Animal Health Research Institute
Assuit Regional Laboratory.

"INCIDENCE OF *LISTERIA MONOCYTOGENES* IN FROZEN BEEF, POULTRY AND FISH IN ASSUIT CITY"

(With 2 Tables)

By

LUBNA M. EBRAHEEM and MANAL H. THABET

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"مدي تواجد ميكروب الليستريا مونوسيتوجينز في لحوم الأبقار والدواجن والأسماك المجمدة في مدينة أسيوط"

لبنى محمد ابراهيم ، منال حسن ثابت

أجريت هذه الدراسة علي ٩٠ عينة ــ بواقع ٣٠ عينة لكل من اللحوم والدواجن والأسماك المجمدة. حيث جمعت العينات من المحلات المختلفه بمحافظة أسيوط وتم عزل ميكروب الليستريا بنسة ٣٣% في اللحوم المجمدة، ٦٠% في الدواجن المجمدة، ٥٣% في الأسماك المجمدة. وتم عد الميكروب حيث وجدت الليستريا بمتوسطات ٢,٩×١٠ ^{٣} ± ١,٩ ، ٥,٦٥×١٠ ^{٢} ± ٤,٧ ، ١٢,٧٥×١٠ ^{٢} ± ٦,٨ / جرام بالترتيب لكل من عينات اللحوم والدواجن والأسماك المجمده علي التوالي تم عزل ميكروب الليستريا مونو سيتوجينز بنسبة ١٦,٦% ، ٣٣% ، ١٣,٣% من كل من العينات السابقه من اللحوم والدواجن والأسماك المجمده بالترتيب فكانت أعلي نسبة له في عينات الدواجن. ولقد أوضحت إختبارات الحساسية للمضادات الحيوية أن كل العترات المعزولة كانت مقاومه للجنتاميسن والأمبيسيلين بنسبة ١٠٠% ولكنها كانت حساسة للكلورامفينيكول ونورفلوكساين بنسبة ١٠٠%، كما أظهرت العترات نسب مختلفة من الحساسية لكل من ستريبتوميسن، تيتراسيكلين، ريفامبين، سيفادروكسيل، سيفوتاكسيم.

SUMMARY

The present study was preformed on 90 frozen samples of meat, poultry and fish (30 of each). The samples were collected from different shops in Assiut city. *Listeria* species were isolated from 33% of frozen meat, 60% of frozen poultry and 53% of frozen fish. Also they were counted in the examined samples, the mean counts were $2.9\pm1.9\times10^3$, $5.65\pm 4.7\times10^2$ and $12.75\pm 6.8 \times10^2$ CFU/g of the examined frozen meat, poultry and fish respectively. *Listeria monocytogenes* was differentially identified from other *Listeria*

species and could be isolated at variable percentages: 16.6% in frozen meat, 33% in frozen poultry and 13.3% in frozen fish. The study revealed that the incidence of *L.monocytogenes* was higher in frozen poultry as compared to both frozen meat and fish. The drug susceptibility characterization of *L.maonocytogenes* cleared that all isolates (100%) were resistant to Gentamicin and Ampicillin but were susceptible (100%) to Chloramphenicol and Norfloxacin, while the other used antibiotics showed different degrees of antimicrobial sensitivity reactions for Streptomycin, Tetracycline, Rifampin, Cefadroxil and Cefotaxime.

Key words: *Frozen beef, frozen poultry, frozen fish, Listeria monocytogens*

INTRODUCTION

Listeria monocytogenes is present in soil, water, vegetables, intestinal contents of a variety of birds, fish, insects and other animals. Human listeriosis is a sporadic disease which is associated with consumption of under-cooked meat, contaminated milk, soft cheese, unwashed raw vegetables and cabbage (Schuchat *et al.,* 1992). Meat and meat products have frequently been contaminated with *L. monocytogenes* and may serve as vehicle of other pathogenic organisms. In human, the illness may range from mild to severe sickness. The sever forms of human listeriosis are present as meningoenceophalitis followed by septic infections and occasionally isolated organ involvement. Death is rare in healthy adults but can occur at a rate as high as 30 % in persons at highest risk (Demetrios *et al.,* 1996). *L. monocytogenes* is the etiologic agent of about 98% of human and 85% of animal cases (Mclauchlin, 1987). Because of its ability to survive and proliferate at refrigeration temperature, *L. monocytogenes* may cause disease through frozen foods (Schillinger *et al.,* 1991). The organism can grow over the temperature range of about 1°C to 45°C and the pH range 4.1 to around 9.6, it may be expected to survive in foods for long periods of time (Ryser *et al.,* 1985). The volume of frozen food consumed (specially frozen meat, poultry and fish) is growing all the time. Consequently, it was decided to examine these foods in order to establish some indication of the incidence of *L. monocytogenes* and assess wheather the level of contamination might pose any risk to consumers.

MATERIALS and METHODS

1- Collection of samples:

A total of 90 samples were collected from different locations in Assiut city for examing the presence of *Listeria monocytogenes*. The samples comprised 30 of each from frozen meat, poultry and fish. Each sample was warped separately and aseptically in strile polyethylene bag, then labelled and transferred as quickly as possible to the laboratory.

2 – Bacteriological analysis:

A – Isolation:

Twenty-fiive grams of each samples were homogenized with 25 of *Listeria* enrichement broth in sterile Moulinex type blender equipped with metallic flask for 1 min, and incubated at 37°C for 48 h. After incubation one loopful was subcultured on *Listeria* selective medium (Oxford agar) according to Oxoid Manual (1990).

B – Enumeration:

Counting of *L. monocytogenes* was achieved by direct plating of decimal dilutions of prepared samples (APHA, 1992) onto plates of Oxford agar. The plates were incubated at 37°C for 24-48 h. and typical colonies presumed to be *L. monocytogenes* were counted.

C – Identification:

Colonies suspected to be *L. monocytogenes* were identified according to koneman *et al.*, (1996) and Quinn *et al.*, (2002) and characterized according to Margolles *et al.*, (2000) by Gram stain, tumbling motility, V.P., catalase, oxidase, haemolysis on horse blood agar and CAMP test. For further confirmation of *L. monocytogenes* the isolates were inoculated into 10 % aqueous stock solution of Mannitol, L. Rhamnose and D. xylose as described by Collee and Miles (1989).

3 – Antimicrobial Susceptibility testing:

All islolates obtained in this study were tested for antimicrobial susceptibility by disc diffusion method as described by Finegold and Martin (1982) using 9 antimicrobial agents, using the following discs, Chloramphenicol (30 μ g), Norfloxacin (10 μ g), Rifampin (5 μ g), Cefadroxil (30 μ g), Cefotaxime (30 μ g) Streptomycim (10 μ g), Tetracycline (30 μ g), Gentamicin (10 μ g) and Ampicillin (10 μ g).

RESULTS

Table 1: Incidence of *Listeria* species and *Listeria monocytogenes* in the examined samples.

Type of samples	No. of samples examined	Positive samples of *Listeria spp*		Positive samples of *L.monocytogenes*	
		No	%	No	%
Frozen meat	30	10	33	5	16.6
Frozen poultry	30	18	60	10	33
Frozen fish	30	16	53	4	13.3
Total	90	44	48.8%	19	21%

Fig. 1: Listeria monocytogenes % in different types of samples

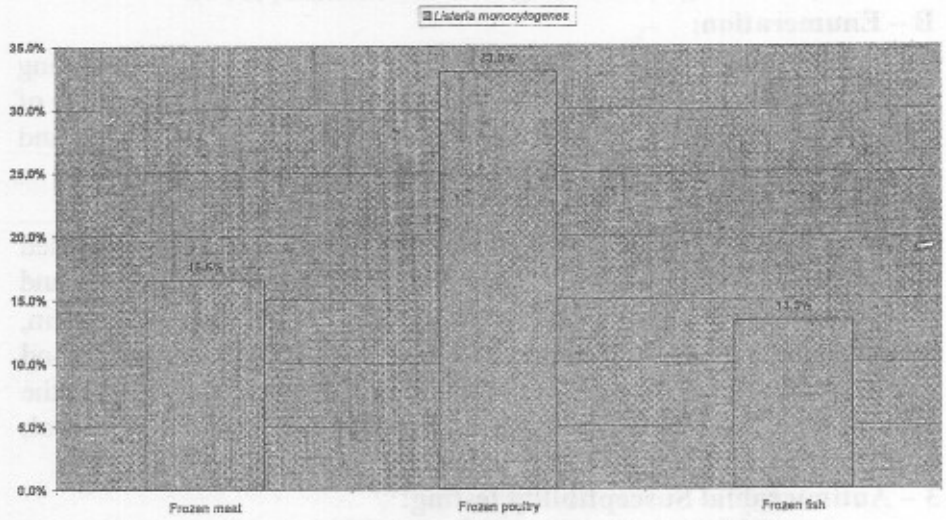

Table 2: Statisitical values of *Listeria* species in the examined samples.

Type of samples	Minimum	Maximum	Mean	±Standard error
Frozen Meat	1×10^3	4.8×10^3	2.9×10^3	1.9×10^3
Frozen Poultry	1×10^2	10.3×10^2	5.65×10^2	4.7×10^2
Frozen Fish	6×10^2	19.5×10^2	12.75×10^2	6.8×10^2

Table 3: Antibiotic sensitivity test for *Listeria monocytogenes* isolates

Antibiotic agent	Frozen meat (5 isolates)		Frozen poultry (10 isolates)		Frozen fish (4 isolates)	
	Sensitive %	Resistant %	Sensitive %	Resistant %	Sensitive %	Resistant %
Chloramphenicol	5(100%)	0(0.0%)	10(100%)	0(0.0%)	4(100%)	0(0.0%)
Norfloxacin	5(100%)	0(0.0%)	10(100%)	0(0.0%)	4(100%)	0(0.0%)
Streptomycin	4(80%)	1(20%)	8(80%)	2(20%)	3(75%)	1(25%)
Tetracycline	4(80%)	1(20%)	7(70%)	3(30%)	2(50%)	2(50%)
Rifampin	2(40%)	3(60%)	4(40%)	6(60%)	1(25%)	3(75%)
Cefadroxil	3(60%)	2(40%)	3(30%)	7(70%)	2(50%)	2(50%)
Cefotaxime	1(20%)	4(80%)	5(50%)	5(50%)	1(25%)	3(75%)
Gentamicin	0(0.0%)	5(100%)	0(0.0%)	10(100%)	0(0.0%)	4(100%)
Ampicillin	0(0.0%)	5(100%)	0(0.0%)	10(100%)	0(0.0%)	4(100%)

DISCUSSION

Results given in Table 1 revealed that 48.8% of *Listeria* spp. were isolated from 44 out of 90 samples of frozen meat, poultry and fish. The percentages of *Listeria* spp. in each product were 33, 60, 53 respectively, while 21% of *L.monocytegenes* were isolated from 19 out of 90 samples, the organism was found in 16.6% of frozen meat, 33% of frozen poultry and 13.3% of frozen fish. (Table 1 and Fig. 1)

The incidence of *Listeria* spp. in frozen meat (33%) was lower than that obtained by Elgazzar and Sallam (1997) and Hassan *et al.* 2001 (73.9%). While the percent of *L. monocytogenes* in the same product was 16.6% which was lower than that recorded by Nicolas and Vidaud (1987) (26.2%), Hassan *et al.* 2001 (75%). On the other hand our result was higher than that recorded by Paul *et al.* (1988) and Scange *et al.* (2000) who recorded 0.59% and 6.3%, respectively.

At the same table the incidence of *Listeria* spp. in frozen poultry was 60% which shown to be intermediary between that

detected by Virendera *et al.* 1995 (82%); Mahmood *et al.* (2003) (17.5%), while the percent of *L. monocytogenes* in the same product 33% was lower than that recorded by Virendra *et al.* (1995) (46%). and Pini and Gilbert (1988) (60%) but higher than (7.5%) which recorded by (Mahmood *et al.*, 2003).

Table 1 reveals that the percent of *Listeria* spp. in frozen fish was 53% which was lower than that obtained by Weagant *et al.* (1988) (61%) and was higher than that recorded by Ronda and Thakor 1992 (35%).

Many investigators detected the presence of *L. monocytogenes* in frozen fish in variable levels as Wong *et al.* 1990 recorded 10.5% Ibrahim and Hassan (2006) recorded 9.3% such results nearly agreed with that obtained in this study 13.3%. While Weagant *et al.*, 1988 recorded 26%.

L. monocytogenes is of greatest concern from the public health point of view. Dalton *et al.* (2004) in their studies about food borne disease outbreaks, found that the most frequently implicated vehicles in 173 outbreaks were sea food and *L. monocytogenes* caused 40% of deaths.

Table 2 shows that the count in *Listeria* spp. in the exmined frozen meat samples ranged from 1×10^3 to 4.8×10^3 with a mean value of $2.9 \pm 1.9\times10^3$ CFU/g. The level of contamination of the examined frozen poultry samples varied from 1×10^2 to 10.3×10^2 with a mean value of $5.65\pm 4.7 \times 10^2$ CFU/g. It is evident from the same table that the count of *Listeria* species in frozen fish samples ranged from 6×10^2 to 19.5×10^2 with a mean value of $12.75\pm 6.8 \times 10^2$ CFU/g.

The difference in the obtained results may be due to the differences between strains, the type of freezing employed the length of time of freezing storage, temperature of freezing also the nature and composition of the food.

The drug susceptibility is one of the important factors of characterization of *L. monocytogenes*. Antibiotic sensitivity testing indicated that Chloramphenicol and Norfloxaicn were the most effective antibiotics, while Ampicillin and Gentamycin were not effective antibiotics. The other used antibiotics showed different degrees of antimicrobial sensitivity reactions (Table 3). Chloramphenicol and Norfloxacin are considered as the antibiotic of choice. Ibrahim and Hassan (2006).

In conclusion the information given by the achieved results revealed that incidence of *Listeria* spp. and *L. monocytogenes* was

higher in frozen poultry than that in frozen meat and frozen fish. This may be due to that frozen poultry are considered as anatural reservoir to *L. monocytogenes*, and also are more liable to be contaminated during their preparation and storage.

The presence of *Listeria* in frozen products supports the statement that freezing has no significant effect on the organism (Sneath *et al.*, 1986).

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