Prevalence Of Listeria Organisms In Meat And Some Meat Products

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ABSTRACT

A total of 250 samples representing 150 samples fresh meat, fresh and frozen sausage, frankfurter, luncheon and shawerma 25 of each and 100 samples frozen minced meat, meat kofta and rice kofta , Hamburger, and hawawshy 20 of each were randomly purchased from shops and supermarkets in Dakahlia Governorate, Egypt and examined bacteriologically for determination of the prevalence of listeria species.

The overall prevalence of Listeria spp. were 7 (28%) of fresh meat, 8 (40%) of frozen minced meat, 7 (35%) of meat kofta, 4 (20%) of rice kofta, 5 (20%) of fresh sausage, 2 (8%) of frozen sausage, 7 (25%) of hamburger, 4 (16%) of frankfurter, meanwhile we couldn't detected Lisreria spp. in luncheon, shawerma and hawawshy.

The prevalence of *L. monocytogenes* was 11 (4.4%) and confirmed in one (4%) of fresh meat, 2 (10%) of frozen minced meat, 1 (5%) of meat kofta, 1 (5%) of rice kofta, 2 (8%) of fresh sausage, 1(4%) of frozen sausage, 2 (10%) of hamburger and 1 (4%) of frankfurter respectively with total incidence of 11 (4.4%), which displayed beta-heamolysis on sheep blood agar and positive CAMP test and they were further identified by PCR technique. Pathogenesity of isolated *L. monocytogenes* to white mice was studied and public health significance of this pathogen and sanitary measures were discussed. *L. monocytogenes* has been recognized as major food born pathogen especially foods which are not exposed to a sufficient heat treatment.

INTRODUCTION

Meat and meat products constitute a valuable part of diet. They are a good source of the first class protein, as well as other nutrients as fat and minerals. However consumption of such palatable and nutritious foods may cause many hazards to the consumers, probably due to contamination with various food–borne pathogens. During the last few years, the factories of meat products were developed and widespread every where, also the retails shops become the sources for many food poisoning disease especially those take away foods. Meat products are very delicious food due to additives of spices and herbs which give pronounced flavour and aroma *(1,2)*.

Human listeriosis is a sporadic disease which associated with consumption of unsufficient cooked meat, contaminated milk, soft cheese, unwashed raw vegetables and cabbage *(3)*. The post processing contamination of ready to – eat meat products with Listeria spp. could be a potential hazard *(4)*. Listeria contamination of fresh, cooked and ready to – eat meat products can take place any time between slaughter, processive and packaging of meat products *(3)*. *L. monocytogenes* the only food born pathogens is recognized in the genous listeria *(5)*.

Listeria is a Gram positive asporogenous coccobacillus which gained increasing attention as a pathogen of public health importance owing to large numbers of food–borne out breaks of listeriosis and of great concern to the food industry, especially in food stored under refrigerated condition where, *L. monocytogenes* is able to multiply *(6)*.

Because of its ability to survive and proliferate at refrigeration temperature *L. monocytogenes* may cause disease through frozen foods *(7)*.

The standard microbiological methods for identification of Listeria species are laborious and time consuming requiring a minimum 5 days to recognize Listeria species and about 10 days to identify L. monocytogenes by confirmation tests *(8)*, while rapid response should be carried out in case of confirmation since it is of principal importance to ensure the safety of foods. In few past years, progressing in biotechnology has resulted in the development of rapid methods that reduce the analysis time and offer great sensitivity and specificity in the detection of pathogens among these, PCR has been increasingly used *(9)*.

MATERIAL AND METHODS

Collection of samples

A total of 250 samples of meat and some meat products were collected from different hygienic levels localities and supermarkets at Mansoura City, then well identified, packed in sterile plastic bags then labelled and immediately transferred to the laboratory where they were bacteriologically screened for presence of listeria spp.

Methodology of isolation of Listeria spp.

Enrichment procedure

Twenty five gm. from each meat or meat product samples were aseptically weighted and added to 225ml. of *Listeria* Enrichment Broth, University of Vermont Medium provided from Biolife (LEBUVMI). The mixture was homogenized by using sterile mixture (New National) at high speed for 2 minutes.

The inoculated enrichment was incubated at 30°C for 24 hrs., then 0.1 of incubated ($LEBUVM_I$) was transferred to 10 ml ($LEBUVM_{II}$) and incubated at 30°C for 24 hours.

Selective plating

A loopful from each of enrichment culture UVM_I and UVM_{II} broth was streaked onto PALCAM agar plates, then incubated at 30°C for 48 hours *(10,11)*.

Confirmation

Colonies showing morphological characters as dew drop-like, black with brown hallow, or dark brown colonies, 1-2 mm in diameter were streaked onto trypticase Soya agar supplemented with 0.6% yeast extract (TSA-YE) and incubated at 30°C for 24 hours till obtaining satisfactory pure separate colonies; which were inoculated into semisolid agar and kept in refrigerator at 4°C for further identification.

Each isolates was checked for Gram staining, catalase, motility, oxidase, H_2S production, blood heamolysis, Vogas-proskour reaction, nitrate reduction and CAMP test.

Genomic DNA extraction *(12)*

Suspected colonies obtained by cultural methods were resuspected in nutrient broth and incubated at 37°C for 24 hours. Bacterial cells were harvested in a micro centrifuge tube, centrifuge at 10,000 rpm for 30 seconds. Bacterial pellets were resuspected and washed in 200µl physiological saline, then recentrifuged at 10,000 for 30 seconds. The cells pellets were resuspended in 200 µl physiological saline, followed by adding 200 µl buffer CB and 20 µl protinase K (20mg/ml).

Thorough mixing of the solution, incubation at 70°C water bath, 10min. cooling to room temperature; adding of 100 µl isopropyl alcohol and mix. The last step solution was then transferred into a spin-column AC, centrifugation at 10,000 for 2 minutes and then flow-through was discarded adding IR Buffer (500 µl), centrifuge at 12,000 rpm for 30 seconds. Flow-through was discarded, adding of WB buffer (700 µl), centrifuged 12,000 for 30 seconds, discarding the flow-through, spin-column was centrifuged at 13,000 for 2 minutes, to remove the ethanol, spin column was placed in the clear centrifuge tube for elution of the extracted DNA.

Elution of extracted DNA

One hundred µl of preheated buffer EB was added into the column, incubated at room temperature for 3 – 5 minutes, centrifuged at

12,000 rpm for 1 minute to recover the purified DNA.

Multiplex PCR for detection of internatin genes *(12)*

Oligonucleotide primers synthesized by Bioteke Corporation (Canada) were used for implication of *Listeria monocytogenes* internalin genes inl A, inl C and inl J.

The inl A primers were intended for species –specific recongestion and inl C and inl J primers were disigned for virulence determination of *L. monocytogenes* (Table 1).

Table 1. Primer sequences and expected product sizes of the multiplex PCR

	Gene	Primer sequence (5'–3')	Expected product size (Pb)
Inl A	Inl A-forward	ACGAGTAACGGGACAAATGC	
	Inl A-reverse	CCCGACAGTGGTGCTAGTT	800 bp
Inl C	Inl C-forward	AATTCCCACAGGACACAACC	
	Inl C-reverse	CGGGAATGCAATTTTTCACTA	517 bp
Inl J	Inl J-forward	TGTAACCCCGCTTACACAGTT	
	Inl J-reverse	AGCGGCTTGGCAGTCTAATA	238 bp

bp= base pair

PCR for detection of listeriolysin O virulence genes (hyl A) *(13)*

The following sequences *(14)*:

hyl Forward: 5'-CGG AGG TTC CGC AAA AGA TG-3' and

hyl A reverse: 5'-CCT CCA GCA GCG TGA TCG ATC TT-3'

Gel electrophoresis

All amplification products were resolved in 1% agarose gel, stained with ethidium bromide, detected under a short wavelength UV light source, and photographed with EDVOTEX gel documentation system. The 1-KE plus DNA Ladder (Invitrogen) was used as molecular size marker.

Pathogencity test

Each strain of well identified of *L. monocytogenes* was isolate from the examined samples grew overnight in tryptic soya broth with 0.6 yeast extract at 37°C, centrifuged and the sediment was resuspended in physiological saline (0.9%) and adjusted to the level used for inoculation (10^8 cell/ml). Each mice were inoculated intrapertonealy with 0.1 ml of one of the bacteria suspensions, the inoculated mice were maintained under observation for evaluation of clinical signs and mortalities. Control mice were inoculated intraperitonealy with 0.1ml of physiological saline. Dead mice were sacrified and from each liver, spleen and brain were collected and screended for presence of *Listeria* using Palcam medium.

RESULTS

Table 2. Prevalence of listeria species in meat and some meat products

Types of examined samples	No. of examined samples	Positive samples	
		No.	%
Fresh meat	25	7	28
Frozen minced meat	20	8	40
Raw meat product			
Meat kofta	20	7	35
Rice kofta	20	4	20
Fresh sausage	25	5	20
Frozen sausage	25	2	8
Hamburger	20	7	35
Frankfurter	25	4	16
Ready to eat			
Luncheon	25	-	-
Shawerma	25	-	-
Hawawshy	20	-	-
Total	250	44	17.6%

No= Number

Table 3. Prevalence of *listeria spp.* among fresh meat and meat products

Type of examined samples	*No. of examined samples*	*+ve sample L. spp*		*Identified listeria spp*													
				L. mono.		*L. innocua.*		*L. welsh.*		*L. seel.*		*L. grayi.*		*L. ivanovii*		*L.murryai.*	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Fresh meat	25	7	28	1	4	2	8			1	4	1	4			2	8
Frozen minced meat	20	8	40	2	10	4	20	1	5			1	5				
Raw meat product																	
Meat kofta	20	7	35	1	5	3	15	1	5								
Rice kofta	20	4	20	1	5	2	10			1	5			1	5	1	5
Fresh sausage	25	5	20	2	8	1	4					1	4			1	4
Frozen sausage	25	2	8	1	4	1	4										
Hamburger	20	7	35	2	10	3	15					1	5			1	5
Frankfurter	25	4	16	1	4	2	8					1	4				
Ready to eat																	
Shawerma	25	-															
Luncheon	25	-															
Hawawshy	20	-															
Total	250	44	17.6	11	4.4	18	7.2	2	0.8	2	0.8	5	20	1	0.4	5	2.0

L. mono: *Listeria monocytogenes* L. innocua*.: Listeria innocua* L. welsh.*.: Listeria welsheneri*

L. seel: *Listeria seelegeri* L. grayi: *Listeria grayi* L. muryi.*.: Listeria murryai*

+ve: positive No: Number

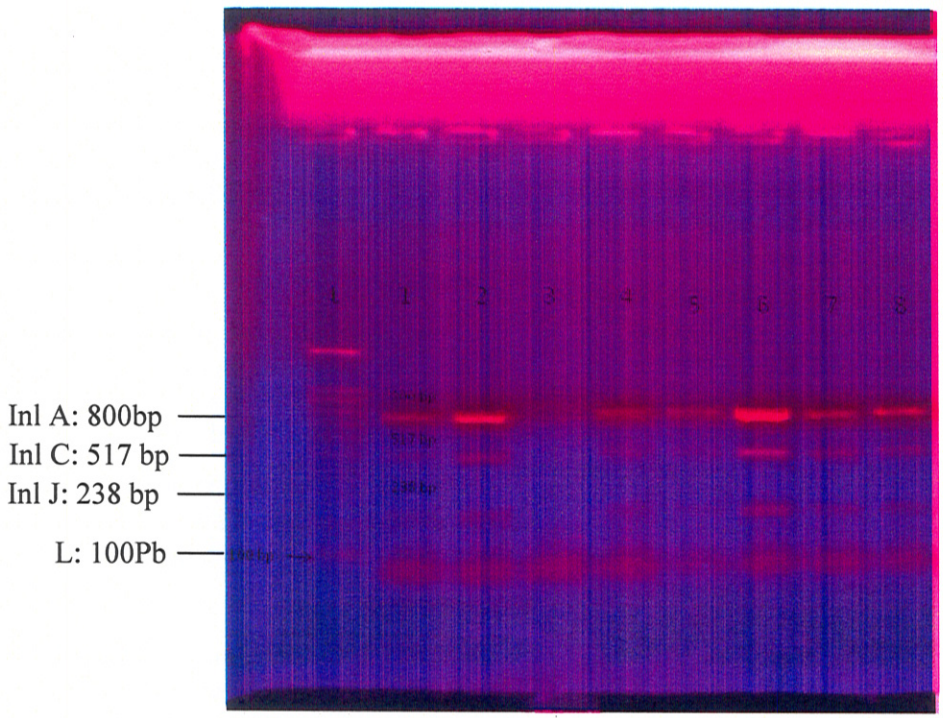

Fig. 1. Agarose gel electrophoresis of inl A, inl C and inl J amplicans obtained from l. monocytogenes suspected isolates L: 100bp ladder, 1 – 2: positive control of L. monocytogenes, 3: negative control, 4 – 8: suspected L. monocytogenes DNA from examined samples

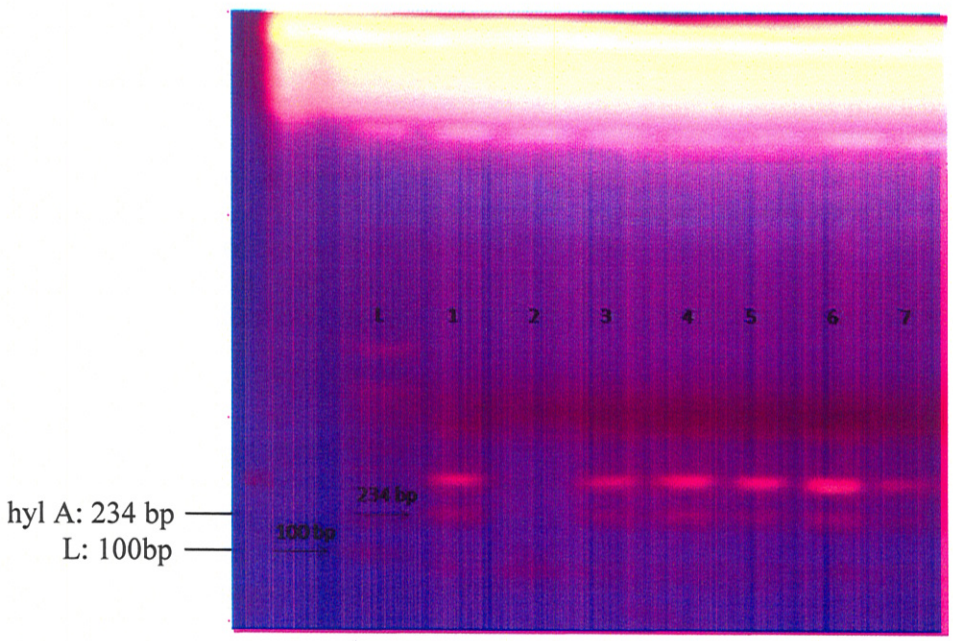

Fig. 2. Agarose gel electrophoresis of hyl A amplicans of listeriolysin O obtained from l. monocytogenes suspected isolates (L: 100bp ladder, 1: positive control of L. monocytogenes, 2: negative control, 3 – 7: suspected L. monocytogenes, DNA from examined samples

DISCUSSION

Prevalence of Listeria spp. From meat and meat products

Listeria has been recognized as one of the major food-born pathogen due to its ability to survive in adverse condition. It is isolated from raw meats (all types), sausage, raw and cooked poultry, milk and raw vegetables. *L. monocytogens* is a bacterium that occurs widely in both the agricultural (soil, plant and water) and food processing environment. The bacterium is resistant to various environmental conditions such as high salt and acidity ***(15)***.

Results given in Table 2 revealed that 44 (17.6%) out of 250 samples of meat and meat products harboured listeria spp. Listeria spp. were recorded by 7 (28%), 8 (40%), 7 (35%), 4(20%), 5(20%), 2 (8%), 7(35%), and 4 (16%) from examined raw fresh meat, frozen minced meat, meat kofta, rice kofta, fresh sausage, frozen sausage, hamburger and frankfurter respectively.

Raw fresh meat

Higher incidence of listeria spp. in raw meat (fresh) was obtained from isolated listeria spp. in the percentage of (42.5%) ***(16)***. Otherwise, lower incidence of listeria spp. was reported isolated listeria spp. (6.6%) out of examined samples ***(17)***. Nearly similar results were reported where L. spp. Was isolated from examined fresh meat samples in a percentage of 25% ***(18)***.

Frozen minced meat

The prevalence of listeria spp. in frozen minced (ground) meat was illustrated in Table 3. It was 8 (40%) out of 20 examined samples. Higher results were reported, ***(19)*** where listeria spp. was isolated from 51 (86.4%) out of 59 samples of minced beef , L. spp. was detected in 8 (80%) out of 10 examined samples of frozen ground beef ***(20)***. On the other hand, lower results of listeria spp. in retail minced meat were reported in a percentage of 18% ***(21)***.

Beef burger (hamburger)

Lower incidence of listeria spp. in beef burger (Hamburger) was also isolated in the percentage of 25% ***(16)*** and in the percentage of 18% out of 44 samples of retail beef burgers ***(21)***. In Table 2 percentage of listeria spp. was 35% of Hamburger samples. Similar percentage listeria spp was isolated from beef burger in the percentage of 30% ***(18)***.

Frozen sausage

Lower incidence of listeria spp. in frozen sausage in 20% of examined samples ***(20)***, higher incidence has been obtained in retail sausage ***(18,21)*** in 35% of examined samples.

In Table 2 prevalence of listeria spp. was 8% of frozen sausage. This result nearly similar value has been (8.2%) has been isolated ***(22)***.

Fresh sausage

In Table 2 the percentage of listeria spp was 20% of fresh sausage samples. These results is higher than that reported (13.3%) of 30 samples ***(23)*** and 3 (10%) of 30 samples ***(24)***.

In case of Meat kofta, 7 samples (35%) were isolated from 20 samples. Nearly similar incidence of L. spp. was obtained 9 (22.5%) of 40 samples of in meat kofta ***(24)***.

Meat kofta

A high percentage of listeria spp. in meat kofta was 4 (40%) of 9 examined samples ***(20)***. This result may be due to the traditional manual preparation of the product in restaurants, applied through contaminated fingers of food workers ***(25)***.

Frozen sausage

The lowest percentage of positive samples was detected in frozen sausage 2 (20%), and this May be attributed to the high susceptibility of *L. monocytogens* to combined chemical preservatives added to sausage ***(26,27)***.

Rice kofta

In case of rice kofta it reported 4 (20%) out of 20 samples, this percentage was lower that that recorded where the organism was isolated 84 (42%) out of 200 of examined samples ***(16)***.

Frankfurter

In case of Frankfurter the percentage was 4 (16%) out of 25 of examined samples. This

was higher than that reported by *(18)* isolated 3 (7.5%) out of 40 samples and *(28)* were isolated 9 (10%) out of 93 packs.

Ready to eat meat products

Luncheon

In Table 2, listeria spp. could not be detected in luncheon examined samples, such result was similar to that previously obtained ***(20,29)***. These results may be due to spices and temperature during manufacture and good hygiene measures.

Shawerma and Hwawshy

Also shawerma and Hwawshy could not be detected for the same reason.

It is evident from the results given in Table 2 11 samples out of 250 examined samples were positive for *L. monocytogenes* (4.4%). *L. monocytogenes* was not isolated from shawerma, luncheon and Hawawshy.

Raw meat

Higher incidence of *L. monocytogenes* in raw meat was reported ***(30)*** where (8.6%) of raw meat were positive for *L. monocytogenes* and ***(31)*** and from 49 (18.4%) of raw meat samples. *L. monocytogenes* was isolated from 39.1% of the examined raw meat samples ***(32)***. In this study, *L. monocytogenes* was isolated from 4% of examined raw samples.

Frozen minced meat

Higher incidences of *L. monocytogenes* in minced meat were reported where it was recovered 20 out of 41 samples of frozen ground beef ***(33)***. Furthermore, isolated *L. monocytogenes* was isolated in the percentage of 45.76% and ***(20)*** isolated 3 (30%) out of 10 examined samples ***(19)***, also, isolated from 19% of ground beef ***(34)***.

In the current study *L. monocytogenes* was isolated 2 (10%) from 20 frozen minced meat samples.

Raw meat products

Meat kofta

In the present work *L. monocytogenes* was isolated from meat kofta by a percentage 5%, one out of 20 examined samples. This result is lower ***(24)*** that reported where 4 (10%) out of (40) raw kofta examined samples. Also the result is higher than that reported one (2.5%) out of (40) examined samples ***(18)***. Higher result was recorded, 4 (40%) out of 10 examined meat kofta samples ***(20)***. *L. monocytogenes* was isolated from rice kofta in the percentage of 5% one out of 20 examined samples. This result is higher than ***(16)*** isolated 2 (1%) out of 200 examined rice kofta samples.

Frozen sausage

Higher incidence of *L. monocytogenes* in frozen sausage was recorded in a percentage of (29.4%) 15 out of 51 samples ***(21)*** and isolated 2 (20%) out of 10 examined frozen sausage samples***(20)***. Our results indicated that *L. monocytogenes* percentage was 4% one out of 25 samples. This lower than previously recorded ***(21)***. Some authors attributed the differences to improvements in the process and factory Hygiene. Lower incidence of *L. monocytogenes* in frozen sausage was recorded where one of 40 samples (2.5%) was contaminated ***(18)***.

Fresh sausage

In Table 5, *L. monocytogenes* in fresh sausage was isolated in a percentage of 8% 2 out of 25 examined samples, this result is lower than that previously cited ***(24)*** one (33%) out of (30) examined sausage samples and higher than (3.8%) of examined sausage sample ***(35)***.

The existence of *L. monocytogenes* in raw meat would a therat only if meat insufficiently cooked or if cross contamination occurred.

Lower incidence of *L. monocytogenes* in raw sausage was reported (1.6%) of examined raw sausage ***(36)***. Higher incidence such (15.6%) has been isolated ***(37)***, while 6.9% were nearly similar to the current study (8%) ***(38)***.

Presence of *L. monocytogenes* in sausage may be attributed either to manufacturing of the product from low–quality materials or post-processing contamination as handling, utensils and equipments ***(17)***.

Hamburger

L. monocytogenes was isolated from beef burger in the incidence of 10% (2 out 20) of examined samples in this study similar incidence ***(18)*** and higher incidence was reported in a percentage of 16% ***(34)***.

Frankfurter

In retail Frankfurter *L. monocytogenes* was recorded in an incidence of 4% (one out of 25) of examined samples in this study. This is higher than that recorded where 2.5% (2 of 93 packs) of frankfurter examined samples ***(28)*** while 3 (7.5%) out of 40 samples ***(18)***.

Ready to eat meat products

Luncheon

L. monocytogenes could not be isolated from luncheon samples in this study, similar results were obtained several authers ***(18,20,23,29)***.

On the contrary, ***(39,40)*** isolated *L. monocytogenes* was isolated in 6% of the examined luncheon samples. However, this lower incidence may be attributed to the addition of spices and heat treatment during manufacture.

The systemic culturing and analysis of products and production facilities may help to identify appropriate interventions to reduce *L. monocytogenes* contamination in food processing plants and used to control of *L. monocytogenes* in meat products ***(41)***.

Shawerma and hawawshy

Also, *L. monocytogenes* could not be isolated from Shawerma and Hawawshy in the present study for the same reasons as luncheon.

PCR technique

The wide application of nucleic acid amplification techniques and the increasing industrial interest of rapid method has led to the development and application of PCR based methods for the detection of microbial pathogens in food ***(42)***.

Analysis the PCR profiles, 11 of listeria isolated strains showed amplified products (Fig. 1) 800bp, 517 bp and 238 bp) which are species specific and 234 bp which is virulence specific of *L. monocytogenes* (Fig. 2). This results suggests the presence of a significant public health hazard linked to the consumption of these meat sale in Mansoura City contaminant with *L. monocytogenes*.

In order to minimize human listeriosis, foods should be cooked to an internal temperature of 70°C for more than 20 minutes to ensure destruction of *L. monocytogenes*. Reheat cooked food thoroughly (70°C) immediate a septic packaging of finished product to avoid post processing environmental contamination. Proper cold storage of meat and meat products (freezing–18°C) and proper personal hygiene of food handlers is advisable ***(43)***.

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الملخص العربي

مدى تواجد ميكروب الليستريا في اللحوم وبعض منتجاتها

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أجريت هذه الدراسة على عدد (٢٥٠) عينة ممثلة في (٢٥) عينة من كل اللحوم الطازجة والسجق (الطازج والمجمد) والفرانكفورت واللانشون والبسطرما و(٢٠) عينة من كل من اللحم المفروم المجمدة وكفتة (اللحم) وكفتة (الأرز) والهامبورجر والحواوشي، تم تجميعها عشوائياً من المحلات المختلفة بمحافظة الدقهلية لبيان مدى تواجد أنواع ميكروب الليستريا.

أظهرت نتائج الفحص أن النسبة العامة لوجود الليستريا في العينات كانت ٢٨%، ٤٠%، ٣٥%، ٢٠%، ٢٠%، ٨%، ٢٥%، ١٦%، في العينات اللحوم الطازجة واللحم المفروم المجمد وكفتة اللحم وكفتة الأرز والسجق الطازج والمجمد والهامبورجر والفرانكفورت على التوالي. كما لم يتم عزلها من العينات المتبقية الأخرى (اللانشون والشاورما والحواوشي)، وقد تم عزل سبع عترات من الليستريا بنسب مختلفة.

كانت النسبة العامة: الليستريا مونوسيتوجين هي (١٠%) في كل من اللحم المفروم المجمد والهامبورجر حيث تم عزل عينيتين إيجابيتين من إجمالي (٢٠) عينة، وكانت (٥%) في كل من كفتة اللحم وكفتة الأرز حيث تم عزل عينة إيجابية من إجمالي (٢٠) عينة وكانت (٤%) في كل من اللحم الطازج والسجق المجمد والفرانكفورت حيث تم عزل عينة إيجابية من إجمالي (٢٥) عينة وكذلك نسبة المونوسيتوجين (٨%) في السجق الطازج حيث تم عزل عينتين إيجابيتين من إجمالي (٢٥) عينة. لهذا يكون إجمالي عزل الليستريا مونوسيتوجين (١١) عينة إيجابية من إجمالي (٢٥٠) عينة بنسبة (٤,٤%) وذلك بناء علي نتائج الاختبارات الكيميائية ونتائجها الإيجابية في اختباري CAMP test and B. heamolysis test وبتقنية تفاعل إنزيم البلمرة المتسلسل PCR.

تم أيضاً دراسة ضراوة معزولات الليستريا مونوسيتوجين على الفئران البيضاء وقد نوقشت الأهمية الصحية والطرق الواجب إتباعها للحد من تلوث اللحوم ومنتجاتها لهذا الميكروب، حيث أنه يسبب مرض الليستريوزيس الذي يصيب الإنسان وخاصة عند تناوله الأطعمة التي لا تتعرض لدرجة حرارة كافية.