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PUBLIC HEALTH ASSESSMENT OF *LISTERIA* SPECIES IN SOME MEAT AND CHICKEN PRODUCTS IN ASSIUT CITY

(With 3 Tables and One Figure)

By

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الأهمية الصحية لليستيريا فى بعض منتجات اللحوم والدواجن في مدينة أسيوط

أشرف محمد عبد المالك ، سهيلة فتحي حسن على ، رأفت حسنين ، مؤمن عبد العظيم محمد ، خالد ابراهيم السايح

أجريت هذه الدراسة على 100 عينة (خمسة وعشرون عينة من كل من اللحوم المستوردة المفرومة واللانشون وأوراك الدجاج المجمدة وصدور الدجاج المخلية المجمدة) تم جمعها من المحلات والسوبر ماركت المختلفة بمحافظة أسيوط في الفترة من شهر يناير إلى شهر يوليو 2009 م وذلك لمعرفة مدى تواجد أنواع ميكروب الليستيريا. هذا بالإضافة إلى فحص 28 عينة من المسحات الشرجية من الأطفال الذين يعانون من حالات إسهال وحمى فى مستشفى طب الأطفال، جامعة أسيوط. وقد أسفرت النتائج عن تواجد الليستيريا بنسبة 32 %، 32 %، 52 % و 56 % في عينات اللحوم المستوردة المفرومة واللانشون وأوراك الدجاج المجمدة وصدور الدجاج المخلية المجمدة على التوالي. وبالنسبة للمسحات الشرجية للأطفال فقد تم عزل الليستيريا بنسبة 7,14 %. تم اختيار 13 سلالة من الليستيريا المعزولة بناءاً على نتائج الاختبارات الكيميائية ونتائجها الإيجابية فى إختبارى beta-haemolysis، CAMP وذلك لتقنية تفاعل إنزيم البلمرة المتسلسل (PCR). باستخدام تقنية الـ (PCR) تم التعرف على الليستيريا مونوسيتوجينز فى خمس عينات من مجموع عينات اللحوم والدواجن المختبرة (100 عينة) بنسبة 5 % والعينات الموجبة تشمل عينة من اللحوم المستوردة المفرومة المجمدة وعينتان من اللانشون وعينتان من أوراك الدجاج المجمدة. ولقد نوقشت الأهمية الصحية والطرق الواجب إتباعها للحد من تلوث منتجات اللحوم والدواجن بهذا الميكروب.

SUMMARY

The present study was conducted for isolation of *Listeria* spp. in some selected meat and chicken products purchased from retail supermarkets in Assiut, Egypt. A total of 100 samples including 25 samples each of minced frozen beef, luncheon, frozen chicken legs and frozen chicken breast fillets were collected over a 7-month period between January and July 2009 and examined for the presence of *Listeria* spp. In addition, 28 stool cultures examined for *Listeria* spp. collected from hospitalized children resident in Assiut Pediatric University Hospital with history of diarrhea or fever. Out of the total 100 meat samples examined, *Listeria* spp. were detected in 8 (32%) of minced frozen beef, 8 (32%) of luncheon, 13 (52%) of frozen chicken leg and 14 (56%) of frozen chicken fillet samples examined, respectively. Regarding the examined stool cultures (28) from hospitalized children with underlying disease in Assiut University hospital, 2 (7.14%) were found positive for *Listeria* spp. For identification of *L. monocytogenes* using polymerase chain reaction (PCR), two primers were selected based on the prfA (transcriptional activator of the virulence factor) gene for *L. monocytogenes*. 13 selected *Listeria* isolates displayed beta-haemolysis on sheep blood agar and positive CAMP test were further identified using PCR. PCR results showed that *L. monocytogenes* were confirmed in one of minced imported frozen meat, two of luncheon samples and two of frozen chicken legs with the total incidence of 5 isolates (5%) from the total 100 examined food samples. This study suggests the presence of a significant public health hazard linked to the consumption of these meat and chicken products sold in Assiut city contaminated with *L. monocytogenes***.** The public health significance of this pathogen and sanitary measures were discussed.

Key words: *Listeria spp, L. monocytogenes, minced beef, luncheon, chicken fillet, chicken legs, children stools, PCR..*

INTRODUCTION

The marked increased of contamination in food industry specially meat and chicken products by pathogenic bacteria has raised a great concern of the public. *Listeria* spp. especially *L. monocytogenes* has been associated with a wide variety of food sources particularly meat and chicken (Endang *et al.,* 2003).

Listeria spp. is ubiquitous bacteria widely distributed in the natural environment. The ubiquitous character of the bacteria inevitably

results in contamination of numerous food products (Farber and Peterkin, 1991).

The genus *Listeria* includes 6 different species (*L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seegligeri* and *L. grayi*). Both *L. ivanovii* and *L. monocytogenes* are pathogenic in mice, but only *L. monocytogenes* is consistently associated with human illness (Seafood Network Information Center, 2007).

Listeria spp. has been isolated from poultry, red meat and meat products in many countries around the world such as Yugoslavia (Buncic, 1991), Belgium (Uyttendaele *et al.*, 1999), New Zealand (Hudson *et al.*, 1992), Australia (Ibrahim and Mac Rae, 1991), and Japan (Ryu *et al.*, 1992), although these foods have not been associated with documented outbreaks of human listeriosis. Detection of *Listeria* spp. in meat is of particular concern in terms of consumer safety, as these organisms are capable of growing on both raw and cooked meat at refrigeration temperatures (Walker *et al.*, 1990).

In the past 25 years, *L. monocytogenes* has become increasingly important as a food-associated pathogen. Because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to food-borne illness. Also, *L. monocytogenes* infections are responsible for the highest hospitalisation rates (91%) amongst known food-borne pathogens and have been linked to sporadic episodes and large outbreaks of human illness worldwide. The ability to persist in food-processing environments and multiply under refrigeration temperatures makes *L. monocytogenes* a significant threat to public health (Jemmi and Stephan, 2006).

Moreover, *L. monocytogenes* is an important food-borne pathogen that can cause septicemia, meningitis (or meningo-encephalitis), encephalitis and gastroenteritis, particularly in children, the elderly and immunosuppressed individuals; it also causes miscarriage in pregnant women. The mortality rate can be as high as 30% (Robinson *et al.*, 2000; Churchill *et al.*, 2006).

Unlike most other enteric 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° C cannot be relied upon to prevent the growth of the organism to dangerous levels (Pal *et al.*, 2008)**.** In addition, because of its ability to survive and proliferate at refrigeration temperature, *L. monocytogenes* may cause disease through frozen foods (Schillinger *et al.*, 1991). Due to

its ubiquitous character, *L. monocytogenes* easily enters the human food chain and may multiply rapidly (Farber and Peterkin, 1991).

The standard microbiological methods for identification of *Listeria* spp. are laborious and time consuming requiring a minimum of five days to recognize *Listeria* spp. and about 10 days to identify *L. monocytogenes* by confirmation tests (Amagliani *et al.*, 2007) while rapid response should be carried out in case of confirmation since it is of principal importance to ensure the safety of foods.

In the 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 for the rapid, sensitive and specific detection of food-borne pathogens (Norton, 2000).

Therefore, the goal of this study was to determine the incidence of *Listeria* spp. and *L. monocytogenes* in minced frozen beef, luncheon, and frozen chicken meats as well as in children stools collected from hospitalized children resident in Pediatric University Hospital in Assiut, Egypt.

MATERIALS and METHODS

Collection of samples:

A total of 100 random samples of meat and chicken products (25 samples each of minced frozen beef, luncheon, frozen chicken legs, and frozen chicken breast fillets without skin) were collected from different retail supermarkets and groceries in Assiut city. The collected samples were transferred directly to the laboratory in an ice box for bacteriological examination.

Preparation of samples:

At the laboratory, frozen samples were thawed by overnight refrigeration. Each sample was aseptically and carefully freed from its casings and mixed thoroughly in sterile mortar.

Children samples:

To identify the occurrence of *Listeria* infections in hospitalized children in Assiut, the cases-control study was conducted. These cases addmitted in Pediatric Univ. Hospital; Assiut Univ., with diarrhea or fever between January and July 2009. A stool culture examined for *Listeria* spp. and the parents of the cases were interviewed, using a standardized questionnaire including addressing the family's consumption of, and purchasing and preparation conditions for, various

foods such as poultry and beef, and their contacts with people having diarrhea.

Isolation *of listeria* spp. (FAO, 1992):

Enrichment procedures

The initial procedure of isolation involves the use of *Listeria* selective enrichment broth (LSEB) to enhance the growth of *Listeria* spp. LSEB base consists of trypticase soy broth with 0.6% yeast extract supplemented with *Listeria* selective supplement (HiMedia laboratories) which contains acriflavin-HCL (15 mg/L), nalidixic acid (40 mg/L) and cycloheximide (50 mg/L). Ten grams of samples as well as swabs from human stools were aseptically added to 90 ml LSEB and mixed thoroughly. All the primary enrichment broths were incubated at 37°C and 30 °C for 24-48 h.

Selective plating

Following the enrichment procedure, a loopful of the homogenate was streaked onto *Listeria* selective agar base (Hi Media laboratories) and Oxford - *Listeria* Selective Agar and the plates were incubated at 35 °C for 24-48 h.

Confirmation: Colonies suspected to be *Listeria* were characterized using Gram stain; catalase reaction; umbrella-shaped motility pattern by using motility test medium; haemolysis on sheep blood agar; fermentation of mannitol, rhamnose and xylose and CAMP test performed according to Bergey's Manual of Systematic Bacteriology (Seeliger and Jones, 1987).

Genomic DNA Extraction

For each *Listeria* strain, a 10-ml culture was grown to mid-log phase in Tryptose Soya (TSY) broth, and 1 ml of cells was pelleted by centrifugation (13.000 ×g for 5 min). The cell pellets were resuspended in 1ml of sterile water. The resuspended cells were re-centrifuged at 12.500 ×g for 15 min. The pelleted cells were then used for DNA extraction.

Genomic DNA from suspected *Listeria* strains was extracted using the Wizard genomic DNA purification kit (Promega, USA) as recommended by the manufactures. Protocol for Gram positive bacteria, cellular lyses was carried out by enzymatic fragment with lysozyme. DNA samples were stored at -20 °C until use.

PCR identification of *Listeria monocytogenes*.

For *L. monocytogenes* PCR identification, two primers were selected based on the prfA (transcriptional activator of the virulence

factor) gene for *L. monocytogenes* as mentioned by (Germini *et al.*, 2009). Primer sequences used in the PCR are listed in Table 1.

Table 1: Oligonucleotide sequences used for identification of *Listeria monocytogenes* by PCR.

Target gene	Primer sequence (5'-3')	Amplified fragment length	Reference
prfA gene	LIS-F: TCA TCG ACG GCA ACC TCG G LIS-R: TGA GCA ACG TAT CCT CCA GAG T	217 bp	Germini *et al.* (2009)

All PCR reactions were performed in a final volume of 25µl using 2µl of extracted DNA as template. Each reaction mixture contained 12.5 µl GoTaq® Green Master Mix (Promega, M7122) 1µl of 500 ρM forward primer (LIS-F);1µl of 500 pM reverse primer (LIS-R) and 8 µl of Ultra-Pure *DNase*/*RNase-Free* Distilled Water (Gibco, Grand Island, NY, USA).

The amplification profile was as follows: pre incubation at 95 °C for 5 min; 40 cycles consisting of dsDNA denaturation at 95 °C for 30 s, primer annealing at 54°C for 30 s, primer extension at 72 °C for 30 s; final elongation at 72 °C for 5 min. Reactions were thermally cycled in a Techne Cyclogene.

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 EDVOTEK Gel documentation system. The 1-KB plus DNA Ladder (Invitrogen) was used as molecular size marker.

RESULTS

Table 2: Isolation rate of *Listeria* spp. from different food and children samples

Type of samples	No. of examined samples	Positive samples	
		No.	%
Minced frozen meats	25	8	32
Luncheon	25	8	32
Chicken frozen fillets	25	11	44
Chicken frozen legs	25	14	56
Children stools	28	2	7.14
Total	128	43	33.59

Table 3: Incidence of *Listeria* spp. in meat and chicken products as well as in children stools.

Listeria spp	*L. monocytogenes*		*L. ivanovii*		*L. innocua*		*L. welshimeri*		*L. seeligeri*		*L. grayi*		*L. murrayi*	
Samples	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Minced meat	1	4	-	-	7	28	-	-	-	-	-	-	-	-
Luncheon	2	8	1	4	-	-	2	8	2	8	1	4	-	-
Frozen chicken fillet	-	-	3	12	2	8	2	8	-	-	4	16	-	-
Frozen chicken leg	2	8	3	12	3	12	3	12	-	-	2	8	1	4
Children stools	-	-	-	-	-	-	-	-	1	3.8	1	3.8	-	-
Total	5	20	7	28	12	48	7	28	3	11.8	8	31.8	1	4

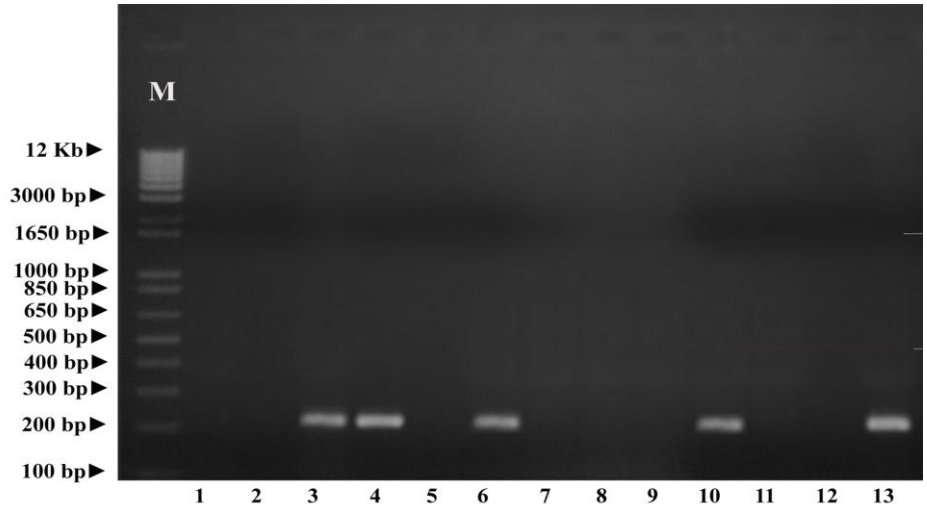

Fig. 1: Agarose gel electrophoresis of PCR products from *Listeria monocytogenes* isolates from examined samples: Lan M, DNA size marker (1-KB Plus DNA Ladder); lane 1, (L 1), lane 2, (L 4); lane 3, (L 3), lane 4, (L 18); lane 5, (F 9); lane 6, (Lu 9); Lan7, (Lu 7); Lan 8, (Lu 13); Lan 9 (F 14); Lan 10, (M 16), Lan 11, (L 11); Lan 12, (H 26); Lan 13, (Lu 18).

L: chicken leg isolate,
F: chicken fillet isolate,
Lu: luncheon isolate,
M: minced meat isolate,
H: Human stool isolate.

DISCUSSION

Meat and chicken products have been frequently contaminated with *L. monocytogenes* and may serve as vehicle of other pathogenic organisms. The frequent occurrence of *L. monocytogenes* in meat and chicken may pose a potential risk for consumers (Mahmood *et al.*, 2003). Human infections primarily result from eating contaminated food and may lead to serious and potentially life-threatening listeriosis (Posfay-Barbe and Wald, 2004).

Out of the 100 meat and chicken product samples examined, 41 (41%) isolates of *Listeria* spp. were recovered. Of these, 13 isolates displayed beta-haemolysis on sheep blood agar and positive CAMP test and identified using PCR.

The wide application of nucleic acid amplification techniques and the increasing industrial interest toward rapid methods has led to the development and application of PCR based methods for the detection of microbial pathogens in food (Germini *et al.*, 2009).

Analyzing the PCR profiles, 5 out of 13 *Listeria* isolated strains showed one amplified product (217 bp), (Fig. 1) which is specific for *L. monocytogenes*. Thus, *L. monocytogenes* were confirmed in one of minced imported frozen meat examined, two of luncheon samples and two of frozen chicken legs with the total incidence of 5 isolates (5%) from the total 100 examined samples (Table 3 and Figure 1). This results suggests the presence of a significant public health hazard linked to the consumption of these meat and chicken products sold in Assiut city contaminated with *L. monocytogenes*.

The percentage of culture positivity of *L. monocytogenes* in meat and chicken products in the present study is in agreement with the reported incidences in other countries such as 5.1% in Ethiopia (Molla *et al.*, 2004), 4.9% in Belgian meat products (Uyttendaele, 1999) and 3.6% in processed meat products in Chile (Cordano and Rocourt, 2001).

When several studies in different countries are compared, *L. monocytogenes* isolation rates seem to vary significantly. This wide variation may be explained in terms of geographic location, isolation methods and kinds of media employed (Akpolat *et al.*, 2004).

Concerning minced imported frozen meat examined in our study, *Listeria* spp. were isolated from 8 (32%) of 25 examined samples. *L. monocytogenes* occurred in one (4%) and *L. innocua* in 7 (28%) of the examined samples, respectively (Tables2 & 3). Similarly, the isolation rate of *L. monocytogenes* in minced beef samples was 5% in a study in

Turkey conducted by Akpolat *et al.* (2004). Nearly, similar results were obtained by other researchers such as Abd El-Aziz (2004) (6%) and Marinsek and Grebenc (2002) who isolated *L. monocytogenes* from 3 of the minced meat samples (6.81 %).

On the other hand, higher records were reported by several investigators as Hassan *et al.* (2001) who found *Listeria* species in 17 (73.9%) of 23 samples of imported frozen beef in Malaysia and Donald *et al.* (1991) who reported that imported frozen beef examined harbored seven *Listeria* spp: 15 *L. monocytogenes*, 18 *L. ivanovii*, 32 *L. innocua*, 2 *L. seeligeri*, 11 *L. grayi*, 7 *L. murrayi*, and 13 *L. welshimeri* isolates, respectively in Canada. Also, Inoue *et al.* (2000) isolated *L. monocytogenes* from 12.2% of the minced meat samples in Japan and Buncic (1991) detected *L. monocytogenes* in 69% of minced meat samples in Yugoslavia.

It is interesting to note that *L. innocua* was isolated predominantly among *Listeria* spp. in minced frozen meat in this study (Table 3). This finding is in agreement with other studies where *L. innocua* was the most common species in raw and cooked meats, while other *Listeria* spp. were less frequently (Choi *et al.,* 2001; De Simon *et al.,* 1992). As similar, *L. innocua* was the most predominantly isolated spp. from a variety of meat samples. It was detected in 83.3% of the raw minced meat, 57.6% of the raw chicken meat, 63.1% of the raw beef, 9.6% of the cooked red meat and 10.7% of the cooked chicken samples (Yucel *et al.,* 2005).

Furthermore, detection of *L. monocytogenes* in foods can be difficult as these bacteria are normally found in very low numbers in the presence of a heterogenous microflora. The most frequent *Listeria* isolates from food are *L. monocytogenes* and *L. innocua*. Several studies have demonstrated that *L. innocua* is found in food more frequently than *L. monocytogenes* (Walsh *et al.,* 1998). The reasons for the higher frequency of recovery of *L. innocua* remain unclear yet. However, this may result from either a naturally higher prevalence or from preferential selection of *L. innocua* during laboratory detection procedures (Gnanou Besse *et al.,* 2005).

Contamination of the meat with *L. monocytogenes* generally occurs after the slaughter and may come from the skin of the animals, the hands of the workers, the equipment and the tools used (Marinsek and Grebenc, 2002).

Regarding luncheon samples as shown in Table (3), *Listeria* spp. isolated were *L. monocytogenes* comprising (8%0) of the samples,

followed by *L. welshimeri* (8%), *L. seeligeri* (8%), *L. ivanovii* (4%) and *L. grayi* (4%). In this research, *L. monocytogenes* was determined in 2 (8%) samples of luncheon meat. Lower incidence was obtained by Gombas *et al.* (2003) who found *L. monocytogenes* in 0.89 of luncheon meat. In contrast, *L. monocytogenes* could not be isolated from luncheon samples as reported by Elgazzar and Sallam, (1997); Mohamed and Ali, (1999); Saad *et al.* (2001).

Cross-contamination, which can occur within the environment of food-processing equipment, is considered to be a possible source of *Listeria* contamination in processed meat such as luncheon. *L. monocytogenes is* able to attach to and survive on various working contact surfaces (Borucki, 2003). One reason may be its ability to form biofilms (Wong, 1998).

Furthermore, during further transformation processes of raw meat into meat products *L. monocytogenes* can be introduced, where the amount depends on the extent of cross-contamination, personal and general hygienic measures and the process parameters (Glass and Doyle, 1989).

In addition, minced/chopped meat products as luncheon, by their nature, undergo extensive processing and handling during their production. This leads to greater opportunities for *L. monocytogenes* contamination (Tompkin *et al.,* 1992; Uyttendaele, 1997).

The ability of *L. monocytogenes* to multiply at refrigeration temperatures could be considered of a significance in food intended for consumption without further cooking as luncheon meat and foods which have received cooking presumed sufficient to eliminate *Listeria*, but nevertheless intended be received further cooking prior to consumption where the potential competitive microflora has been largely eliminated and thus even low numbers could pose a potential hazard if proper storage conditions are not adhered to (Schuchat *et al.,* 1992).

Frozen chicken breast fillet examined harbored variable rates of four *Listeria* species: 3 (12%) *L. ivanovii*, 2 (8%) *L. innocua*, 4 (16%) *L. grayi* and 2 (8%) *L. welshimeri* isolates, respectively (Table 3).

In this study, *L. monocytogenes* was not isolated from any of the 25 samples of frozen chicken breast fillet examined. On the other hand, *L. monocytogenes* was determined in 9 samples of mechanically-deboned chicken meat (15.78 %) (Marinsek and Grebenc, 2002). Also, Hindy (2006) isolated *listeria* spp. from 28% of chicken fillet, from which 8% were *L. monocytogenes*.

It is also important to comment that the presence of any *Listeria* spp. may be indicative of poor hygiene and cross-contamination scenarios which could favour the persistence of *L. monocytogenes* (Azevedo *et al.*, 2005).

Of the 25 frozen chicken legs samples examined, 14 (56 %) were found to be contaminated with *Listeria* species (Table 2). *L. monocytogenes* could be detected in 2 (8%) samples of chicken legs examined in the present study. This obtained result was in agreement with other reports as in the study by Arslan *et al.* (1999), who mentioned that *L. monocytogenes* was found at 15%, 10% and 5% in wing, leg and washing water, respectively, of 20 chickens. In the contrary, several studies showed that the rates of *L. monocytogenes* varied between 23% and 60% (Pini and Gilbert, 1988; Skovgaard and Morgen, 1988).

Poultry can harbour *L. monocytogenes* in their intestinal tract and as such are a potential source of contamination (Capita *et al.*, 2002). Therefore, higher incidence of *Listeria* in chicken meat products could be attributed to contamination caused by chopping board, mincing machine, knives, cleaning cloth, other working surfaces and more human contact (Lowry and Tiong, 1985).

Moreover, *L. monocytogenes* has been strongly implicated particularly in the contamination of foods stored at low temperatures. Storage of such products under such low temperature conditions may allow the growth of significant numbers of these organisms leading to food-borne illnesses among consumers (Walker *et al.*, 1990; Beumer *et al.*, 1996).

Concerning the examined 28 stool cultures from hospitalized children with underlying disease in Assiut Univ. hospital, 2 (7.14%) were found positive for *Listeria* species from which one *L. seeligeri* and one *L. grayi*.

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 aseptic packaging of the 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 (Mahmood *et al.*, 2003).

It was significantly important for public health to detect *Listeria* spp., and particularly *L. monocytogenes*, in meat products sold in Assiut, since consumers are frequently exposed to these products. Therefore,

meat and meat products must be thoroughly cooked or grilled before consumption so *L. monocytogenes* is likely to be eliminated.

In conclusion, this study has demonstrated the presence and distribution of *L. monocytogenes* and other *Listeria* spp. in a variety of meat and chicken products in Assiut city. The study also suggests the need for improved food safety through the implementation of hygienic measures at all levels from production to consumption.

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