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PREVALENCE OF ENTERIC BACTERIA PRODUCING TOXINS IN ICE-CREAM AND KAREISH CHEESE IN PORT-SAID CITY MARKETS

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ABSTRACT

The present study estimate the enteric bacteria in ice-cream and Kareish cheese. The study included 120 samples of ice-cream and Kareish cheese (60 of each), collected randomly from supermarkets, shops and street vendors in different districts of Port-Said city. All samples were examined for presence of Enterobacteriaceae spp. (on Violet Red Bile Glucose agar medium). Enterobacteriaceae strains were isolated in a percentage of 60% and 80% from group B (unpacked-shop) and group C (unpacked- street vendors) of ice-cream samples respectively, while the Enterobacteriaceae strains were isolated in a percentage of 40% and 93.3% from group B (unpacked-shop) and group C (unpacked- street vendors) of Kareish cheese samples respectively. The percentage of isolation of *E. coli* was 13.3% from group C (unpacked- street vendors) of ice-cream samples , while it was isolated in 6.6% from group B (unpacked-shop) and 60% from group C (unpacked- street vendors) of Kareish cheese samples. *Salmonella* spp. have been isolated in a high percentage (13.3%) from group C (unpacked- street vendors) of Kareish cheese samples and more than that of ice-cream (6.6%). The confirmation of the colonies was done biochemically and were identified up to species level. *E.coli* isolates from Kareish cheese in group C (unpacked- street vendors) were belonged to Poly(1) O:01, O55:K59 (B5), O124:K72 (B17) and in group B (unpacked-shop) were untyped, while *E.coli* isolates from ice-cream in group C (unpacked- street vendors) were untyped. *Salmonella* isolates from ice-cream and Kareish cheese were *S.* Typhimurium and *S. paratyphi* B. Studying the *E.coli* and *Salmonella* isolates for ability to produce toxin by using infant mouse assay was carried out. The isolated serotypes constitute public health hazards to consumers.

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Key words: *Microbiological examination, dairy products, enteric bacteria producing toxins, Public health.*

INTRODUCTION

Ice-cream and Kareish cheese are considered the most popular Egyptian dairy products. Ice-cream is a milk product, which contains a variety of ingredients in addition to milk, cream and sugar. The richness in nutritive constituents of ice-cream has been realized by all but the production and handling of this food is very complex and associated with problems. Ice-cream is a nutritionally enriched congealed dairy product consumed by all age groups particularly children, during summer (Sharif *et al.,* 2005). Although ice-cream is a nutritious food for human but also an excellent medium for the growth of many microorganisms due to high nutrient value, almost neutral pH (pH~ 6-7) and long storage duration of ice-cream. Contaminated ice-cream causes several outbreaks of gastrointestinal diseases in a number of countries in Asia, Europe and North America (Yaman *et al.,* 2006). Primary sources of microbial contamination of ice-cream include water and raw milk (raw materials),whereas secondary sources include flavoring agents, colouring substances, sanitizer utensils ,handling and from contaminated air during processing (Khalil *et al.,* 2009).

Kareish cheese is one of the most popular soft, fresh, lactic acid, low salt type of cheese in Egypt. Kareish cheese manufacture and handling techniques in Egyptian markets are still primitive and unhygienic. Many contaminants find their way to raw milk, from which they gain access to dairy products (Al-Khatib and Al-Mitwalei, 2009).

Foodborne diseases are common and widespread global problem. Several outbreaks have been reported as a result of eating contaminated dairy food that may look, taste and smell perfectly normal but is in fact contaminated with large number of harmful bacteria (CDC, 2009).

Enterobacteriaceae spp. have been implicated in many cases of food poisoning outbreaks (Koneman *et al.,*

1994). *Escherichia coli* is an important organism in the microbiology of the food, besides being involved in foodborne gastroenteritis, it is considered a good indicator of possible fecal contamination of dairy products El-Bagoury and Mosaad, (2002) and Maher *et al.* (2001).

Salmonella is considered among the most important enteric foodborne pathogen whose presence in the food constitutes a severe health hazard. Many outbreaks of human illness have been associated with the consumption of raw or inadequately heat treated milk or their dairy products (Ellis *et al.,* 1998).

Therefore, this study was carried out to determine the prevalence of Enterobacteriaceae spp. and pathogenic strains in commercially soled ice-creams and Kareish cheese in Port-Said city as well as the ability to produce enterotoxin was studied. Also the public health significance of the isolated microorganisms were discussed.

MATERIALS and METHODS

1- Collection of samples:
This study was carried out on 120 dairy products (60 ice-cream and 60 Kareish cheese samples) collected randomly from supermarkets, shops and street vendors in different districts of Port-Said city. All of the samples were collected in sterile plastic bags and transported to the laboratory on ice – box and divided to three groups (A, B and C) for microbiological examination.

2- Preparation of the samples:
The technique recommended by APHA (1992) was used for samples preparation. Twenty five grams of Kareish cheese samples and 25 ml of ice-cream (after thawed) were aseptically added to 225 ml of Buffered peptone water and then homogenized in a stomacher for 2 min to form 1:10. Ten fold serial dilution were prepared using sterile 0.1% peptone water.

3- Microbiological examination:
a- Isolation and identification of Enterobacteriaceae according to FDA (2002).
b- Isolation and identification *Salmonella* spp. according to ISO (2002). c- Isolation and identification *E.coli* according to APHA (1992).
d- Serotyping of *Salmonella* isolates: Colonies were serologically confirmed using diagnostic polyvalent (O, H) and monovalent *Salmonella* anti-sera according to (Kauffmann and Das- Kauffmann 2001).
e- Serotyping of *E. coli* isolates: Colonies were serologically confirmed using diagnostic polyvalent (O, K) anti-sera according Sanderson *et al.* (1995).

4- Detection of toxin produced by isolates:
Enterotoxin assay:
The ability to produce enterotoxin was assayed by the infant mouse test according to the technique described by (Pai and Mors, 1978) and (Stavric *et al.,* 1992).

1-Preparation of culture filtrate:
E. coli strains were inoculated into 25 ml of specific media, incubated at room temperature at 22-26 °C, (as well as *Salmonella* isolates were tested as mentioned above) on a rotatory shaker (200 r.p.m) for 48 hours, centrifuged at 12000 x g for 10 minutes. The supernatant were filtered through Millipore membrane filter (Pore size 0.22µm), stored at - 20 °C, till used. A part of the sterile medium was used as a negative control.

2- Infant mouse assay:
A volume of 0.1 ml of culture filtrate was injected through the abdominal wall into milk-filled stomach of each of 2 mice (for each serotype) which were 2 to 4 days old. Another 2 infant mice were injected by 0.1 ml saline and were used as negative control. After 4 hours, the mice were killed and the entire intestine was removed. The intestine and the remaining body were weighted to calculate the ratio of (intestine weight) / (remaining body weight). A ratio greater than 0.083 was recorded as positive test for enterotoxin.

RESULTS

Table 1: Incidence of Enterobacteriaceae in examined ice-cream (60) and Kareish cheese (60) samples.

examined samples	Type		Enterobacteriaceae	
			No.	%
Ice-cream	Group A	(n=30)	0	0%
	Group B	(n=15)	9	60%
	Group C	(n=15)	12	80%
Kareish cheese	Group A	(n=30)	0	0%
	Group B	(n=15)	6	%40
	Group C	(n=15)	14	93.3%

Group A = Packed (market).
Group B = unpacked (shop).
Group C= unpacked (street vendors).

Table 2: Incidence of Enterobacteriaceae strains isolated from the examined ice-cream (60) and Kareish cheese (60) samples.

Enterobacteriaceae Strains	Ice-cream						Kareish cheese					
	Group A n=30		Group B n=15		Group C n=15		Group A n=30		Group B n=15		Group C n=15	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
E.coli	0	0%	0	0%	2	13.3%	0	0%	1	6.6%	9	60%
Klebsiella pneumoniae	0	0%	2	13.3%	3	20%	0	0%	2	13.3%	1	6.6%
Klebsiella oxytoca	0	0%	1	6.6%	2	13.3%	0	0%	1	6.6%	1	6.6%
Proteus mirabilis	0	0%	6	40%	4	26.6%	0	0%	2	13.3%	1	6.6%
Salmonella sp.	0	0%	0	0%	1	6.6%	0	0%	0	0%	2	13.3%

Group A = Packed (market).
Group B= unpacked (shop).
Group C= unpacked (street vendors).

Table 3: Serodiagnosis of *E.coli* and *Salmonella* isolated from the examined ice-cream (60) and Kareish cheese (60) samples.

Types of examined samples	*Ecoli*			*Salmonella*		
	Strain/ Antigenic structure	No.	%	Strain / Antigenic structure	No.	%
Ice-cream Group A n=30	-	0	0%	-	0	0%
Ice-cream Group B n=15	-	0	0%	-	0	0%
Ice-cream Group C n=15	Untyped	2	13.3%	*B S. paratyphi* O:1,4,(5),12 H_1:b,H_2:1,2	1	6.6%
Kareish cheese Group A n=30	-	0	0%	-	0	0%
Kareish cheese Group B n=15	untyped	1	6.6%	-	0	0%
Kareish cheese Group C n=15	Untyped	3	20%	*B S. paratyphi* O:1,4,(5),12 H_1:b,H_2:1,2	1	6.6%
	Poly(1)O:01	3	20%	*S.* Typhimurium O:1,4,(5),12 H_1:i,H_2:1,2	1	6.6%
	O55:K59 (B5)	2	13.3%			
	O124:K72 (B17)	1	6.6%			

Group A = Packed (market).
Group B= unpacked (shop).
Group C= unpacked (street vendors).

Table 4: Detection of enterotoxin producing *E. coli* and *Salmonella* strains isolated from the examined ice-cream (60) and Kareish cheese (60) samples using infant mouse assay.

Types of examined samples	*E.coli*	Positive of enterotoxigenic *E. coli*		G/W ratio mean	*Salmonella*	Positive of enterotoxigenic *Salmonella*		G/W ratio mean	Control	G/W ratio mean
	No.	No.	%		No.	No.	%		No.	
Ice-cream Group C n=15	2	1	50%	0.084	1	0	0%	0.079	2	0.079
Kareish cheese Group B n=15	1	0	0%	0.079	0	0	0%	-		
Kareish cheese Group C n=15	9	5	55.5%	0.086	2	0	0%	0.079		

Group B= unpacked (shop).
Group C= unpacked (street vendors).

DISCUSSION

The International Commission on Microbiological Specification for foods has classified cheese and ice-cream as a high risk potential hazard (Abou-Donia, 1984). This could be confirmed by its implication in several outbreaks of food poisoning (Bryan, 1988). The microbiological qualities of packed and unpacked ice-cream and Kareish cheese are shown in Table 1. Current results revealed that Enterobacteriaceae were isolated in a high percentage 93.3% and 80% of Kareish cheese and ice-cream samples in group C (unpacked – street vendors) respectively than those isolated from (unpacked – shop). The results nearly agree with those obtained by Fadel and Jehan (2009) who isolated Enterobacteriaceae in 100% and 75% of Kareish cheese and ice-cream samples respectively. Enterobacteriaceae are distributed worldwide. The presence in large number indicates faecal contamination of food, inadequate processing and post- processing contamination Koneman *et al.* (1994).

Table 2, showed the incidence of Enterobacteriaceae strains from examined ice-cream and Kareish cheese (unpacked-shop) and (unpacked – street vendors). *E.coli* (0%-13.3%), *Klebsiella pneumoniae* (13.3%-20%), *Klebsiella oxytoca* (6.6%-13.3%), *Proteus mirabilis* (40%-26.6%) and *Salmonella* spp. (0%- 6.6%) from examined ice-cream (unpacked-shop) and (unpacked – street vendors) respectively. These results agree with that recorded by Khalil *et al.* (2009) as they could isolate *E. coli* (100%), *Klebsiella* (85%), *proteus* (45%) and *Salmonella* spp. (15%) from ice- cream samples who explained that many opportunities exist for contamination of ice-cream from the hands of workers and other equipments. During processing of ice-cream. Furthermore, the microbiological quality of ice-cream during retail marketing mainly depends upon the post production, handling of the product as well as efficiency and sanitary conditions during storage. The lack of efficient storage under warm tropical climatic conditions causes a chance of temperature abuse during transport and distribution of ice-cream Kanbakna *et al.* (2004). Also *E.coli* (6.6%-60%), *Klebsiella pneumoniae* (13.3%- 6.6%), *Klebsiella oxytoca* (6.6%-6.6%), *Proteus mirabilis* (13.3%-6.6%) and *Salmonella* spp. (0%- 13.3%) from examined Kareish cheese (unpacked-shop) and (unpacked – street vendors) respectively. These results agree with that recorded by Fadel and Jehan (2009) as they could isolate *E. coli* (44.4%), and *Klebsiella pneumoniae* (11.1%) only from Kareish cheese samples, while they couldn't detect *Klebsiella oxytoca* and *Proteus mirabilis*. El-Prince and Hussein (2000), Khalil *et al.* (2009) and Amin, (2004) as they could isolate *Salmonella* spp. from ice-cream samples, while Delia *et al.* (1980) failed to detect *Salmonella*. On the contrary, There is no much available data about the prevalence of *Salmonella* in Kareish cheese, however, (Abd El- Atty and Meshref 2007; El-Kosi, 2001 and Amin, 2004) detected *Salmonella* spp. in (4%) , (3.33%) and (10%) in Kareish cheese respectively. On the other hand (Bahout and Moustafa 2006; El-Kholy 1989 and Fadel and Jehan 2009) failed to recover *Salmonella* from Kareish cheese samples. presence of some pathogenic microorganisms especially *Salmonella* due to using raw milk in the production accompanied by improper sanitary practices during manufacturing, handling and selling. The difference between our results and the previous studies may be attributed to sampling techniques, sources of sampling, handling of samples and types of media. Because of the public health hazards of these microorganisms, the Egyptian standards for ice- cream and Kareish cheese proposed that *E. coli* and *Salmonella* spp. should not be detected in the ice-cream and Kareish cheese (Ministry of Industry and Technological Development, 1993; Ministry of Industry and Technological Development, 2000) (Table 2).

Contamination represents a public health risk due to the possible presence and transmission of pathogens. The mode of transmission of all these bacteria is fecal-oral route and or via common house flies. The results suggested negligence such as poor sanitation during the preparation and/or storage of these products. These include the observed dirty premises and utensils used, the use of bare hands in preparing the products (personal communication with the handlers) (Fadel and Jehan 2009). In this study the most important genera *Escherichia coli* and *Salmonella* spp.

Table 3, showed the E.coli could be isolated and identified as 13.3% untyped from ice-cream in group C, and E.coli which isolated from Kareish cheese were identified as 6.6% untyped in group B, while in group C E.coli strains were identified as 20% untyped, 20% of Poly(1) O:01, 13.3% of O55:K59 (B5) and 6.6% of O124:K72 (B17) (Hassan *et al.,* 2009). *Salmonella* spp. were identified as *S. paratyphi B* from ice-cream in group C (unpacked- street vendors), But *Salmonella* isolated from Kareish cheese samples were *S.* Typhimurium and *S. paratyphi* B. in group C (unpacked- street vendors) (Amal and Mohammed 2003; Ekbal and Azza 2006 and Mahmoud, 2004). The production of enterotoxin of the isolated *E. coli* and *Salmonella* spp. from ice-cream and Kareish cheese samples were shown in Table (4). *E. coli* strains isolated from ice-cream samples in group B (unpacked-shop) and group C (unpacked -street vendors) in a percent of (50%), (55.5%) were enterotoxin producing and caused accumulation of fluids in the intestinal tract of the injected infant mice respectively. While *Salmonella* isolated in this study were unable to produce enterotoxin (Stavric *et al.,* 1992 and Wallis *et al.,* 1986).

In conclusion, our results revealed out that Egyptian Kareish cheese and ice cream products sold in Port-Said city markets and street-vendors are contaminated with Enterobacteriaceae sp. These isolates constitute public health hazards to consumers. Periodical examination of dairy products to ensure safety for consumers must be practiced. The isolation of bacterial pathogens from dairy products reflects bad hygienic standards and necessitates regular inspection of them for prevalence of foodborne pathogens. Adoption of reward and punishment policy may help to improve their hygienic standards. Overall, good quality raw materials used in product processing, adoption of Good Manufactured Practices (GMP) and strict personal hygiene are the way to ensure safety and high quality dairy products.

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مدى تواجد الميكروبات المعوية المنتجة للسموم في الأيس كريم والجبن القريش بأسواق مدينة بورسعيد

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أجريت هذه الدراسة علي مائة وعشرون عينة من الأيس كريم والجبن القريش (60 عينة من كل صنف) التي جمعت من مصادر مختلفة من المحلات والباعة الجائلين في مدينة بورسعيد لتقييم حالتها الميكروبيولوجية. حيث اشتملت هذه الدراسة علي عزل الميكروبات المعوية المنتجة للسموم (ميكروب الايشيريشيا كولاي O157 وعائلة الميكروبات المعوية) بالإضافة إلي تصنيفها فأسفرت الدراسة عن النتائج التالية : وفحصت العينات لوجود الميكروبات المعوية التي تم عزلها بنسبة 60 % و 80 % من عينات الأيس كريم الغير مغلفة والمجمعة من كل من المحلات والباعة الجائلين علي التوالي و 40 % و 93.3 % من عينات الجبن القريش الغير مغلفة والمجمعة أيضا من كل من المحلات والباعة الجائلين علي التوالي. وقد صنفت الى الايشيريشيا كولاي ، البروتيس ، الكليبسيلا والسالمونيلا. تم عزل ميكروب الايشيريشيا كولاى من 10 عينات من الجبن القريش الغير مغلفة والمجمعة من كل من المحلات والباعة الجائلين علي التوالي بنسبة 13.3% و60%. كما تم عزل ميكروب الايشيريشيا كولاى من عينتين فقط من الأيس كريم الغير مغلف من الباعة الجائلين بنسبة 13.3% وبتصنيفها سيرولوجيا وجد أنه ، O124:K72(B17) ، Poly (1)O:01 ،O55:K59(B5) وعترات غير مصنفة. كما كانت نسبة عزل ميكروب السالمونيلا عالية 13.3 % من الجبن القريش الغير مغلفة من الباعة الجائلين عن نسبة عزلها من الأيس كريم الغير مغلف من الباعة الجائلين 6.6 % وبتصنيفها سيرولوجيا وجد أنها سالمونيلا تيفيميوريم وسالمونيلا باراتيفي بي. أما بالنسبة لنتائج اختباري النشاط الإفرازي للسموم داخل الأمعاء وجد أن ميكروب الايشيريشيا كولاى المعزولة من عينات الأيس كريم والجبن القريش المباعة لدي الباعة الجائلين كانت قادرة علي إفراز السموم بنسبة 50 % (الأيس كريم) و 55.5 % (الجبن القريش) عـ لي الـ توالـ ي. وكانت نتيجة عترات السالمونيلا المعزولة من الأيس كريم والجبن القريش سلبية. هذا وقد نوقشت الأهمية الصحية والاقتصادية للميكروبات المعزولة وكذلك الاجراءات التي يمكن اتخاذها لتحسين جودة هذان المنتجان.