J. Food and Dairy Sci., Mansoura Univ., Vol. 5 (10): 725 - 730, 2014

Listeria spp. IN READY-TO-EAT DAIRY PRODUCTS FROM RETAILERS AND SMALL DAIRY SHOPS

Metwally, A. M. M. [1] and Fatma H. M. Ali[2]

[1]Dairy Science and Technology Department, Faculty of Agriculture, Cairo University, Giza, Egypt

[2]Food Hygiene and Control Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt

ABSTRACT

Three hundred samples of six different dairy products, collected from retailers and small dairy shops in Giza and Beni-Suef governorates, were examined for the presence of *Listeria* spp. Twenty-one percent of the samples contained *Listeria* spp. The ratio was between zero percent for yogurt and 42% for ice cream. *Listeria innocua*and*L. grayi*were most dominant in the samples, only few samples contained *L. monocytogenes*. These small shops and retailers have a good share of the Egyptian market. These findings pose a serious situation for public health aspect.

Keyword: Listeria, Dairy, Pathogenic,Listeriosis, cheese, ice cream, yoghurt

INTRODUCTION

Listeria monocytogenes and other species are opportunistic intracellular pathogens that have become important cause of human foodborne disease. It causes large outbreaks with a mortality rate of 9 – 44%. In US, there is 1600 illness and 260 death annually due to listeriosis. The microorganism is found in the soil, water, animal feed and feces and in foods particularly in ready-to-eat foods. The microorganism tolerates extreme conditions of low pH, low temperature and high salt.

Milk is contaminated from either infected animals by direct excretion into milk or the unsanitary conditions prevailing on the farm or processing plants. Contaminated surroundings such as contaminated water, animal feed or feces, bulk milk filters or milk spill area on the floor beside bulk tanks or biofilms on milk contact surface, are sources of contamination.

Traditionally, listeriosis was considered a raw milk disease. However, recent outbreaks in the US and other countries occurred from soft and other chesses made from pasteurized milk.These findings make it vital to routinely test for this pathogen in Egypt where the unsanitary conditions prevails through the whole cycle of milk production, storage and distribution. Actually, literature about the presence of the pathogen in Egypt is scarce. For example, in 2013 outbreak of food poisoning after students eating in dormitory of Al-Azhar University where 300 students were hospitalized were claimed to be listeriosis. Recently, El-Shenawy*et al*., (2011) tested for *Listeria* spp. in street-vended ready-to-eat foods in Egypt. They found that 24% of the tested samples of dairy products contaminated with the pathogen. The dominant species were *L. monocytogenes* and *L. innocua*. Some samples contained up to 10^3 CFU/g viable cells.

Therefore, this research was carried out to test for the presence of such a pathogen in number of dairy products.

MATERIALS AND METHODS

Collection of samples:

Dairy products were sampled from Giza and Beni-Suef governorates retailers, street-vended products and small dairy shops. Samples were collected aseptically in sterile sample polyethylene bags and preserved in icebox during transportation. Samples were stored under refrigeration until analysis. Fifty samples were collected from each of the following products: Kariesh Cheese, Talaga soft cheese, Buttermilk cheese, fresh cream and yoghurt. Analysis started within 24 after sample collection.

Isolation of Listeria spp.

Enrichment and isolation methods accepted by FDA and developed by Hichens and Jinneman, 2011 were followed. A representative sample of 25 g was homogenized with 225 ml of sterile selective enrichment broth supplemented with nalidixic acid, cycloheximide, and acriflavine hydrochloride (Lovett *et al*., (1987). The homogenate was incubated at 30˚C for 24 h and 48 h, then the broth was aseptically streaked on the selective media (*Listeria* Oxford Medium base). The inoculated plates were incubated at 30˚C for 24 – 48 h. *Listeria* colonies are black with a black haloon esculin-containing media. Five typical colonies were transferred to trypticase soya agar with yeast extract for purification, incubated at 30˚C for 24 – 48 h, for further identification.

Typical *Listeria* spp. colonies, on the above selective agar plates were then selected for further identification to the species level, using a battery of tests as recommended by APHA, 1992. The tests included the Gram-Staining reaction, production of catalase, tumbling mobility at 20 – 28˚C andhemolytic activity on blood agar (defibrinated horse blood). CAMP test (Christie-Atkinson-Munch-Peterson test) (synergistic lysis of red blood cells) against *S. aureus* (NCTC 1803) and *R. equi* (NCTC 1621), each on one plate of nutrient agar with a thin layer (3-4 ml) of 5% sheep blood agar was also performed. The organism was tested by streaking at right angle within 1-2 mm of the standard organism. After incubation at 37˚C for 18 h, hemolysis reaction of tested organism was examined. Carbohydrates fermentation of glucose (+), rhamnose (+) and xylose (-) and hydrolysis of Aesculin was also determined (Jeyaletchumi*et al.,* (2010), Lovett (1987), Karen *et al.,* (2012), OIE Terrestrial Manual 2014).

Listeria Identification

The following tests were used for *Listeria* identification.

Table (1): Principal characteristics of the main *Listeria* species.

Test	***Listeria*spp.reaction**
Gramstain	Positive
Cellmorphology	Short(0.4-0.5µm×0.5-2.0µm)nonsporeformingrod withafewperitrichousflagella. In old cultures, it may appear cocci.
Growthconditions	Aerobicandfacultativeanaerobic
Motility	Positivetumblingmotilityin a hanging drop from nutrient broth incubated at20–28°C.
Catalase	Positive
Oxidase	Negative
Aesculinhydrolysis	Positive
Urease	Negative

Table (2):Differentiation of main *Listeria* species

Species	β haemolysis	Production ofacidfrom			Christie,Atkins,Munch-Petersen(CAMP) reactionon sheepblood with	
		L-Rhamnose	D-Xylose	D-Mannitol	*S.aureus*	*R.equi*
L. monocytogenes	+	+	–	–	+	–
L.innocua	–	V	–	–	–	–
*L.ivanovii*subsp.*ivanovii*	+	–	+	–	–	+
Lseeligeri	(+)	–	+	–	(+)	–
L.welshimeri	–	V	+	–	–	–
*L.grayi*subsp.*grayi*	–	–	–	+	–	–

V:variable;(+): weakreaction;+: >90%positivereactions;–:noreaction.

Statistical Analysis

The results were analyzed by meansof the Chi-square test of independenceto determine the relationshipbetween the type of the dairy product and the presenceof Listeria spp.Statistical analysis was performed byrunning the SPSS 20 (IBM Corp., Copyright© 2011) package on a personal computer.

RESULTS AND DISCUSSIONS

Fifty samples of each of three types of soft chesses were collected, namely Domiati Cheese (Talaga type), Kariesh cheese and buttermilk cheese and tested for *Listeria* contamination, results are shown in Table (3). These soft cheeses contain high moisture of almost 50%, a matter that would help in microorganisms' growth.

Table (3): Incidence of*Listeria* species in soft cheeses.

Listeria sp.	Number of positive samples		
	Domiati Cheese (Talaga)	Kariesh Cheese	Buttermilk Cheese
L. mono	0	2	0
L. innocua	2	4	2
L. welshimeri	0	1	0
L. grayi	3	7	8
L seeligeri	0	0	0
L. ivanovii	0	0	1
Total Positive Samples	5 (10%)	14(28%)	11 (22%)

Number of samples of each product is 50

The products varied in percentage of contamination. The percentage of positive samples were 10, 28, and 22% for Talaga chees, Kariesh cheese and Buttermilk cheese, respectively. The percentage of contamination varied according to product methods of processing and method of preservation. Talaga cheese contained the lowest contamination because the cheese usually contains high percentage of sodium chloride, and is preserved in salted whey. The high percentage of contamination in the other cheeses

compared to Talaga cheese could be expected because the sanitary measures usually followed are less stringed than Talaga cheese. Separating milk for Kariesh cheese and the collection of buttermilk from the butter churn are steps difficult to control and it vulnerable for contamination. Moreover, they do not contain the high salt contents nor preserved in brine solution as to Talaga cheese.The three products also differ in the dominant *Listeria* spp.*L. grayi*predominated in the three cheeses followed by low presence of *L. innocua*. Kariesh cheese was the only cheese contained *L. monocytogenes* and *L. welshimeri*.

Another three products were sampled, namely fresh cream, ice cream and yoghurt. Table (4) shows the percentage of contaminated samples.

Table (4): Incidence of*Listeria* species in different dairy products.

***Listeria* sp.**	**Number of positive samples**		
	Fresh Cream	**Ice Cream**	**Yoghurt**
L. mono	1	0	0
L. innocua	4	6	0
L. welshimeri	2	3	0
L. grayi	3	9	0
L seeligeri	0	1	0
L. ivanovii	0	2	0
Total Positive Samples	10 (20%)	21 (42%)	0 (0%)

Number of samples of each product is 50

Again, there were differences between samples according to their method of manufacturing, heating, storage conditions and composition. Yoghurt was free of the pathogen since its processing steps controls contamination, which include milk heat treatment, lactic acid culture and the developed acidity. On the other hand, 42% of ice cream samples were contaminated, this high percentage points out the dangerous situation. Ice cream processing involves an aging step in which the mix is kept overnight under refrigeration step which gives the pathogen a chance to grow if present. Moreover, number of ingredients are added to ice cream, which could be a source of contamination particularly if fruit particles are used. Twenty percent of cream samples contained the pathogen. Separation process, unclean utensils and might be improper heat treatment could be the reason behind this contamination. These contamination percentages were close to those of El-Shenawy*et al*., (2011) except ice cream, which contained higher contamination. This means that the situation in Egypt has not improved since 2011. *L. innocua*and*L. grayi*were the dominant species in the products. In general, the presence of *L. monocytogenes* was not common in the tested products.

The statistical analysis revealed that there is a significant relationship ($P< 0.01$) between the type of dairy products and the presence of *Listeria* spp. Such results confirm what was previously pointed out that the

differences between these dairy products depend mainly on the steps of processing and their chemical composition.

The results indicated the potentially hazardous situation and failure of hygienic standards in processing, handling and storage of dairy products in Egypt. This is a dangerous situation particularly that those dairy products are widely consumed with consumers of different ages and different health conditions. It is known that infection dose of *Listeria* spp. for the disease to appear can vary depending on several factors of which, the level of contamination, the immune stats of individual and the virulence of the strain ingested, are most determining factors. Therefore, number of countries issued permissible limit of the pathogen. The limiting number for *L. monocytogenes* in ready-to-eat food in UK and European Union is less than 100 CFU/g testing at the end of shelf life. However, US adopted zero tolerance. These findings are also a warning for the governmental authorities to implement different measures to avoid poisonous outbreaks.

REFERENCES

El-Shenaway, M.; El-Shenawy M; Manes J. and Soriano J. M. (2011). Listeria spp. in Street-Vended Ready-to-Eat Foods. Interdisciplinary Perspectives on Infectious Diseases. Volume 2011, Article ID 968031, 6 pages.

Hitchins A.D. & Jinneman K. (2011). Chapter 10. Detection and Enumeration of Listeria monocytogenes. In:Bacteriological Analytical Manual. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm.

Jeyaletchumi, P.; Tunung, R.; Maegaret, S. P., Son, R. Farinazleen, M. G. and Cheah, Y. K. (2010). Detection of Listeria monocytogenes in foods, Review Article. International Food Research J. 17:1-11.

Karen H.; Drummond N.; Murphy M.; Butter F.; Buckly J. and Jordan K. (2012). A case of bovine raw milk contamination with *Listeria Monocytogenes*. Irish Veterinary J.

Lovett, J. (1987) *Listeria* isolation. In: Bacteriological Analytical Manual, AOAC, Arlington, AV.

Lovett, J., D. W. Francis, and J. M. Hunt. 1987. Listeria monocytogenes in raw milk: detection, incidence, and pathogenicity. J. Food Prot. 50:188.

OIE Terrestrial Manual 2014, Chapter 2.9.7 *Listeria Monocytogenes* (1-18).

بكتريا الـ *Listeria spp.* في منتجات الألبان من المحلات الصغيرة والباعة الجائلين

أحمد محمد محمد السيد متولي[١] وفاطمة حسن محمد علي[٢]
[١]قسم الألبان – كلية الزراعة – جامعة القاهرة – الجيزة – مصر
[2]قسم مراقبة الأغذية – كلية الطب البيطري – جامعة بني سويف -مصر

ثلاثمائةعينة من ستة أنواع مختلفة من منتجات الألبان، تم تجميعها من محلات الألبان الصغيرة والباعة الجائلين بمحافظتي الجيزة وبني سويف، تم اختبار تلك العينات لتحديد مدى تواجد بكتريا *Listeria* spp. بهم. وجد أن ٢١% من العينات كانت تحتوي على بكتريا *Listeria* spp. تراوحت نسبة تواجد البكتريا ما بين 0% كما في الزبادي الي 42% في الأيس كريم. نوعي البكتريا *Listeria innocua* and *L. grayi* كانتا هما السائدتان في أغلب العينات، ولكن النوع *L. monocytogenes* تواجد بكميات قليلة. تشير النتائج الي وجود خطر حقيقي على الصحة العامة لمستهلكي مثل هذه المنتجات.