

## **ISOLATION AND IDENTIFICATION OF LISTERIA MONOCYTOGENES IN READY TO EAT (RTE) FOOD**

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### **ABSTRACT:**

**Listeria monocytogenes constitutes one of the major food borne diseases worldwide. One of the sources of Listeria monocytogenes infection in humans is the consumption of contaminated ready to eat (RTE) meat foods. The objective of the model was to determine concrete options to mitigate the risk of L.monocytogenes illness associated to the consumption of RTE meat-based foods. Based on the collected data, a Quantitative Microbial Risk Assessment model was built up. The model considered three main risk exposure pathways namely the consumption of bovine meat within the household, the consumption of bovine meat outside the home, as well as the consumption of goat meat (grilled goat brochettes) of Coimbatore city. According to the model, the risk of L.monocytogenes illness associated to the consumption of RTE meat based foods Coimbatore city inhabitants appeared to be relatively low (ranging from 1.7 to 3.4% depending of the risk exposure pathway) and was found to be increasing with the meat consumption level in different socio-economical category of the consumers. The analysis of risk mitigation scenario revealed that an efficient control of L.monocytogenes illness associated to the consumption of RTE meat based foods can be achieved through a simultaneous application of control measures at different levels of the meat chain, in particular at the preparation stage within households and collective catering establishments.**

### **Keywords:**

Quantitative Microbial Risk Assessment, meat, L.monocytogenes, risk factor, bacterial contamination, food, Coimbatore.

### **INTRODUCTION:**

Listeria monocytogenes is a bacterium that causes listeriosis, a disease that can have severe consequences for groups of the population. It can cause miscarriages in pregnant women

and be fatal in immunocompromised individuals and the elderly. In healthy people, listeriosis generally only causes a mild form of illness [1]. *L. monocytogenes* can be found throughout the environment. It has been isolated from domestic and wild animals, birds, soil, vegetation, fodder, water and from floors, drains and wet areas of food processing factories. It has been found in both raw and processed food samples, including dairy products, meats, vegetables and seafood [2,3]. Infection with this bacterium often produces non-specific initial manifestations; without prompt antibiotic therapy, it can have severe clinical consequences, occasionally including death [4,5].

The present study was the first research contribution to shed light on the risk of *L. monocytogenes* illness attributable to the consumption of RTE meat based foods in Coimbatore city. However, it cannot pretend having fully explored all risk factors along the meat production chain. In fact, the used risk assessment model started and appeared to be hampered by the lack of data for some model variables. Furthermore, epidemiological studies comparing *L. monocytogenes* strains isolated from RTE meat at different levels of the chain to those isolated from human listeriosis patients should be conducted to confirm the contribution of RTE meat-based-foods to the human *L. monocytogenes* in Coimbatore city inhabitants. Additionally, the present risk assessment study dealt only with *L. monocytogenes* species though there are other bacterial pathogens of food safety interest in the meat chain. Therefore, this study should be a starting point for further microbiological risk assessment studies on RTE meat or other food commodities in Coimbatore.

## **MATERIALS AND METHODS:**

### **Collection of samples:**

Fifty retail ready to eat (RTE) meat products samples were collected in Coimbatore City, Tamilnadu state. Selected households were grouped into three (3) socio-economical categories namely households with low, medium and high income according to the Rwandan mutual health insurance scheme [6].

Considering the meat consumption rate of 89% and the proportion of different socio-economical categories in the population of Coimbatore city (21% for low income, 75% for middle income and 4% for high income households), the population consuming meat was estimated to 212,211; 757,898 and 40,421 inhabitants respectively in low, medium and high-income households of Coimbatore city. A total of 270 samples were collected. The meat samples were transported to the laboratory, within approximately 2 h, in a cold box with freeze packs. At the laboratory, samples were stored frozen at -30°C and microbiologically analysed within 24 h.

### **Isolation and identification of *L. monocytogenes*:**

Ready to eat meat products samples were tested for the presence of *L. monocytogenes* following the procedure recommended by using the International Organization for Standardization [7] procedure. The *L. monocytogenes* was confirmed by various preliminary confirmation tests and biochemical tests like Haemolysis test, Catalase test, oxidase test, Christie, Atkins, Munch-Petersen (CAMP) test and Carbohydrate utilization test, pH of samples, titratable acidity, salt content, moisture content and water activity were also determined.

### **Conduct of the survey:**

The survey was conducted to assess the consumption of meat at the household level. The questionnaire was divided into two sections. The first section provided the socio-demographic information of the household, namely the physical address and the socio-economic category of the household, as well as the number and the age of the household members consuming meat. The second section supplied information on the consumption of meat within the household, including the meat-type preferences, the frequency of consumption, and the quantity of meat consumed. In each selected household, the consumption of meat was monitored for a period of 30 days between April and August 2014.

### **RESULTS AND DISCUSSION:**

#### **Isolation and identification of *L.monocytogenes*:**

Among the *Listeria* species, *L. monocytogenes* is the most commonly associated with human listeriosis. The mean *Listeria* counts for the samples in this study were  $7.7 \times 10^4$  CFU/g and  $8.6 \times 10^4$  CFU/mL, for meat, respectively. Although these values represent total *Listeria* counts and not *L.monocytogenes* counts, they are still a cause for concern, considering that *L.monocytogenes* was the second most predominant species isolated from the food samples. According to international standards for acceptable levels of *L.monocytogenes* in foods, foods with a shelf life of less than five days or foods with the potential to support the growth of *L.monocytogenes* should not contain more than 100 CFU/g of food; and when the food is intended for infants, there should be complete absence of *L.monocytogenes* per 25 g of the food [8]. The high frequency of *L. monocytogenes* in this study and the high total *Listeria* counts suggest a possibility that the *L. monocytogenes* counts in these products could well exceed the acceptable limit of 100 cfu/g of food (Table-1). ALOA agar medium has proved to be a useful and significantly better assay than other media (Oxford agar, UVM agar and PALCAM agar) for the isolation and differentiation of *L.monocytogenes* from non-pathogenic *Listeria* species, because *L. monocytogenes* colonies on ALOA agar exhibited clear halo zone. After the enrichment procedure, the inoculum was placed on PALCAM agar (HiMedia) and incubated for 48 h at 37°C. The gray-green colonies surrounded by diffuse black zone on PALCAM agar were picked up and further purified on Tryptone Soya Yeast Extract agar (TSYEA).

Subsequently, pinpoint colonies of TSYEA were subjected to identification procedures which included Gram's staining followed by a microscopic examination, catalase test, and oxidase test. Evaluation of the physico-chemical properties of the samples showed that the pH and water activity (aw) values of the meat samples were  $4.5 \pm 0.1$ ,  $0.76 \pm 0.0$  and  $3.6 \pm 0.1$ ,  $0.94 \pm 0.0$ , respectively. The characteristic Gram-positive, coccus bacillary or short rod-shaped organisms which were catalase positive and oxidase negative, were sub-cultured in Brain heart infusion (BHI) broth at 25°C for 12-18 h. Subsequently, the cultures showing typical tumbling motility were considered as "presumptive" *Listeria* isolates, which were in turn subjected to detailed biochemical tests (Table-2) viz.; the identification of *L.monocytogenes* showed the presence of catalase, sugar fermentation, NaCl, haemolysis test and CAMP. It showed the absence of oxidase. Although conventional method of selective culture media are confirmed by the Microbact [9] had examined the factors of colonization of broiler chickens with *L. monocytogenes* (orally inoculated) did not colonize chickens as easily as did *Salmonellae* or *C. jejuni*.

In Current study, the presence of *L. monocytogenes* in processed RTE meats could be explained by the inadequate heat treatment to destroy the growth of *L. monocytogenes* or as a result of post process contamination. Many studies have reported that the prevalence rate in RTE meats ranged from 1.8 to 48%) [10,11]. The growth of *L. monocytogenes* in meat is highly dependent on the temperature, the pH of the meat, the type of tissue and the type and amount of background micro flora present. [12] found that the growth of *L. monocytogenes* in meat was highly dependent on product type and pH. The organism was able to grow well in meat products with a pH value near or above 6.0 while it grew poorly or did not grow in meats that have pH-5.0. The isolation of virulent *L. monocytogenes* from the RTE product, RTE meat, in this study is a significant cause for concern. RTE meat is considered to be a complete food and is relatively cheap. Therefore, in a country with a large population of low-income earners, it comes highly recommended as a means of reducing malnutrition and is widely consumed across demographic groups, including pregnant women, infants and the elderly, who are among the groups most at risk for listeriosis. The results from this study highlight a need for the development and implementation of food safety policies and standards to guide the production and distribution of RTE foods in India [13].

A survey of this study showed the results of household category and daily intake of meat products (Tale-3). Previous studies also reported that 939 home refrigerators conducted by Audits International in the United States of America in 1999 [14] provided data for considering the impact of home storage temperatures on the levels of *L. monocytogenes* at consumption. A cumulative distribution of the data was used without any model fitting. The 5th, 50th and 95th percentile temperatures were 0.5, 3.4 and 6.9°C. This distribution had approximately 1.4% of the refrigerators operating above 10°C, where growth of *L. monocytogenes* would be relatively rapid. The distribution of refrigerator temperatures may differ in important ways between different countries. Thus European temperatures appear to average 6.6°C [15].

## **CONCLUSION:**

The results from this study highlight a need for the development and implementation of food safety policies and standards to guide the production and distribution of RTE meat foods in Coimbatore. Producers and consumers of RTE meat products should also be made aware of the need to observe the highest possible standards of hygiene during production of RTE and foods to reduce the risk of listeriosis and other food-borne diseases.

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**Table-1: Utilization of meat from various animal species within retail establishments of Coimbatore city**

n		Animal species
		Goat/mutton
Small-scale	39	39 (100.0a)
Medium scale	69	69 (100.0a)
Large-scale	42	42 (100.0a)
<b>Total</b>	150	150 (100.0[w])

Values are numbers (percentage) of retail establishments selling meat of a given animal species. In the same column, different superscript letters (a, b, c) indicate a significant ( $p \leq 0.05$ ) difference. Values with the same superscript letters (w, x, y, z) are not significantly different ( $p \leq 0.05$ ).

**Table-2: Identification of L.monocytogenes using biochemical tests**

Pathogen	haemolysis	Catalase	Motility	CAMP	NaCl	Oxidase	Sugar fermentation	Carbohydrate utilization
<b>L.monocytogenes</b>	+	+	+	+	+	-	+	-

+ denotes presence

- denotes negative

**Table-3: Meat dietary intake in Coimbatore city inhabitants.**

<b>Risk exposure pathway</b>	<b>Household category</b>	<b>Daily intake</b>
<b>RTE food consumption within the household</b>	Low-income households	14.2
	Middle income households	34.3
	High income households	96.0
<b>RTE food consumption outside the household</b>	Low-income households	0.4
	Middle income households	11.2
	High income households	53.2
<b>Goat Meat consumption outside the household</b>	Low-income households	0.2
	Middle income households	39.5
	High income households	62.5

Values are the means daily meat intake (g/pers. day) for different categories of households in Coimbatore city