

Prevalence of *Listeria* in ice creams in Baghdad province

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Accepted – November – 2010

Summary

The aim of this study was concerned on the prevalence of *Listeria spp.* in the ice cream samples collected from the different pastry shops and downtown stores of Baghdad province during December 2009 till April 2010. A total of 50 ice cream samples (25 locally produced samples from raw and imported powdered milk and 25 imported samples from different origins) were analyzed and processed according to the standard protocols of Food and Drug Administration (FDA) and International Organization for Standardization (ISO) with some modifications. Growth of *Listeria spp.* was determined in 7 (14%) of total samples as 4 (16%) isolates from locally produced ice creams , from which one isolate was confirmed as *Listeria monocytogenes*, and 3 (12%) isolates from imported ice creams. The findings of this study suggest that *Listeria* contaminates some dairy products and *Listeria* infections are not a potential risk in Baghdad province, but it is strongly recommended that these products are monitored carefully.

Key Words: *Listeria monocytogenes*, *Listeria spp.*, ice cream, food microbiology.

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التحري عن الليستيريا في المثلجات اللبنية في مدينة بغداد

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الخلاصة

أستهدفت الدراسة التحري عن تواجد جرثومة الليستيريا في المثلجات اللبنية المباعة في الأسواق المحلية في مدينة بغداد للمدة من كانون الأول 2009 ولغاية نيسان 2010. جمعت 50 أنموذج بواقع 25 أنموذج من المثلجات اللبنية المصنعة محليا من مصادر حليب مختلفة المنشأ (حليب خام و حليب مجفف مستورد) و 25 أنموذج من المثلجات اللبنية المستوردة من مناشأ مختلفة من العالم, وعولمت النماذج حسب الطرائق القياسية لل FDA و ISO مع بعض التحويرات. أظهرت النتائج عزل وتشخيص جرثومة الليستيريا بواقع 7 عزلات (14%) من 50 أنموذج: 4 عزلات (16%) من نماذج المثلجات اللبنية المحلية كان منها النمط المصلي *Listeria monocytogenes* و 3 عزلات (12%) من نماذج المثلجات اللبنية المستوردة. نستنتج من هذه الدراسة تلوث بعض منتجات الحليب بجراثيم الليستيريا وينصح بمراقبة المنتجات اللبنية المصنعة محليا و المستوردة لضمان الصحة العامة.

Introduction

Listeria especially *Listeria monocytogenes* are gram-positive, rod-shaped bacteria found in soil, stream water, sewage, plants and food especially in raw, unpasteurized milk and its products as well as, in low grade quality and post-thermal treated products of food especially milk (1,2,3&4). They are responsible for *listeriosis*, a rare but potentially lethal food borne infection ranging from flu-like syndrome or gastroenteritis to deadline infection of nervous system causing meningitis and abortion in pregnant women (3). *Listeria* species are incredibly and hardy able to grow in refrigerator at 4c° to 37c° the body's internal temperature (3). The presence

of any *Listeria* spp. in food may be an indicator of poor hygiene (2). Ice cream, a dairy product consumed with pleasure by the general populace, especially children due to its contents of sweeteners, aromatic compounds and various fruits, is known to be the richest in terms of content among the dairy products (5). Milk and dairy products under low temperature especially ice cream, constitute a potentially risk for *listeriosis* (5&6). Unfortunately, in Iraq country most of the consumed milk and dairy products are not prepared under hygienic conditions (1). Ignorant producers utilize milk that is most often not boiled enough in the production of their products, as well as, random importation of low quality milk and dairy products from different origins; this obviously affects the health of the consumers (1). Quality of ice cream depends on both extrinsic factors that include manufacture procedure, and intrinsic factors that include proportion of ingredients used (7). Ice cream, a milk based product is good media for microbial growth due to its high nutritive value, almost neutral pH value and long storage duration (6&7). Primary sources of microbial contamination to the ice cream include water and raw milk whereas secondary sources include flavoring agents, utensils and handling (7). Although pasteurization, freezing and hardening steps in production can estimate most of the microbial hazards, but still numerous health hazards are persistent due to various conditions (8). Many psychrophiles and psychrotolerant microorganisms like *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus species*, *Salmonella species*, *Shigella species*, *Streptococcus spp.*, *Pseudomonas spp.*, *Campylobacter spp.*, *Brucella spp.* and coliform bacteria are generally present in ice cream (4). There are few studies on the presence of *Listeria* spp. in ice cream, so that, this study was designed for determining the level of contamination of imported and locally produced ice cream samples from across our region.

Materials and Methods

Collection of Ice Cream Samples: This study was conducted on ice cream sold in different locally markets in Baghdad province. The origins of the milk samples used for producing the ice creams were different from locally raw, unpasteurized milk to imported milk powder. A 50 ice cream samples (25 locally produced and 25 imported) were collected into sterile containers and transported under cooling to the laboratory of food hygiene in Baghdad Veterinary College during December 2009 till April 2010.

Isolation of the *Listeria* species: The ice cream samples were thawed to below room temperature and a 25 ml from each was mixed with a 225 ml Trypton Soya Yeast Extract Broth (HiMedia, India) according to standard protocols, in which a portion of analytic sample was added to 9 portions of *Listeria* enrichment broth (9,10&11). For a homogenous distribution, the sample in the medium was shaken for 2-3 minutes inside stomacher. The inoculated media were then incubated at 30 C° for 24-48 hours. After 24 and 48-hour enrichment processing, the culture was made on the *Listeria* Selective Agar (PALCAM, Oxoid). The planted plates were incubated for 48 hours at (35-37) C° under aerobic conditions. At the end of the incubation, they were evaluated as typical colonies with black haloes of 1-3 mm in diameter. The typical *Listeria* colonies observed on the culture medium were selected and cultured separately in Trypton Soya Yeast Extract Agar (HiMedia, India). After that, the colonies were

checked morphologically and their purity controlled by gram staining. Later, the separated colonies were then subjected to carbohydrate fermentation tests (mannitol, D-xylose, rhamnase), catalase activity, oxidase activity, modified motility test with triphenyltetrazolium chloride salts (Umbrella formation), esculin hydrolysis. The colonies isolated as *Listeria* were then subjected to β -hemolysis in a 7% sheep blood agar for species identification. Confirmation of *Listeria* isolates was done by Latex Agglutination test (Oxoid, UK), and differentiation of *Listeria monocytogenes* from other *Listeria* spp. was done by Oxoid Biochemical Identification System (O.B.I.S. mono test kit, Oxoid) (12).

Results

Results revealed that out of the 50 ice cream samples, 7 samples (14%) were contaminated with different *Listeria* species as 4 (16%) isolates from locally produced ice creams , from which one isolate was confirmed as *Listeria monocytogenes*, and 3 (12%) isolates from imported ice creams as shown in table (1).

Table (1). Isolation of *Listeria* species from Baghdad province.

Sample Type	Number	<i>Listeria spp.</i>	<i>Listeria monocytogenes</i>	Total
Ice creams	50	6 (12%)	1 (2%)	7 (14%)
Locally produced	25	3 (12%)	1 (4%)	4 (16%)
Imported	25	3 (12%)	None	3 (12%)

Listeria monocytogenes appeared on chromogenic PALCAM (Polymyxin Acriflavin Lethium Chloride Ceftazidime Aesculin Mannitol) agar as grayish-green, smooth, convex, circular or creanated colonies with entire margins, black haloes of 1-3 mm in diameter and sunken center; after 5 days, they became brown-black, enlarged in size with black haloes of 3-5 mm in diameter lock like bull or fish eyes and medium became cherry-red; while other *Listeria spp.* appeared as grayish-whitish and some types as grayish-yellowish with black centers (13). The results of biochemical tests, *Listeria* latex agglutination test and O.B.I.S. mono test to differentiate *Listeria monocytogenes* from other *Listeria spp.* are summarized in table (2).

Table (2). *Listeria* biochemical's and confirmation tests.

Test	<i>Listeria monocytogenes</i>	<i>Listeria spp.</i>
Gram-positive rod	+	+
Umbrella formation	+	+
β -Hemolysis	+	-
Catalase activity	+	-
Oxidase activity	+	-
esculin hydrolysis	+	+
Rhamnose fermentation	+	V
D-Xylose fermentation	-	V
Mannitol fermentation	-	V
<i>Listeria latex agglutination</i>	+++	+
O.B.I.S.mono	+	-

+ = positive test, - = negative test, v = variable results and +++ = strongly fast positive result

Discussion

Listeria species are found in almost every medium in the environment including soil, excrement, canalization waters, plants, animals, animal feeds and foods; they contaminate milk by various means from these sources (14). These microorganisms, because its psychrotrophic genetic ability can grow and contaminate of ice cream due to its a complex food containing sugar, emulsifiers and fats(15).In our country, studies on the prevalence of *Listeria* in ice cream are limited in number, I found *Listeria spp.* especially *Listeria monocytogenes* in raw, unpasteurized milk and soft cheese from different animal species (1).This study revealed isolation of *Listeria spp.* from locally produced and imported ice cream samples either due to poor hygienic measurements and poor surveillance on product or contamination during production, processing, transportation and storage. From several studies on the prevalence of *Listeria spp.* in milk and dairy products, various *Listeria spp.* including *Listeria monocytogenes* have been isolated (16).Other countries such as USA showed that in a factory dealing with frozen milk and dairy products in California, 922 samples were taken from the different divisions of the factory, *Listeria spp.* were isolated in 111 (12.04%) samples, and of the 39 examined factories, *Listeria monocytogenes* was encountered, in only 5, whilst 13 of them contained *Listeria innocua*, in 9 of them

both species were encountered, and in 12 factories no isolates were encountered (17). Farber (18), in a study in which 394 ice cream samples were examined, found the incidence of *Listeria monocytogenes* to be 0.25% (12). Pak . (19), in a study on dairy products in 2002, found the incidence of *Listeria monocytogenes* to be 4.9%. In the same study, whilst the incidence of *Listeria monocytogenes* was 7.6%, Laciari . (20), in their study in Spain in 1999, investigating the presence of *Listeria spp.* in different food items, failed to isolate *Listeria spp.* in pasteurized milk, cheese and chocolate milk products but *Listeria welshimeri* was isolated from ice cream samples. Casadei (21), in 1998 and Manifreni . (22), in 1993 in their studies on ice cream samples couldnot isolate the known pathogen *Listeria monocytogenes*. In 1999, Laciari . (20), in their study on ice cream samples reported that *Listeria monocytogenes* could not be isolated.

In conclusion, since *Listeria* species were found to a large extent in our ice cream samples and *Listeria monocytogenes* was encountered, it should be mandatory to perform routine controls to screen ice cream samples for detection of *Listeria spp.* Our findings suggest that *Listerial* infections are not a potential risk in Baghdad province; it is strongly recommended however that these products are monitored carefully. The results obtained showed differences between the hygiene and bacteriological quality of commercial and homemade ice creams. The implementation of Hazard Analysis and Critical Control Points (HACCP) Programs in ice cream industries is essential for addressing food safety for population of Baghdad province, as well as intervention and education of people involved in the production of house made ice creams and monitoring the imported ice creams.

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