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STUDIES ON LISTERIA MONOCYTOGENES IN IMPORTED FROZEN MEAT AND MACKEREL FISH IN ASSUIT GOVERNORATE

(With 3 Tables)

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دراسة عن ميكروب الليستريا مونوسيتوجينز في اللحوم المجمدة المستوردة وسمك الماكريل المستورد في محافظة اسيوط

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تمت هذه الدراسة علي عدد ٩٠ عينة-٤٥ عينة من اللحوم المجمدة المستوردة، ٤٥ عينة من سمك الماكريل المجمد المستورد حيث جمعت هذه العينات من اسواق السمك والمحلات ذات المستويات الصحية المختلفة بمدينة اسيوط. تم عزل ميكروب الليستيريا بنسبة ١٦(٣٥,٦%) في اللحوم المجمدة المستوردة و٢٦(٥٧,٨%) من اسماك الماكريل المجمد المستورد وان متوسطات عدد الميكروب كانت ٣,٢×١٠^{٣}، ١١,٣×١٠^{٤} لكل جرام علي الترتيب. وتم عزل ميكروب الليستريا مونوسيتوجينز نسبة ٨(١٧,٨%) و٦(١٣,٣%) في كل من عينات اللحوم المجمدة واسماك الماكريل المستورد حيث ظهرت النسبة الاعلي لميكروب اللستريا مونوسيتو جينز في عينات اللحوم المجمدة المستوردة ولقد تم عمل اختبار حساسية للمضادات الحيوية للعترات المعزولة واظهرت العترات حساسية للنور فلوكساسين والكلورمفنيكول بنسبة ١٠٠% ولكنها حساسة بنسب مختلفة لكل من ريفامبين ، تتراسيكلين ، سيفا دروكسيل، سيفوتاكسيم، والاستربتومايسين. كما اظهرت مقاومة نسبة ١٠٠% لكل من الاميسيلين، الريماكتان ، الجراميسين، الكاناميسين، قد تم مناقشة الاهمية الصحية لهذا الميكروب حتي لا تشكل خطرا علي صحة المستهلك.

SUMMARY

This study was carried out on 90 imported frozen samples of meat and mackerel fish (45 each). These samples were obtained from different shops and markets of varied sanitary levels at Assuit city. *Listeria* species were isolated from 35.6% of frozen meat and 57.8%of frozen mackerel fish with an average counts of 3.2×10^3 and 11.3×10^4 /g respectively. *Listeria monocytogenes* was differentially identified from

other *Listeria* species and could be isolated at variable percentages 17.8% and 13.3% in the imported frozen meat and mackerel fish samples. The study revealed that the incidence of *Listeria monocytogenes* was higher in imported frozen meat as compared to frozen mackerel fish. Antibiogram for the. *Listeria monocytogenes* revealed that the isolates were susceptible (100%) to norfloxacin and chloramphenicol and showed different degrees of antimicrobial sensitivity reactions for rifampin, tetracycline, cefadroxil, cefataxime and streptomycin while all isolates (100%) were resistant to ampicillin, rimactan, garamycin and kanamycin. The hygienic and public health importance of the isolated organisms were discussed.

Key words: *Listeria spp., Frozen meat, mackerel fish, antibiogram.*

INTRODUCTION

Listeria species including *L.monocytogenes* are ubiquitous microorganisms, which can found in the environment and contaminated food. There are six species of *Listeria: L.monocytogenes, L.ivanovii, L.seeligeri, greyi, L.innocua and, L.welshimri*. Two of these species, are considered to be pathogenic to human especially the young, olderly, and immunocompromised (Jorgenses, and Huss, 1998) while *L.ivanovii* is pathogenic to animals as reported by Farber, and Peterkin, (1991). Several reports have described the presence of *L.monocytogenes* in vegetable, dairy and meat products, as well as in fish and sea food (Fletcher, *et al.,* 1994.,. Simon, *et al.,* 1992., Farber, *et al.,* 1989).

L. monocytogenes grows at low oxygen condition and refrigeration temperatures and survives for long periods in the environment, on foods, and in the house hold refrigerator (FAD, 1999; Ryser and Marth, 1991). Food borne disease by *Listeria monocytogenes* has recently been reported in many countries although contamination by this bacteria is also known to be gradually spreading among the marketed foods of Japan, several isolates of *Listera monocytogenes* from meats and fish were obtained from different Japan shops (Cossart *et al.,* 2003; Okutani, *et al.,* 2004). In Japan wide contamination by *L.monocytogenes* has also been found in marketed meats, fish and processed foods (Okutani *et al.,* 2004).

The ingestion of *Listeria* species especially *L. monocytogenes* in food can pose a significant health risk, with high reported mortality rate for fetuses and immunocompramized patients. Thus it is important to

prevent the cont ination of foods like frozen beef, and sea foods with *Listeria* species, emphasizing the need for reliable procedures to test the presence of the pathogens in such food (Gian Franceschi, *et al.*, 2003 and Mena, *et al.*, 2004). Ben Embrek, (1994), Loncarevic, *et al.* (1998), Ebraheem and Thabet, (2007) reported the incidence of *Listeria monocytogenes* in frozen beef, poultry, fish and sea foods. 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 severe forms of human listeriosis are meningoencephalitis followed by septic infections and occasionally isolated organ involvement (Demetrios, *et al.*, 1996 and Lida *et al.*, 1998). *Listeria* is also of major veterinary importance, and the primary clinical manifestations in cattle are encephalitis and mastitis (Schuchat, *et al.*, 1992). *Listeria* has been reported to be able to grow over a wide temperature range and are capable of growth under a variety of oxygen conditions, thus, this organism can survive and grow in refrigerated foods, since the organism can grow at refrigerator food there is also concerv that this may contribute to the increased *Listeria* population (Pinner *et al.*, 1992). Because its ability to survive and proliferation at refrigeration temperature *L. monocytogenes* may cause disease through frozen foods (Schillinger *et al.*, 1991). Consequently, it was decided to examine these foods in order to establish some indication of the incidence of *L. monocytogenes* and assess whether the level of contamination might pose any risk to consumers.

MATERIALS and METHODS

I- Collection of samples:

Ninety random samples of imported frozen meat and mackerel fish (45 of each) were collected from different fishery markets and shops in Assuit city to be investigated for the presence of *Listeria monocytogenes*. All samples were collected in sterile polyethylene bags and were transferred to the laboratory in an ice box then were kept at 0-4°c until tested within 24h.

II- Bacteriological examination:

a- Isolation of *L. monocytogenes* from samples:

According to FAO (1992), twenty five g from each sample were added to 225ml of Listeria enrichment broth (L.E.B.) then incubated at 30°C for 48h. The enrichment culture was streaked on PALCAM selective agar and incubated at 30°C for 24-48h.

b- Enumeration of *L. monocytogenes* from the examined samples:

Counting of *L. monocytogenes* was recorded by direct plating of decimal dilutions of the prepared samples (APHA.1992) onto plates of PALCAM agar. The plates were incubated at 35C° for 24-48h. and typical colonies presumed to be *Listeria monocytogenes* were counted

c- Identification of *L. monocytogenes* from the examined samples:

Colonies suspected to be *L. monocytogenes* were identified according to Koneman *et al.* (1996); Margoles *et al.* (2000) Quinn *et al.* (2002); 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 incubated into 10% aqueous stock solution of mannitol, L.Rhamnose and D xylose as described by Collee and Miles (1989)

III- Antibiotic sensitivity tests for *listeria* isolates:

All isolates obtained in this study were tested for antimicrobial susceptibility by disc diffusion method as described by Fimegold and Martin.(1982) using 11 antimicrobial agents, using the following discs, Tetracycline (30ug), Rifampin (5ug), Cefadroxil (30ug) Cefotaxime (30ug), Ampicillin (10ug), Rimectan (30ug), Kanamycin (30ug), Garamycin (30ug), Stereptomycin (10ug), Norfloxacin (10ug), and Chloramphenical (30ug).

RESULTS

Table 1: Incidence of *Listeria* species and *listeria monocytogenes* in imported frozen and mackerel fish samples.

Type of samples	No.of examined samples	Positive samples of *Listeria* species		Positive samples of *L. momocytogenes*	
		No.	%	No.	%
Imported frozen meat	45	16	35.6	8	17.8
Imported frozen mackerel fish	45	26	57.8	6	13.3
Total	90	42	46.7	14	15.6

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

Type of samples	Minimum	Maximum	Mean
Imported frozen meat	1×10^3	4.9×10^3	3.2×10^3
Imported frozen mackerel fish	5×10^4	18.4×10^4	11.3×10^4

Table 3: Antibiogram sensitivity test for *Listeria monocytogenes* isolates.

Antibiotic agent	Frozen mackerel fish (6 isolates)		Frozen meat (8 isolates)	
	Sensitive %	Resistant %	Sensitive %	Resistant %
Tetracycline	3 (50%)	3 (50%)	5 (62.5%)	3 (37.5%)
Rifampin	5 (83.3%)	1(16.7%)	7 (87.5%)	1 (12.5%)
Cefadroxil	4 (66.7%)	2 (33.3%)	6 (75%)	2 (25%)
Cefotaxime	4 (66.7%)	2(33.3%)	5 (62.5%)	3 (67.5%)
Amipicillin	0 (0.0%)	6(100%)	0 (0.0%)	8 (100%)
Rimectan	0 (0.0%)	6(100%)	0 (0.0%)	8 (100%)
Kanamycin	0 (0.0%)	6(100%)	0 (0.0%)	8 (100%)
Garamycin	0 (0.0%)	6(100%)	0 (0.0%)	8 (100%)
Streptomycin	4 (66.7)	2 (33.3%)	6 (75%)	2 (25%)
Norfloxacin	6 (100%)	0 (0.0%)	8 (100%)	0 (0.0%)
Chloramphenicol	6 (100%)	0 (100%)	8 (100%)	0 (0.0%)

DISCUSSION

The recorded results in Table (1) revealed that 46.7% of *Listeria* spp. were isolated from 42 out of 90 samples of imported frozen meat and imported frozen mackerel fish. The percentages of *Listeria* spp; in each product was 35.6% and 57.8% respectively, while 15.6% of *Listeria monocytogenes* were isolated from 14 out of 90 samples. The organism was found in17.8% of imported frozen meat and 13.3%of frozen mackerel fish (Table1). The percentages of *Listeria spp.* in imported frozen meat (35.6%) was higher than that recorded by Ebraheem and Thabet (2007), (33%), while the obtained results were lower than that reported by Elgazzar and Sallam (1997); Hassan *et al.* (2001) (73.9%)

On other hand, the incidence of *L. monocytogenes* in the same product was 17.8% which was higher than that recorded by Paul *et al.* (1998), Scange *et al.* (2000) Ebraheem and Thabet (2007), who recorded

16.6% , 0.59?% and 6.3% respectively, while the result in this study was lower than that recorded by Nicolos and Vidand (1987) (26.2%) and Hassan *et al.* (2001) (75%) at the same table the incidence of *Listeria spp.* in frozen mackerel fish was (57.8%) which was higher than that obtained by Ebraheem and Thabet (2007) (53%), Ronda and Thaker (1992) (35)% but was lower than Weagant *et al.* (1988) (61%). Many investigations detected the presence of *L. monocytogenes* in frozen fish, Ebraheem and Thabet (2007) recorded 13.3%, 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* of greatest concern from public health point of view. Dalton *et al.* (2004) in their studies about food borne diseases outbreaks, found that the most frequently implicated vehicles in 17.3 outbreaks were seafood and *L. monocytogenes* caused 40% of deaths.

Table (2) shows that the count of *Listeria* spp. in the examined imported frozen meat samples ranged from 1×10^3 to 4.9×10^3 with mean value of 3.2×10^3/g. It is evident from the same table that the count of *Listeria* spp. in the imported frozen mackerel fish samples ranged from 5×10^4 to 18.4×10^4 with a mean value of 11.3×10^4/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 and also the nature and composition of the food.

The drug susceptibility is one of the important factors of characterization of *L. monocytogenes* antibiogram sensitivity test indicated that Norfloxacin and Chloramphenicol were the most effective antibiotics, while Kanamycin, Garamycin and Ampicillin were not effective antibiotics. The other used antibiotic showed different degrees of antimicrobial sensitivity reaction (Table3). Norfloxacin and choramphenicol are considered as the antibiotic of choice (Ibrahim and Hassan, (2006) Ebraheem and Thabet, (2007). The presences of *Listeria* in frozen products supports the statement that freezing has no significant effect on the organism (Sneath, *et al.*, 1986).

In conclusion the information given by the achieved results revealed that the incidence of *Listeria* spp. was higher in frozen mackerel fish than that in frozen meat. The main purpose of this study was carried out to investigate the incidence of *L. monocytogenes* in imported frozen meat and mackerel fish in Assiut city. The public health importance as well as some recommended measures for improving the quality of such products were disscused.

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