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DETECTION AND ENUMERATION OF LISTERIA MONOCYTOGENES IN MINCED BEEF AND LIVER IN ASSIUT GOVERNORATE

(With 2 Tables)

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اكتشاف وعد ميكروب الليستريا مونوسيتوجينز فى اللحوم المفرومة والكبده في محافظة أسيوط

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أجريت هذه الدراسة على تسعين عينة (خمسون عينة من اللحوم المفرومة و أربعين عينة من الكبده) تم جمعها من المطاعم والسوبر ماركت المختلفة بمحافظة أسيوط وذلك لمعرفة مدى تواجد ميكروب الليستريا والتعرف عليها بكتريولوجياً (بواسطة الاختبارات البيوكيمائية والسيريولوجية المختلفة) وتحديد العدد الكلى لها. وقد أسفرت النتائج عن تواجد ميكروب الليستريا بنسبة ٤٠% و ٤% فى عينات اللحوم المفرومة والكبدة على التوالى. وقد وجد أن نسبة عزل ميكروب الليستريا مونوسيتوجينز تمثل ٨٠% و ١٠٠% من مجموع العينات الإيجابية فى كل من اللحوم المفرومة والكبدة على التوالى . كما وجد أن متوسط العدد الكلى لميكروب الليستريا مونوسيتوجينز هو ٥,٢ × ١٠^{٤} و ١,٦ × ١٠^{٣} فى اللحوم المفرومةوالكبدة على التوالى. ولقد استخدمت طريقتان للإغناء والإخصاب " Enrichment " فى هذه الدراسة. وتم دراسة ضراوة ميكروب الليستريا منوسيتوجينز على الفئران البيضاء ولقد نوقشت الأهمية الصحية والطرق الواجب اتباعها للحد من تلوث اللحوم المفرومة والكبدة بهذا الميكروب .

SUMMARY

A total of ninety samples (50 of minced beef and 40 of liver) were collected from randomly selected supermarkets and restaurant at Assiut city and examined for the presence of Listeria spp. Listeria spp. was detected in 40% and 4% of both minced beef and liver respectively. L. monocytogenes was recovered from 80% and 100% of the all positive samples of both minced beef and liver. The average number of colony

forming unit/gram (CFU/g) of L.monocytogenes were $5.2X10^4$ and $1.6 X10^3$ in both minced beef and liver respectively. Two methods of the enrichment for the isolation of Listeria spp. were performed. Pathogenicity of L. monocytogenes to mice was studied. The public health importance as well as recommended sanitary measures were discussed.

Key words: Listeria Monocytogenes, Minced.

INTRODUCTION

The role and importance of Listeria monocytogenes as an agent of foodborne disease is becoming increasingly apparent. L. monocytogenes, which is pathogenic for both humans and animals, can cause abortion and mastitis in demostic animals (Gitter et al. , 1980), abortion in pregnant women and often fatal (30% mortality) meningitis and encephalitis in newborn infants and immunocompromised adults (Hyslop, 1974). This pathogen can be transmitted from infected animal to humans (Odegaard et al., 1952) and also can be transmitted to human through consumption of food of animal origin.

Meat and meat products have frequently been found to be contaminated with L. monocytogenes (Johnson et al., 1988) and may serve as vehicles of this pathogenic bacterium. The frequent occurrence of L. monocytogenes and its ability to survive and proliferate at refrigeration temperatures provokes accumulation of this pathogen in contaminated meat products during cold storage, and hence presents a potential risk for consumers (Doyle, 1988).

L. monocytogenes is an ubiquitous organism. It can be shed from intestine of animals and man without any apparent symptoms (Gracey and Collins,1992). Hence, the presence of the organism during the processing of meat products become unavoidable (Lowry and Tiong, 1988).

Although L. monocytogenes are found in meat and meat products, there is relatively little published infromation on the number of this organism (Johnson et al., 1990). Therefore, the main aim of the present study was to obtain information on the incidence of contamination and number of L. monocytogenes in both minced beef and liver at Assiut Governorate by using two methods of enrichmnet prior to isolation.

MATERIAL and METHODS

Sampling:

Ninety (90) samples (fifty samples of minced beef and forty samples of liver) were collected from randomly selected supermarkets and restaurants at Assiut Governorate and represented various meat plants. Each sample was wrapped separately and aseptically in sterile polyethylene bag, then labelled and transferred under refrigeration (2-7°C) as quickly as possible to the laboratory where isolation and enumeration procedures were performed.

Enumeration and isolation of L. monocytogenes:

Within 2 h. of purchase, 25 g of each sample was blended in 225 ml (1:10 dilution) of sterile Listeria selective enrichment broth (LSEB, Oxid) using sterile blender at high speed for 3 min. Serial 10- fold dilutions in 0.1% peptone water from all samples (from 10^{-1} to 10^{-7}) were made and by surface plating techniques, 0.1 ml from each dilution was transferred and evenly spread over dry surface of Listeria selective agar (Oxford formulation, Oxid) (LSA) and incubated at 37°C for 48 h. The residual suspension was also incubated at 30°C for 48h. to determine the presence of Listeria. After incubation, one loopful was subcultured on LSA and incubated at 37°C on 24 h. (Frederick, and Vanderlinde 1992). Presumptive colonies (Listeria hydrolyzes aesculin, producing black zones around the colonies on LSA) were picked on tryptone soya agar supplemented with 0.2% yeast extract and 0.2% glucose (TYSG), incubated at 30°C for 24h. and confirmed according to Seeliger and Jones (1986), through Gram staining reaction (+) oxidase (-), catalase (+), motility at 21°c in motile agar media (+umbrella top shape), Urease(-ve) and Kliger iron agar (+glucose and Lactose fermentation without gas and -ve hydrogen sulphide production). Confirmed colonies of Listeria were further identified into species through detection of β-.heamolysis on 5-10% sheep blood agar, carbohydrate fermentation and nitrate reduction (Quinn et al.,1994).

Serological slide agglutination test was done on all isolates thought to be L.monocytogenes using commerically prepared antiserum (Difco) O poly 2302-50-0 (antiserum contain agglutinins for all serotypes of L.monocytogenes).

All initial samples diluted in 225 LSEB from which Listeria were not isolated, were re-examined after 2,4,6 and 8 weeks of storage at 3°C or until Listeria was detected. Cold-enrichment samples were surface plated on LSA plates and incubated 48h at 37°c. Colonies resembling Listeria were confirmed as previously described (Ryser et al, 1985).

Pathogenicity test (Seeliger and Jones 1986):

All isolates confirmed as L. monocytogenes were grown over night at 30°C in 10 ml of LSEB, centrifuged and pellet was resuspended in 1 ml physological saline. Swiss white mice weighting 16-20 gm were each inoculated I/P with 0.1 ml of bacterial suspension. The inoculated mice was maintained under observation for evaluation of clinical signs and mortalities. The dead mice were scarified and the liver, spleen and brain were collected and processed for L.monocytogenes isolation using LSEB and LSA.

RESULTS

The obtained results were recorded in Tables 1 and 2.

Table 1: Numbers and percentage of Listeira spp. recovered from minced beef and liver by using two enrichment methods.

Samples	No. of examined samples	L. moncyto-genes	L.innocua	L.wels-himeri	Total listeria spp
Minced beef.	50				
-Incubated at 30°C for 48h.		6 (12%).	1 (2 %)	1 (2 %).	8 (16 %)
-After cold enrichment		10 (20%)	2 (4%)	-	12 (24 %)
Total		16 (32%)	3 (6%)	1 (2%)	20(40%)
Liver	40				
-Incubated at 30°C for 48h.		-	-	-	-
-After cold enrichment.		2 (5 %)	-	-	2 (5%)
Total	90	2. (5%)			2 (5%)

Table 2: Number of L. monocytogenes (CFU/g) in positive samples of minced beef and liver

Samples	Minimum	Maximum	Average
- Minced beef	2×10^3	6.3×10^6	5.2×10^4
- Liver*	2.1×10^2	2×10^4	1.6×10^3

* Counted after cold enrichment.

DISCUSSION

Foodborne listeriosis nowday is represented a serious public health problem in many countries since the fatality rate is high (ICMSF, 1996).

In this study Listeria spp. was detected in 40% and 4% of the minced beef and liver respectively,obtained from different supermarkets and restaurants over 4 months period at Assiut city (Table 1). These obtained results were in agreement with recent surveys who found that the prevalence of Listeria in minced meat and other products requiring cooking before consumption ranged from 8 to 92% (Johnson et at, 1990,Grau and Vanderlinde 1992, and El-Gazzar and Sallam, 1997). Höhne et al. (1975) isolated L.monocytogenes by cold enrichment from 8 of, 342 liver and intestinal lymph nodes samples of apparently healthy slaughter animals, while Temper (1961) failed to isolate Listeria spp. from 331 liver, spleen and kidney samples.

In this study, it was observed that higher incidence of Listeria spp. on minced beef samples (40%) than on liver samples (4%) (Table 1). This is mainly due to suggesting contamination of minced beef by cutting boards, knives, other work surfaces, additional processing steps and human contact during preparation of such product in restaurants (Lowry and Tiong, 1988, and Kerr et.al. 1993).

Results given in Table (1) point out that L.monocytogenes was detected more frequently in both minced beef (80 %, 16/20) and liver (100% 2/2) than other Listeria spp. Similar finidings were reported by Luppi et at. (1988) and Grau, and Vanderlinde, (1992). On the other hand Breer and Schopfer (1988) recorded that the isolation of L.innocua from meat and meat products was common, and often the incidence of this organsim was higher than that of L.monocytogenes. This species differences may be attributed to differences in geographic distribution of

Listeria, variation in animal husbandry and feeding practices or variation in methods of isolation (WHO 1988).

The data presented in Table (1) shows that (14/22) of all positive samples (12 from minced beef and 2 from liver) were obtained after cold enrichment at 4°C for 2-8 weeks, while only (8/22) samples were obtained after enrichment for 48 h at 30°C. These results substantiate what have been reported by Hayes et.al. (1986) and Johnson et.al., (1990), they reported that the best method for detecting Listeria was cold enrichment followed by surface plating on selective media. Quinn et al. (1994) demonstrated that L. monocytogenses is one of the few pathogenic bacteria able to grow and multiply under refrigeration temperature, and this psychrotrophic nature of Listeria classes it as a very dangerous foodborne pathogen especially during the cold storage of food (Doyle, 1988 and Johnson et al., 1990).

The pathogenicity of isolated strains of L.monocytogenes in this work induced 100% mortality at 2-4 day post I/P inoculation. On P.M examination haemorrhagic foci in liver, spleen and brain were observed. The organism was recovered from spleen, liver and brain of all dead mice. These results were compatible with those reported by Marco et al. (1992).

The average number of CFU/g of L.monocytogenes were 5.2 X 10^4 and 1.6X10^3 for both positive samples of the minced beef and liver respectively. These findings agree with that reported by Grau and Vanderlinde, (1992) in corned beef. But this number is very high if compared by the standard recorded by Netten et al. (1991). The authors reported that for public health reasons, a standard has been suggested enforcing the absence of L.moncyrogenes in meat and meat products.

This study confirms the view of L.monocytogenes being a frequent contaminant of meat products especially minced beef, so methods should be implemented to prevent this strain from entering and/or multiplying in minced beef e.g cleaning and sanitizing in restaurants should be adequate, water used should be free from this pathogen, and handling the final product just before marketing should occur in an area far away where raw materials are brought and not by the same raw material handlers.

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