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**PREVALENCE OF SOME FOOD POISONING
ORGANISMS IN RAW MILK AND ICE CREAM WITH
SPECIAL REFERENCE TO ENTEROPATHOGENIC
ESCHERICHIA COLI.**

(With 3 Tables)

By

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مدى تواجد بعض ميكروبات التسمم الغذائي باللبن الخام و الأيس كريم مع
الإشارة الخاصة للميكروب القولوني العسوي الممرض

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اجرى البحث على خمسة وسبعون عينة من كل من اللبن الخام والأيس كريم بنوعيه صغار المنتجين وهم الباعة الجائلين وإنتاج المصانع الكبرى بواقع ٢٥ عينة لكل منهم وقد جمعت العينات عشوائيا من أماكن متفرقة بمدينة الإسكندرية وذلك لفحصها معمليا ومدى تلوثها بميكروبات التسمم الغذائي. وقد أسفرت النتائج عن تواجد الميكروبات المحبة للبرودة ومجموعة القولونية المعوية ، ميكروب المكور السبحى و ميكروب المكور العنقودى بمتوسط عدد كلوى كالتالى (٤ و٩ و١٠^٨ ± ١٠^٨ × ١٠^٨) و (٥ و٧ و١٠^٥ ± ١٠^٥ × ٢ × ١٠^٥) و (٣ و١٠^٢ ± ١٠^٢ × ٠,١ و ١٠^٢ × ١,٠) و (١,٠ و ١٠^٤ ± ١٠^٤ × ٠,٥ و ١٠^٤ × ٥,٦) و (٠,٥ و ١٠^٢ × ٢,٢ ± ١٠^٢ × ٥,٦) و (٠,٥ و ١٠^٣ × ١٦,٣٨ ± ١٠^٣ × ١,٥٣ و ١٠^٣ × ٨,٩٧ ± ١٠^٣ × ١,٣١) و (٠,٥ و ١٠^٢ × ١,٦٧ ± ١٠^٢ × ١,٦٤) و (١٠^٢ × ١,٦٧ ± ١٠^٢ × ٦,٩١) و (١٠ × ١,٦٧ ± ١٠ × ٧,٣٠) و (١٠ × ١,٦٧ ± ١٠ × ٧,٣٠) من عينات اللبن الخام والأيس كريم بنوعيه على التوالى كما تم عزل وتصنيف كلا من الميكروبات القولونية العسوية الممرضة ، والميكروبات القولونية المعوية و ميكروبات السيدوموناس ، و ميكروبات اليرسينيا والايروموناس هيدروفيليا بنسب مختلفة كما أمكن عزل ميكروبات المكور العنقودى والمكور السبحى بنسب مختلفة ايضا. ثم تم عمل تصنيف سيرولوجى لميكروبات القولونية العسوية الممرضة وعزل ٨ عترات منها هذا وقد تم مناقشة النتائج من الناحية الصحية والاقتصادية وتوضيح الاجراءات الصحية الواجبة لضمان جودة المنتج وحماية المستهلك.

SUMMARY

75 samples of raw milk, small scale ice cream and large scale ice cream (25 each) were collected from different supermarkets and street vendors in Alexandria city, Egypt and examined bacteriologically for the presence of

some food poisoning organisms. Psychrotrophic, Coliform, Staphylococcus and Enterococci were present with a mean value of (9.4×10^8 , 7.5×10^5 and 3×10^2), (1.0×10^4 , 5.6×10^2 and 0.0), (16.38×10^3 , 8.9×10^3 and 0.0) and (6.9×10^2 , 1.6×10^2 and 7.3×10) in the examined raw milk, small scale and large scale ice cream samples, respectively. *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., *Yersinia* spp., *Aeromonas hydrophila* improving the quality of the product were discussed. *Staphylococcus aureus* and Enterococci could be isolated at varying percentages from the examined samples. The Enteropathogenic *E. coli* could be isolated from 96% and 20% from raw milk and small scale ice cream samples and identified serologically as O₁₁₉ : K₆₆, O₁₁₂ : K₆₆, O₁₁₁ : K₅₈, O₁₁₄ : K₉₀, O₇₈ : K₈₀, O₁₂₄ : K₇₂, O₁₂₈ : K₆₇ and O₂₆ : K₆₀. The public health significance and economic importance of existing food poisoning organisms as well as suggestive measure for improving the quality of the product were discussed.

Key words: Milk, ice cream, *E. coli*, *Aeromonas*, *Yersinia*

INTRODUCTION

Milk is a complex biological fluid, containing a wide variety of constituents and possessing unique physical characteristics. It consists largely of lipids, proteins, carbohydrates, minerals and vitamins, so it is a well-known vehicle of a number of human pathogens whatever, it is raw or processed (Aisha *et al.*, 2002).

Ice cream is considered as one of the most favourite frozen dairy products all-over the world. It is a delicious, highly nutritious food and has a therapeutic value for persons suffering from irritation and infection of mouth and throat due to its coldness (Robinson, 1994).

Although, ice cream is a wide spread dairy food consumed by people of all ages, yet it may be subjected to contamination by various microorganisms at different stages of manufacturing, handling and packaging. Furthermore, the processing of ice cream requires a variety of heat treatment, handling and frequently adding substances as sugar, fruits, raw eggs or other products of animal origin. These additives may contribute to food poisoning outbreaks gastroenteritis in human beings or other bacterial problems either by adding microflora or by creating conditions favourable for the growth and survival of bacteria (Kumari *et al.*, 1996; El-Prince and Hussein, 2000).

In addition, ice cream has been incriminated as a transmitter of other pathogenic microorganisms. *Klebsiella*, *E. coli*, *Serratia*, *Proteus*,

Pseudomonas, *Yersinia*, *Citrobacter*, *Enterobacter*, *Aeromonas* species were the most predominant organisms previously isolated by Abdel-Haleem (1995); El-Bagoury (1996); Kumari *et al.*, (1996) and Mansour *et al.*, (2000) from ice cream samples in varying percentages. The presence of these microorganisms in higher percentages have been implicated in many cases of food poisoning and other foodborne diseases (Varnam and Evans, 1991).

The most psychrotrophic bacteria in milk were readily killed by heat treatment but many species produced heat resistant extra-cellular proteinases and/or lipases which remained active in milk products (Cogan, 1980).

Proteinase enzymes produced by psychrotrophic bacteria cause un-desirable proteolytic changes, objectionable flavour and quality defects in dairy products after storage (Bigalke, 1985; Mansour *et al.*, 2000).

The growth of *Staphylococcus aureus* in milk constitutes a potential public health hazard since many strains of *Staphylococcus aureus* produce enterotoxins that cause food poisoning if ingested. Neither the absence of *Staphylococcus aureus* nor the presence of small numbers is complete assurance that a food is safe. Conditions inimical to the survival of *Staphylococcus aureus* may result in diminishing operation or death of viable microbial cells, while sufficient toxins remain to elicit symptoms of staphylococcal food poisoning (AOAC, 1984; Lancette and Tatini, 1992).

Aeromonas spp. consists of three species, *A. hydrophila*, *A. caviae* and *A. sorbia*, these species collectively referred to as motile or mesophilic aeromonads. Milk and milk products as ice cream have been surveyed by several investigators for the occurrence of *A. hydrophila* (Khalil, 1997; El-Prince, 1998; Ahmed *et al.*, 2001). Two types of diarrhea have been attributed to these organisms, a "cholera like" illness characterized by watery stools and a less common "dysentery like" illness, characterized by blood and mucus in the stool. Both types of diarrhea are usually mild and self-limiting, but either may be chronic or sever (Palumbo *et al.*, 1992; Abd El-Hady and Halawa, 1999). These organisms cause gastroenteritis and several diseases such osteomyelitis; Septicemia; meningitis; endocarditis; skin infection; cellulitis, wound infections, pneumonia, urinary tract infections, endocarditis and ear infections (Koneman *et al.*, 1994). *Aeromonas hydrophila* produces a number of potential virulence factors including enterotoxins, cytotoxins, haemolysins, lipases and proteases. Therefore it posses a highly

significant public health problem as well as economic importance (Trust and Chipman, 1979 and Abd El- Hady and Halawa, 1999).

Yersinia organisms are Gram-negative, psychrotrophic milk-borne enteric pathogens. These organisms are widespread in the environment and are indigenous to the gastrointestinal tracts of warm-blooded animals including dairy cattle (Marshall, 1992; Hussein and Ahmed, 2002; Bahout and Moustafa, 2004).

Y. enterocolitica causes enterocolitis in young children and adults while older children and young adult may be accompanied present with symptoms of appendicitis including fever, abdominal pain, tenderness in the right lower quadrant and leucocytosis. However, serious cases may occur with rectal bleeding and perforation of the ileum (Rabinovitz, 1987) Moreover, there may be secondary immunologically mediated complication such as arthritis, erythema nodosum and to a lesser extent Reiter's syndrome, glomerulonephritis, myocarditis, exudative pharyngitis and septicemia (Butler, 1998; Amal and Sayed, 2003).

Hazardous bacteria that grow at refrigeration temperature include *Y. enterocolitica* and *Aeromonas hydrophila* are newly recognized as food borne disease organisms. These bacteria are a potential threat for food held at refrigeration temperatures, and they grow much faster at higher abuse temperatures (Corlett, 1989). Although, psychrotrophic bacteria could be destroyed by freezing, bacterial spores and many vegetative cells can survive freezing. Thus some psychrotrophic bacteria will grow in frozen foods as ice cream, if the food is subjected to temperature abuse, (Kornacki and Gabis, 1990; Mira *et al.*, 2001).

Pseudomonas species are widely distributed in nature, they have been found in external environmental conditions surrounding dairy animals such as water, soil, sewage, air, grass, hay, feces and bedding materials. These organisms represent the most common psychrotrophs that contaminate milk and cause variety of defects including fruity, rancid, bitter and putrid flavor as well as color defects (Kraft, 1995; Al-Ashmawy *et al.*, 1997; Pearson *et al.*, 2000)

P. aeruginosa is considered all over the world as one of the dangerous organisms causing different diseases and capable of secreting many extracellular products such as lipopolysaccharide (LPS), fibrinolysin, haemolysins, exotoxins and enterotoxins. These products have a major role in the virulence of pathogenic strains of *P. aeruginosa* (Khalil, 1992; Champagne *et al.*, 1994; Beuchat, 2000; Aly and Zaki, 2001).

Escherichia coli (*E. coli*), is incriminated as an etiologic agent of foodborne illness involving a variety of foods. The organism contaminates food through both direct and indirect sources, as it is commonly found in the gastrointestinal tract of man and animals (Gad El-Said *et al.*, 2005). *E. coli* strains involved in foodborne illness could be placed into five groups which are referred to as diarrhoeogenic groups: enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enterohaemorrhagic *E. coli* and enteroadherent-aggregative *E. coli* (El-Bagoury and Hammad, 2004; Hassan and Abosrea, 2005). They are frequently associated with outbreaks of diarrhea affecting persons at different ages specially infants and elderly (Todd, 1990).

Enterotoxigenic *E. coli* (ETEC) is considered among the most prevalent organisms associated with infantile diarrhea in developing countries and a leading cause of mortality among children less than five years of age (Ryan, 1991). ETEC produces diarrhea by the elaborated in a heat-labile enterotoxin (LT), a heat-stable enterotoxin (ST) or both toxins, that act on the intestinal mucosa resulting in fluid outpouring into the, intestinal lumen (El-Shishnagui and Nazem, 1999; Al-Hawary, 2005).

The present study was planned to reveal the incidence of some food poisoning organisms in raw milk and ice cream and high light the public health importance and suggested measure for their control in these products.

MATERIALS and METHODS

Collection of Samples:

Seventy five random samples of raw milk and ice cream (25 raw milk samples, 250 ml of each milk sample were collected in sterile screw capped glass jars, 25 each of small scale and large scale producers) were collected from different localities in Alexandria city, Egypt. The collected samples were transferred to the laboratory with a minimum of delay, in a thermos containing ice to be immediately examined..Each sample of ice cream was left to stand in a water bath at 40°C for not more than 15 minutes to be melted.

Bacteriological examination:

1-Coliform count (MPN) and isolation:

The count was determined by using 3-tube MPN technique as described by Harrigan (1998). Streaks from positive tubes (gas after 48h.) were evenly spread onto violet red bile lactose agar plates. The plates were incubated at 30°C for 48 h. and five colonies were selected

Table 2: Statistical analytical results of different bacterial groups in examined samples.

Bacterial Counts/ml	Raw milk			Ice cream Small scale			Ice cream Large scale		
	Min	Max	Mean±S.E	Min	Max	Mean±S.E	Min	Max	Mean±S.E.
Psychrotrophic	9×10^3	6.8×10^{10}	$9.4 \times 10^5 \pm 1.1 \times 10^5$	3×10	1.4×10^7	$7.5 \times 10^5 \pm 2.4 \times 10^5$	10×4	2×10^3	$3 \times 10^2 \pm 0.1 \times 10^2$
Coliform (MPN/ml)	0.3×10^2	11×10^4	$1 \times 10^4 \pm 0.5 \times 10^4$	0.3×10^2	0.5×10^3	$0.56 \times 10^2 \pm 2.2 \times 10^2$	-	-	-
Staphylococcus aureus	8.95×10^3	26.5×10^3	$16.38 \times 10^3 \pm 1.53 \times 10^3$	3.42×10^2	21.4×10^3	$8.97 \times 10^3 \pm 1.31 \times 10^3$	-	-	-
Enterococci (count/ml)	3.7×10^2	1.14×10^3	$6.91 \times 10^2 \pm 1.67 \times 10^2$	8×10	3.7×10^2	$1.64 \times 10^2 \pm 0.38 \times 10^2$	3×10	2×10^2	7.3×10 $\pm 1.67 \times 10$

Table 3: Frequency distribution of Serotyping of Enteropathogenic *E. coli* isolates recovered from raw milk and ice cream (small scale).

<i>E. coli</i> Serotype	Raw milk		Ice cream (small scale)		Strain characteristics
	NO	%	NO	%	
O ₁₁₉ :k ₆₆	7	29.16	2	40	Untypable
O ₁₁₂ :k ₆₆	5	20.83	1	20	Untypable
O ₁₁₁ :k ₅₈	4	16.66	1	20	Enteropathogenic
O ₁₁₄ :k ₉₀	2	8.33	-	-	Enterotoxigenic
O ₇₈ :k ₈₀	2	8.33	-	-	Enterotoxigenic
O ₁₂₄ :k ₇₂	2	8.33	1	20	Enteroinvasive
O ₁₂₈ :k ₆₇	1	4.16	-	-	Enterotoxigenic
O ₂₆ :k ₆₀	1	4.16	-	-	Enterohaemorrhagic
Total	24	100	5	100	

DISCUSSION

Ice cream is the most popular frozen dairy products widely used all over the world due to its high nutritive value and its palatability to consumers at all ages. However, it is worth to mention that ice cream may constitute a serious public health hazard due to its contamination with a variety of pathogenic microorganisms which may gain entrance to ice cream through using inferior quality raw material, insufficient heat treatment of milk or contaminated equipment used for its preparation and distribution.

Results reported in Table (1), reveal that the incidence of different Coliforms organisms could be isolated in variant percentages. These organisms were identified in raw milk, small scale ice cream and large scale ice cream as *E. coli* 96%, 20% and 0%, *Klebsiella oxytoca* 20%, 8% and 0%, *Klebsiella pneumoniae* 8%, 4% and 0%, *Enterobacter geroviae* 8%, 4% and 0%, *Enterobacter agglomerans* 4%, 0% and 0% and *Enterobacter aerogenes* 4%, 4% and 0%, respectively. Similar microorganisms could be isolated by El-Bassiony *et al.* (1985), El-prince and Hussein (2000) and Mansour *et al.* (2000).

Pseudomonas aeruginosa could be isolated from raw milk, small scale ice cream and large scale ice cream samples at varying percentages 44%, 20% and 12%, respectively (Table 1). Similar results of raw milk samples were recorded by Aly and Zaki (2001) with a rate of 48.57%. Comparatively, higher than those reported by Khalil (1992) and Madeha and Zaki (2003) with incidence of 17.03% and 16.67%, respectively. On the other hand, the obtained prevalence of small and large scale ice cream were lower than those detected by Mansour *et al.* (2000) with a

rate of 29.4% and 20%, respectively. The ability to produce extracellular hydrolytic enzymes (Proteases, Lecithinases, and Lipases) is common to *P. aeruginosa* (Madeha and Zaki, 2003). Those products play some role in its pathogenicity (Seddik *et al.*, 1987).

Yersinia enterocolitica isolated from the examined small scale ice cream with incidence (4%) Table (1)., similar finding have been reported by Mira *et al.*, (2001) was isolated *Yersinia enterocolitica* from small scale ice cream with incidence of 4%. Lower incidence was recorded by El-Prince and Hussein (2000) with a rate of 1%. Moreover, *Yersinia enterocolitica* could be isolated from 4 (16%) of the examined raw milk samples. Nearly similar results were obtained by Bahout and Moustafa (2004) with a rate of 14% and comparatively higher than those reported by Mercado and Ibanez (1986) and Mira *et al.* (2001) with incidence of 5.5% and 8% in finished product is most likely due to post pasteurization contamination. Also contamination of ice cream samples obtained from street vendors with species of Enterobacteriaceae could be taken as an index of fecal contamination and could be attributed to the unsanitary practices, poor hygienic quality of ingredients used and/or absence of pasteurization during manufacturing processes (Hussein and Ahmed, 2002).

Aeromonas hydrophila could be isolated from 60% of the examined raw milk samples. This result was higher than those reported by Abdel-Khalek (1997), Mira *et al.*, (2001) and Nashwa and Isis (2001) with a rate of 40%, 36% and 25%, respectively. On the other hand, *A. hydrophila*, *A. caviae* and *A. sobria* were recorded to be 60%, 40% and 28% of the examined milk samples which are lower than that reported by E-Shorbagy and Al- Ganzoury (2002) with a rate of 75%, 50% and 37.5%, respectively. The higher incidence of *Aeromonas* species in raw milk could be a result of bad sanitary measures. *A. sobria* was more virulent in the cytotoxic activity than the *A. caviae* and *A. hydrophila* (Janda and Abott, 1998). Table 1 indicates that *A. hydrophila*, *A. caviae* and *A. sobria* were detected in 16%, 20% and 8% of the examined small scale ice cream, respectively. The obtained results are in a agreement with that reported by Ahmed *et al.* (2001) with incidence of 17.3%, 22.7% and 6.7%, respectively. Strains of *Aeromonas species* can cause gastroenteritis where some individuals and consumers of ready to eat foods in Italy, are regularly exposed to many genetically distinct strains of *A. hydrophila*, *A. caviae* without evident signs of malaise. Therefore, few of these strains if any likely to be pathogenic (Villari *et al.*, 2000).

Staph. aureus could be detected in raw milk with incidence of 12%. The obtained results are lower than that reported by Aisha *et al.*, (2002) with incidence of 64%. While, *Staph. aureus* could be isolated in small scale ice cream samples with incidence of 4%. This result was agree to Mansour *et al.* (2000) with a rate of 4%.

Enterococci species could be isolated from examined raw milk and (small and large scale) ice cream samples with incidence of 8%, 4% and 4%, respectively. The obtained results of small and large scale ice cream are nearly similar results recorded by Mansour *et al.* (2000) with incidence of 4% and 2%. Although microbial growth doesn't occur in frozen foods held below -10°C, large numbers of proteolytic enzymes remain active at the usual storage temperatures for frozen products, consequently the deterioration in their quality during the frozen storage will be occurred. Therefore, freezing will be slow but not arrest the development of enzymatic spoilage after it has begun (ICMSF, 1980).

The data recorded in Table (2) recorded the Psychrotrophic, Coliform, Staphylococcus, and Enterococci counts. Nearly similar counts were reported by El-Shorbagy and Al-Ganzoury (2000); Mansour *et al.* (2000) and Aly and Zaki (2001).

The results recorded in Table (3) showed the most prevalent serogroups of entero-pathogenic *E. coli* revealed from the examined raw milk could be identified as O₁₁₉: K₆₆; O₁₁₂: K₆₆; O₁₁₁: K₅₈; O₁₁₄: K₉₀; O₇₈: K₈₀; O₁₂₄: K₇₂; O₁₂₈: K₆₇ and O₂₆: K₆₀, with the frequency percentages of 29.16, 20.83, 16.66, 8.33, 8.33, 8.33, 4.16 and 4.16, respectively. In ice cream (small scale) Table (3), 3 strains of enteropathogenic *E. coli* serotypes were belonged to: O₁₁₂: K₆₆; O₁₁₁: K₅₈ and O₁₂₄: K₇₂, with the same frequency percent (20%), but two strains were belonged to O₁₁₉: K₆₆, with percent of (40%). These isolated serovars were belonged to Enteropathogenic *E. coli* (EPEC) as O₁₁₁, O₁₂₈. Enterotoxigenic *E. coli* (ETEC) O₁₁₄ and O₁₂₈. Enterohaemorrhagic *E. coli* (EHEC) as O₂₆ and O₁₁₁. Enteroinvasive *E. coli* (EIEC) as O₁₂₄ (Abram, 1995), who added that *E. coli* O₁₁₂ and O₁₁₉ serovars caused epidemic children enteritis. The same findings could be detected by (El-Bagoury and Hammad, 2004; Gad El-Said *et al.*, 2005; Hassan and Abosrea, 2005).

Pathogenic *E. coli* is one of the most significant groups of bacteria causing diarrhoea and extra-intestinal infections in humans and domestic animals. Five distinct groups of the organisms have been described associated with enteric disease of human. These include the toxin-producing strains, enterotoxigenic, enterohaemorrhagic and

enteroinvasive and the non-toxin producing strains, enteropathogenic and enteroinvasive *E. coli*. These strains have been proposed as contributors to enteric and extra-intestinal disease in both human and animals (DeRycke *et al.*, 1990; Al-Hawary, 2005).

The public health importance of enteropathogenic *E. coli* in gastroenteritis was reported by Tulloch *et al.*, (1973). A milkborne outbreak of *E. coli* enteritis among children has been reported in USSR (Matsievskii *et al.*, 1971). Enteropathogenic *E. coli* (EPEC) which were an important cause of infantile diarrhoea were originally incriminated epidemiologically as pathogens for infants and young children and Enterohaemorrhagic *E. coli* (EHEC) a cause of haemorrhagic colitis and haemolytic uremic syndrome (Levine, 1987).

Environmental contamination, bad hygienic measures during milking and transportation are the main sources of raw milk contamination which must be diminished as possible. Sanitary precautions in connection with handlers, utensils and surroundings during milking and processing must be adopted. Adequate cooling of raw milk, as well as efficient measures to preclude the multiplication of the organisms during preparation, service and storage of dairy products must be applied.

Although the results obtained show lower contamination rates of pathogenic microorganisms in large scale ice cream than small scale ice cream, both of them constitute a high-risk hazard to consumers. The finding of the study warrant the need to undertake safety measures to avoid potential threats and apply educational programs for both small and large scale ice cream producers about the risk of contamination, how to prevent it and how to apply strict hygienic measures during production, storage and distribution of ice cream. Moreover, regulation of small scale producing ice cream should be a part of a strategy to enhance producing of save and high quality ice cream in Alexandria city.

It is clearly evident from the previous results, that ice cream samples of street vendors were of inferior quality. Therefore, using of high quality raw ingredients, efficient heat treatment, proper cleaning and sanitation of equipment as well as good hygienic measures during manufacturing, handling, storage, transportation and distribution of ice cream are necessary to prevent its contamination and safeguard consumers against infections.

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