. Unlike *L. moncytogenes* which causes listeriosis in humans and animals, *L. ivanovii* is exclusively an animal pathogen and accounts for about 10% Listeria infections in animals (Jones, 1992).

The organism could be rod-shaped, coccoid or filamentous. The shape depends on nutrients, environmental and cultural conditions (Hass and Kreft, 1988). Among pathogenic bacteria, there are three distinctive properties that are peculiar to the genus *Listeria* which are of interest. These properties include the ability of the genus to survive and grow within a very broad temperature range (0^0C-44^0C), wide pH range (5.0-9.5), its ability to tolerate sodium chloride concentration of 5-25% and a variety of other toxic chemical such as tellurite, acridine dyes, lithium chloride, nalidixic acid and cycloheximide. *Listeria monocytogenes* has been isolated from cattle, sheep, pigs, chickens, chicks and a variety of other species (Gray and Killinger, 1966).

In addition, *L. monocytogenes* causes diseases in some 60 or more species of wild and domesticated, warm and cold blooded animals. The organism has been considered as zoophilic, and the various syndromes were ascribed to zoonosis (Gray and Killinger, 1966; Blenden et al., 1987). This study was aimed at evaluating the bacteriological quality of conventional chicken meat, compare the level of contamination of conventional chicken wings and thighs from four (4) locations. The aim of this study was to determine the occurrence of *Listeria monocytogenes and other Listeria* species in conventional chicken meat sold in some selected markets in Rivers State, Nigeria.

2. Methods

2.1. Study area; Retailer outlet in Rumuola, Aluu, Rumuosi and Choba in Rivers State, Nigeria. According to census data released in 2006, the state has a population of 5,185,400, making it the sixth-most populous state in the country. Its capital, Port Harcourt is the largest city and is economically significant as the centre of Nigeria's oil industry.

2.2. Sample collections: Sixteen conventional chicken meat comprising wings(8) and Thigh(8) were purchased from some selected retailer outlets at Rumuola, Aluu, Rumuosi and Choba in Rivers State, Nigeria and immediately transported in ice containers to the Food Microbiology Laboratory, University of Port Harcourt and analysed within two hours of collection.

2.3. Sample preparations: Twenty-five (25) grams of each wing and thigh samples were aseptically cut and placed in sterile plastic stomacher bags containing 225ml of buffered peptone water (Oxoid) and half strength Fraser broth, stomached for 2 min using Stomacher and this initial blend is stored for 1hr 5 min in order to recover stressed microorganisms. The enrichment culture was then incubated at 30^0C for 24 or 48h.

2.4. Culturing, Enumeration, and Isolation: Secondary enrichment was carried out on the 24h culture by aseptically transferring 0.1ml into 10ml full-strength Fraser broth containing Fraser supplement and incubated at 35^0C for 24 or 48h. Decimal dilutions is prepared and plated in duplicate on PALCAM (Oxoid). In order to increase the sensitivity of the technique, 1 ml of the first decimal dilution will also be plated (using one 140-mm plate or three 90-mm Petri dishes). Characteristics darkening of the medium by *Listeria species* due to aesculin hydrolysis was checked out for. The plates is incubated for 24 or 48 h at 37^0C, and typical colonies is counted and isolated in TSAYE for further confirmation as described by Awaisheh (2010). Using sterile pipette, 0.1ml of the secondary enriched broth with characteristic darkening of medium by *Listeria* species due to aesculin hydrolysis was aseptically transferred onto polymyxin acriflavin lithium chloride aesculin mannitol agar (PALCAM) containing supplement and carefully streaked over the surface of the agar. This was then incubated at 37^0C for 24-48h. Five typical Listeria colonies, about 2mm in diameter, grey-green with a black sunken centre and a black halo on a cherry-red background on PALCAM agar were aseptically inoculated onto Tryptone soy agar with yeast extract (TSAye) slants pending confirmation.

2.5. Identification of Listeria: Identification of the isolates was based on their morphology on PALCAM agar, Gram staining, catalase test, oxidase test, haemolysis of sheep blood and the production of acid from xylose, rhamnose and mannitol then the colonies of the bacteria present are identified using various biochemical tests.

3. Results

A total of 16 conventional chicken wing and thigh meat samples were collected. From the results obtained, the total bacterial count on plate count agar ranged from 5.6×10^6 - 5.6×10^7 cfu/g in refrigerated chicken wings and from 7.0×10^6 – 8.8×10^8 cfu/g in refrigerated chicken thighs (Figures 1-3) and 34 isolates were identified (Table 1 and Figure 5). Of the 34 isolates obtained, 18 were identified as *L. monocytogenes,* 6 were *L. innocua*, 5 were *L. invanovii*, 3 were *L. murrayi and 2 was L. welshimeri*. *L. monocytogenes* was most prevalent followed by *L. innocua,* then *L.invanovii* (Table 1 and Figure 4).

The Total viable count (TVC) for conventional chicken wings and thighs is presented in Table 1. And the percentage occurrence of the *Listeria* isolates from the various conventional/refrigerated chicken meat samples is presented in Figures 1-3.

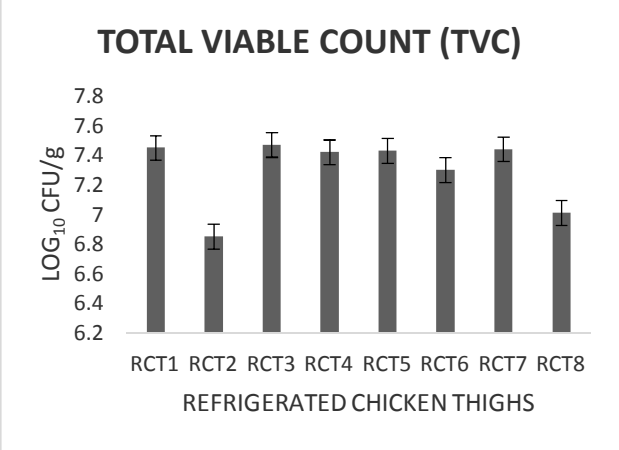

Figure 1: A bar showing the total viable count of conventional/refrigerated chicken thigh.

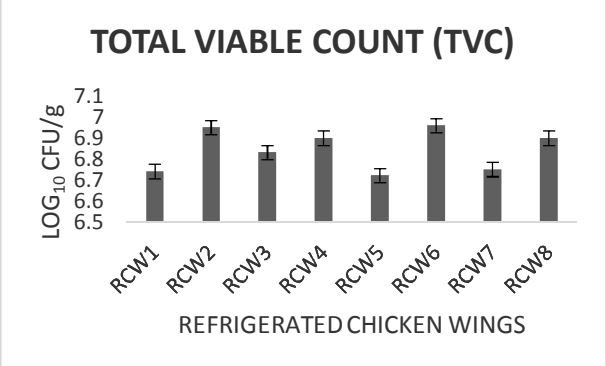

Figure 2: A bar chart the total viable count of conventional/refrigerated chicken wings.

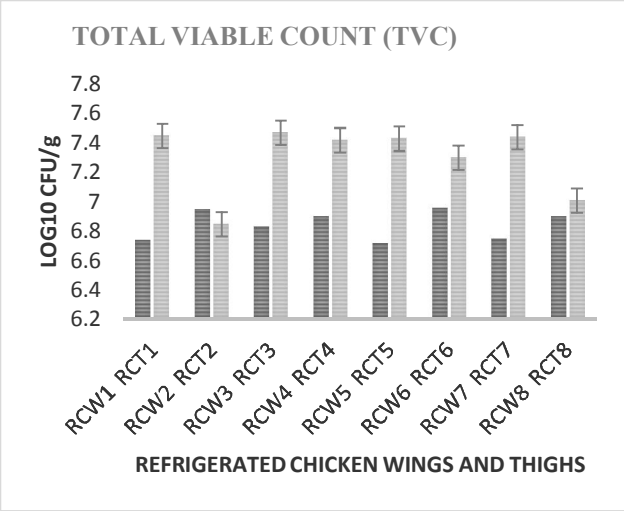

Figure 3: A bar showing the comparison of total viable count of conventional/refrigerated chicken thighs and wings.

Figure 4: A bar showing the number of Listeria spp isolated from different locations.

Table 1: Occurrence of *Listeria species* in Refrigerated Chicken Samples

Species	No. (%)	Chicken Wings (%)	Chicken Thighs (%)
L. monocytogenes	18(53.0)	4(22.2)	14(77.8)
L. invanovii	5(15.0)	2()	3()
L. murrayi	3(9.0)	1()	2()
L. innocua	6(17.0)	2()	4()
L. welshimeri	2(6.0)	1()	1()
Total	34(100.0)	10(29.4)	24(70.6)

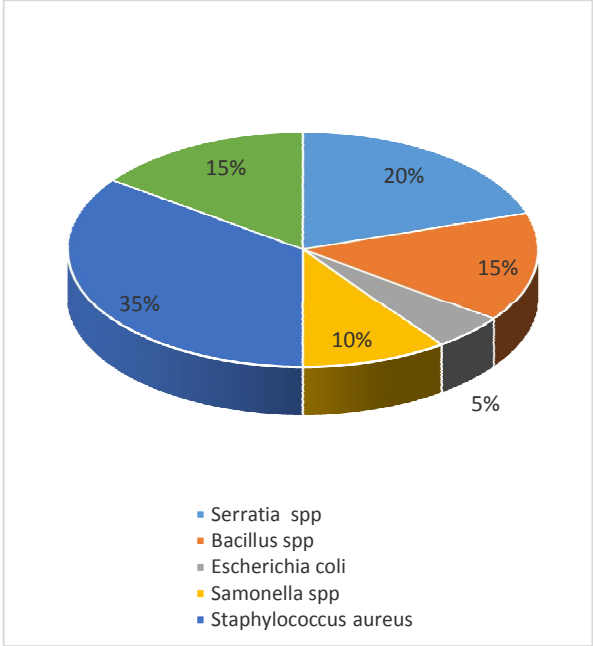

Figure 5: A pie chart showing the percentage occurrence of the various organisms isolated from conventional /refrigerated chicken meat.

4. Discussion

The study on the microbiology of Refrigerated chicken samples from market within Port Harcourt metropolis for *Listeria* species was conducted mainly to determine the state of hygienic practices which may determine incidences of cross-contamination of refrigerated chicken meat by *Listeria* from the poultry farms and the environment by farmers and traders, so as to ascertain the safety of these foods consumed by the people in the study areas. The *Listeria* species isolated from the conventional/ refrigerated chicken meat were identified as *L. monocytogenes* (53%), *L. innocua* (17%), *L. invanovii* (15%), *L. murrayi* (9%) and *L. welshimeri* (6%). *Listeria* were present in all 16 samples examined. The predominant species among the isolated *Listeria* strains was *L. monocytogenes* (56%), followed by *L. innocua (28%),* then *L. invanovii* (12%). This finding was not in agreement with earlier reported by Franco et *al.* (1994), who stated that the occurrence of *L. innocua* usually supersede that of *L. monocytogenes* in terms of number. It is however possible that since the five characteristic colonies on PALCAM agar were picked randomly, more of *L. monocytogenes* were picked in this study.

This finding is different from other similar investigations. Kwiatek (1993) reported 60% isolation of Listeria. In a study by Capita et al. (2001), 95% of the samples contained Listeria species among which they recognized 32% as *Listeria monocytogenes* and

66% as *Listeria innocua*. The study of refrigerated chicken proved that *L. monocytogenes* was present in 62% of the samples. Variation among similar studies may be a direct result of the ability of *L. monocytogenes* to grow in a wide temperature range, from -1.5 to 45ºC. Poor sanitary conditions during handling and processing may also contribute to higher incidence of contamination. Another possible factor that should be investigated is the source of poultry. Differences between sampling and isolation methods should also be considered in the context of differences between results (Kalender, 2003). Sources of contamination differ but may be from poultry feces as *L. monocytogenes* has been isolated from the fecal samples of chickens (Takasi et al., 1991; Ojeiyi et al., 1996) in many countries.

In a study by Kalender (2003) he concluded that animal feces can represent a source of *L. monocytogenes* contamination of carcasses at abattoirs. This constitutes a serious hazard to human health as it may lead to outbreaks of human listeriosis. The unusual growth and survival properties of *L. monocytogenes* and its ability to adhere to various food contact surfaces (Mafu et al., 1990) each contribute to the complexity of eliminating the organism from this environment. Therefore, to reduce or eliminate the potential for cross contamination of foods from the processing environment, attention must be focused on detection contamination sources and practices. It is however possible that since the five characteristic colonies on PALCAM agar were picked randomly, more of *L. monocytogenes* were picked in this study.

TVC is accepted to be the most important microbial group associated raw food as indicator for wholesomeness. The mean counts of TVC revealed that all of the means in the conventional chicken samples were higher. This contamination may be considered as indicator for the degree of sanitation during handling, processing, packaging and transportation or during storage and retailing in retailer stores Relatively higher counts than that found in this study were recorded by Abu-Ruwaida et al. (1994), Altalhi and Albashan (2004) and Ahmed and Dalia (2005). The standards stipulated by the guidelines of PHLS (2000) stated that the TVC in raw chickens should not exceed 6 log CFU/g, respectively.

Therefore, the information obtained from present study may be useful for the consumers of meat and meat products. Ensuring that food is safe from *L. monocytogenes* requires the collaboration of government, food industry and consumers. This include the government's efforts to monitor and enforce laws maintaining good hygienic practices in poultry farms, abattoirs and markets so as to control food contamination, the food manufacturer's role to maintain good manufacturing practices (GMPs), and the consumer's responsibility to follow safe food handling practices and consumption guidelines.

These efforts combined with a better understanding of the pathogenic process of *L. monocytogenes* will lead to improved control in foods. The components of the environment are the atmosphere, the land, water, humans and other animals. All this interrelate in a balanced natural form or pattern. However, these interrelationships are mostly disturbed by human actions and activities. These actions, although are for development often bring about disadvantages to human health. While it is estimated that 90% of *L. monocytogenes* infection to man and animals are through food, there is still much to learn about how the environmental factors operate. Thus, health education on the effect of soil, possible farm produce and water pollution by *L. monocytogenes* need to be advocated in Nigeria

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