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Prevalence of *Listeria monocytogenes* in Fresh and Raw Fish, Chicken and Beef

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Authors' contributions

This work was carried out in collaboration between all authors. Author LJA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EPO and JGE managed the analyses of the study. Author HEE managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

There has been recent outbreaks of food poisoning associated with *Listeria monocytogenes*. Most of the outbreaks were attributed to fresh meat and dairy products, and processed foods. This study was therefore carried out to examine the prevalence of *Listeria monocytogenes in* fish, beef *&* chicken sold in area markets in Calabar. A total of two hundred and fifty five (255) samples; fresh raw fish, chicken and beef were obtained from six different markets and were analyzed for possible contamination with *Listeria monocytogenes* using compact Dry LS plate. Out of the two hundred and fifty five (255) samples analysed, the results obtained showed one hundred and sixty two (63.5%) were positive. Beef showed a prevalence rate of 88% while chicken and fish showed prevalence rates of 64.7% and 37.7% respectively. The highest bacterial count was recorded in beef ($7.2x10^3$ cfu/g) and lowest in fish (1.08×10^2 cfu/g). The market locations also played a role as the Akim market beef showed the highest load followed by Goldie market and Mayne avenue market.

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The frequency of occurrence of *L. monocytogenes* was highest for Watt market with 73.8% followed by Uwanse market with 71.4%. The least occurrence was Marian market. The *L. monocytogenes* counts (cfu/g) according to locations for beef, chicken and fish showed significance ($p < 0.05$). However, comparison of the counts beef with chicken, beef with fish, and chicken with fish did not show any significance ($p > 0.05$). The occurrence and safety challenge of this organism in foods as well as the high mortality rate associated with listeriosis have made the pathogen a serious public health concern. For the fact that *L. monocytogenes* was isolated from these meat products is of serious concern to the health of consumers of these meats. Documentation of food borne disease outbreaks and the causative agents is therefore necessary.

Keywords: Listeriosis; compact dry Ls plate; contamination; entero-invasive gastrointestinal pathogens.

1. INTRODUCTION

It is well documented that the contamination of food with pathogens is a major public health concern worldwide [1,2]. *Listeria monocytogenes* has been associated with a wide variety of food sources particularly meat and chicken [3]. Listeria is a widely distributed bacterium in nature and commonly found in soil, sewage, water and animals. The ubiquitous character of the bacteria inevitably results in contamination of numerous food products [4].

The genus listeria is a Gram positive, non-spore, rod-shaped bacteria occurring singly or in short chains and not capsulated [5]. It contains six (6) species: *L. monocytogenes, L. innocua, L. seeligeri, L. welshimeric, L. ivanovii* and *L. grayi.* Of which *L. ivanovi* and *monocytogenes* are pathogenic for mice and other animals. However, only *L. monocytogenes* is commonly associated with human listeriosis [6,7]. *Listeria* spp has been isolated from poultry, red meat and meat products in many countries around the world [8,9].

The detection of *Listeria monocytogenes* in food is of particular concern in terms of consumer safety as this organism is capable of growing on both raw and cooked meat at refrigeration temperature [10]. In the past 25 years, *L. monocytogenes* has become increasingly important as a food associated pathogen because of its high case fatality rate [11]. Listeriosis is a relatively rare but serious disease with the highest hospitalization rate amongst known food borne pathogens of 91% and fatality rate of ranging from 20 to 30 [12]. Outbreaks of Listeriosis occur sporadically in chickens, turkey, pigeons and other avian species. Young birds are more susceptible [13]. Listeriosis is a relatively rare forborne illness, but can be life threatening with high fatality rates. It is mainly associated with the consumption of processed foods [14], raw beef, fish, milk, and vegetables [15]. The species *L. monocytogenes* is widely studied in developed countries and is known as an entero-invasive gastrointestinal pathogen [16]. There are no strains of *L. monocytogenes* with unique properties that lead to persistence and there are no mechanisms that can protect the organism when present in acidic juices, yogurt, salad dressing and modified CO_2 atmosphere [17]. Humans can become infected when contaminated food is ingested because the acidic stomach environment and its surface protein can help the organism to attach to the gut and multiply in the host's cell cytosol.

Moreover, *L. monocytogenes* is an important food borne pathogen that cause septicemia, meningitis and gastroenteritis particularly in children, the elderly and immune suppressed individuals. It causes miscarriage in pregnant women [18-20]. Unlike most other pathogens, *L. monocytogenes* is notable for its ability to grow at refrigeration temperatures. This has considerable significance for food safety, as it means that chilling to 4℃ cannot be relied upon to prevent the growth of the organism to dangerous level [21].

The consumption of food contaminated with *L. monocytogenes* has been identified as the main transmission route for this pathogen in both humans and animals. *Listeria moncytogenes* has been isolated from all categories of food [22]. There are many reports about the isolation and detection of *L. moncytogenes* and from meat, fish and seafood [23,24], and raw poultry [5,10,33], raw vegetables, [25], raw milk and cheeses, [26].

The standard microbiological methods for identification of Listeria species are laborious and time consuming, requiring a minimum of five (5)

days to recognize listeria spp and about 10 days to identify *L. monocytogenes* by confirmation test [27]. It is therefore necessary to use a chromogenic medium that will give results within 24 hrs.

Despite an increasing rate of *Listeriosis* reported in several European countries in recent years [28] and other outbreaks in the United States [29], Canada [30,31] and China [32], the occurrence and prevalence of the organism in food borne disease in Nigeria is hardly reported. There is limited information on the status of food borne *Listeriosis* in the public health sector in Nigeria. In view of the health risk posed by *L. monocytogenes* in beef, chicken and fish and the frequent consumption of these foods, the present study was undertaken to evaluate the prevalence of this organism in beef, fish and chicken sold in Calabar. The findings of this work will enable people to appreciate the possible health implications of eating *Listeria monocytogens* contaminated beef, fish and chicken.

2. MATERIALS AND METHODS

2.1 Materials

The materials employed for this research study followed proper analytical procedures. The materials used were glass wares, equipments, media and reagents, compact Dry Ls plate, ATCC 19114 and lots of other miscellaneous materials.

2.2 Compact Dry Ls Plate

Compact Dry LS (NISSUI pharmaceutical Co ltd. Tokyo, Japan) are ready to use, chromogenic plates for specific isolation and detection of *Listeria monocytogenes*.

2.3 Sample Collection

A total of two hundred and fifty five (255) food samples consisting of (85) raw meat, (85) raw chicken and (85) raw fish were obtained from the area local markets – Marian market located along Marian Road and Akim market located between IBB and Etta Agbo roads in Calabar Municipal Council, Watt market, Mayne avenue market, Uwanse market and Goldie market all located in Calabar South Local Government from September to November 2015. The samples were aseptically collected and each placed in food grade containers and put in pre cooled cool box at 4℃ and immediately transported to the microbiology laboratory of the faculty of science at University of Calabar, Calabar. In the Laboratory a code number was given to each sample and processed immediately.

2.4 Microbial Analysis of Samples

A 20 g representative portion from each sample was introduced aseptically to sterile stomacher bag containing 180 ml of peptone water (primary enrichment medium) to obtain a 1:10 sample dilution. The samples were then homogenized for 1 min at 260 rpm in the Seward Stomacher Model 400, (Seward Limited, U.K.). The cap of the compact dry plate was then opened and 1 ml of the specimen was inoculated in the middle of the plate. Each sample was inoculated in triplicates. The specimen diffuses automatically and evenly into the sheet and transforms the dried sheet into a gel within seconds. The cap was put back on the plate and necessary information needed written on the memorandum section. The capped plates were then turned upside down and put in the incubator for incubation at 37℃ for 24-48 hrs (NISSUI Pharmaceutical CO Ltd, Japan). After incubation, the number of distinct colored colonies (blue) on the plates were counted and the averages recorded [33].

2.5 Confirmation Test

The blue appearing colonies were sub cultured to purify. The pure cultures were then stocked and used for various tests such as catalase, motility, hemolysis, glucose, maltose, lactose, CAMP test with *Staphylococcus aureus*, esculin hydrolysis and mannitol. The isolates were also Gram stained. For the hemolysis test, sheep blood agar plates were prepared and inoculated with the isolates and incubated for 37℃ for 24 hrs. A reference strain from American Type Culture Collection Center ATCC 19114 was obtained and sub cultured. This strain was tested along with the isolates as control.

2.6 Statistical Analysis of Data

Triplicate readings were subjected to Analysis of Variance (ANOVA) at 95% level of significance to test for significance and presented as Mean(±Sd). Further, *L. monocytogenes* counts in the different sampled meats and fish were compared using student t-test. Values with p-values ≤ 0.05 were considered significance. All analysis were performed using Microsoft Excel 2010.

3. RESULTS AND DISCUSSION

3.1 Results

The mean *L. monocytogenes* counts per gram of the samples (Beef, chicken and fish and locations respectively are shown in Table 1. Table 2 shows the confirmation biochemical test results while the percent prevalence rate of the organism (*Listeria monocytogenes*) in the samples are shown in the Table 3 and Table 4 shows the frequency of occurrence of *L. monocytogenes* at different markets on the meat products.

3.2 Discussion

The results show the contamination levels and prevalence rates of *Listeria monocytogenes.* As can be seen from the results, this organism was isolated from the 3 samples. The loads of *L. monocytogenes* for the various samples are much higher than the results obtained by Wu [34]. This could be due to the environmental differences and market types where these products were bought. The open markets here are usually infested with flies and the slaughter tables are usually not disinfected before or after use. This is in line with the environmental studies in Nigeria by Tambuwal [35], Kawo [36], Ikeh [37], and Adetunji and Ishola [38] who enumerated Listeria on meat tables before and after sales of meat in Ibadan municipal abattoir in Nigeria and found an increase in Listeria counts after meat sales. There are numerous direct or indirect sources of contamination, including animals or insects, soil, water, dirty equipment, and human handling [39]. These give room to cross contamination which may affect ready- to-eat foods. The average prevalence rate for the analyzed samples is 63.5%. For the types of samples and locations, the *L. monocytogenes* loads are higher than the limits of 100cfu/g recommended by The Microbiological criteria in European Union while United States of America recommends zero tolerance.

Table 1. Mean *Listeria monocytogenes* counts for each sample type and locations

Samples	Locations	Average counts (cfu/g)
Beef	Marian market	$7.2x10^2 \pm 0.02$
	Watt market	$4.8 \times 10^2 \pm 0.01$
	Uwanse market	$4.1 \times 10^2 \pm 0.03$
	Akim market	$1.88 \times 10^3 \pm 0.02$
	Goldie market	$2.8 \times 10^2 \pm 0.01$
Chicken	Marian market	$2.6 x10^2 \pm 0.02$
	Watt market	$4.3 \times 10^2 \pm 0.01$
	Akim market	$1.8 \times 10^2 \pm 0.04$
	Mayne avenue	$3.0 \times 10^2 \pm 0.02$
	market	1.72×10^3 0.03
	Goldie market	
Fish	Marian market	$1.08 \times 10^2 \pm 0.04$
	Watt market	$6.0 \times 10^2 \pm 0.01$
	Uwanse market	$2.6 \times 10^2 \pm 0.03$
	Mayne avenue	$1.16 \times 10^3 \pm 0.03$
	Goldie market	$1.69 \times 10^3 \pm 0.02$

Mean of 3 determinations

The prevalence of *Listeria monocytogenes* in raw beef, fish and chicken as presented in Table 3 reflects the ubiquity of this organism. The results obtained is in line with the work of Indrawattana [9], where they reported 15.4% incidence of *Listeria monocytogenes* in raw meat, raw chicken, raw fish and raw milk. The presence of *L. monocytogenes* in these products can be considered important in ready-to-eat products

Table 2. Biochemical test results

	Lac	Cat	Glu	Mal	Mot	Esc	Man	Hem	Grm rxn	CAMP test with *S. aureus*
L. monocytogenes	-	+	acid	+	+	+	-	β-hem	+	+
L. innocua	-	+	acid	+	+	+	-	-	+	-
L. ivanovii	-	+	acid	+	+	+	-	-	+	-
L. grayi	-	+	acid	+	+	+	+	-	+	-
L. welshimeri	-	+	acid	+	+	+	-	-	+	-
L. seeligeri	-	+	acid	+	+	+	-	+	+	-
L. monocytogenes ATCC 19114	-	+	acid	+	+	+	-	+	+	+

Key: Lac = lactose, Cat = catalase, Glu = glucose, Mal = maltose, Mot = motility, Esc = esculin, Man = mannitol, Hem = hemolysis, Grm rxn = Gram reaction, CAM = CAMP

Table 3. Prevalence of *Listeria monocytogenes* in beef, chicken and fish

Samples	Number of samples collected and examined	Positive samples contaminated	Percentage (%)
Beef	85	75	88.24
Chicken	85	55	64.71
Fish	85	32	37.65

because cross contamination can lead to food poisoning. It has been demonstrated that normal pasteurization process is effective in the destruction of this pathogen, so conventional cooking would also be expected to eliminate this organism [40]. The potentials of these food products to be contaminated can be either directly or via surfaces and equipments that may become contaminated with *Listeria monocytogenes*. Previous studies had also demonstrated that colonization of refrigerators by *L. monocytogenes* is a potential source of contamination of food products [41]. The high incidence rate in beef sample obtained in this study is not surprising as previous workers reported 80-86% contamination of raw meat samples with *Listeria monocytogenes*. Despite the world-wide reports of outbreaks of food-borne listeriosis, the occurrence of Listeria is still not widely reported in Nigeria [42]. From Table 1, it can be seen that counts (cfu/g) according to locations for beef, chicken and fish showed significance ($p< 0.05$). However, comparison of the counts of beef with chicken, beef with fish, and chicken with fish did not show any significance ($p > 0.05$).

Table 4. Frequency of occurrence of *L. monocytogenes* at different markets on the meat products

Location	No. of samples	No. positive	% occurrence
Watt	42	31	73.8
Marian	43	20	46.5
Goldie	42	27	64.3
Uwanse	42	30	71.4
Akim	43	22	51.2
Mayne av	43	23	53.5

4. CONCLUSION

The presence of this organism in these food products if undercooked could be a potential risk for consumers. It is pertinent to note that listeriosis is a poorly reported disease in Nigeria. Different criteria or recommendations for admissible levels of *L. monocytogenes* in food products have been established in different countries. For example, absence in 25 g of food in USA and Italy is recommended. However, a requirement for the absence of *L. monocytogenes* in some food products is considered realistic by several countries [28]. A tolerance of below 100 cfu/g of food at a time of consumption has been accepted in Germany, Netherland, France and the UK. This might present a serious problem of public health particularly in those products that support the growth of the organism. Based on the above, one can say that the results obtained in this study show that the *L. monocytogenes* levels on the meats exceed the permissible levels. If proper hygiene is not maintained in the homes while preparing these meats for consumption, food poisoning can result because of cross contamination from the knives and other kitchen utensils.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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