Dept. of Food Hygiene.
Fac. Vet. Med., Assiut University

OCCURRENCE OF ENTEROBACTERIACEAE IN ICE CREAM WITH SPECIAL REFERENCE TO SALMONELLA SPECIES

(With 3 Tables and 1 Figure)

By

ENAS EL-PRINCE and ASMAA A.A. HUSSEIN*
*: Animal Hygiene & Zooonoses Dept., Fac. Vet. Med., Assiut University
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تواجد الميكروبات المعوية في الآيس كريم مع إشارة خاصة لميكروب
السالمونيلا

إيناس البرنس ، أسماء حسين

يعتبر الآيس كريم من منتجات الألبان الواسعة الانتشار السهلة الهضم، ذات القيمة الغذائيـــة العالية، و الذي ينتشر تداوله بين فئات كثيرة و أعمار مختلفة، و يتم إنتاج الآيس كريم فـــي مصر من عدة مصادر منها الباعة الجائلين و هذا النوع يكثر إنتاجه في المنــاطق الشـــعبية و المدن الصغيرة و القرى. و تحت ظروف الإنتاج و كذلك التوزيع و التداول قـــد يتعـــرض الآيس كريم للتلوث بالعديد من الميكروبات مثـــل البكتريـــا المعويـــة، و منـــها ميكـــروب السالمونيلا، و التي تشكل خطورة لا يستهان بها على صحة المستهلك لذلك أجريـــت هـــذه الدراسة و التي شملت فحص مائة عينة من الآيس كريم المنتج من الباعة الجائلين بالمناطق الشعبية خلال أشهر الصيف و ذلك لمعرفة مدى تواجد ميكروب السالمونيلا و الميكروبـــات المعوية الأخرى *Enterobacteriaceae* باستخدام نوعين مـــن *Enrichment broth* وهي *selenite F. broth* و *Rappaport-Vassiliadis* ثم عزلها من كل منهما علـــى مستبتتين و هي *Brilliant Green* و *XLD*. و قد أثبـــتت النتـــائج وجـــود ميكـــروب السالمونيلا في ٤ عينات بنسبة ٤% و التي تم تصنيفها إلى S.ohio بنســـــبة ٣ % و *S. typhimurium* بنسبة ١ % و رغم أن هذه النسبة قليلة إلا أنها قد تشكل خطورة على صحة الإنسان حيث أنها قد تسبب في حدوث مرض السالمونيلا و الذي يعد من الأمـــراض المشتركة و الذي ينتشر حيث الظروف الصحية و البيئية السيئة، و يعتبر تلوث الغذاء الآدمي بهذا الميكروب من أسباب انتشار المرض بين الآدميين خاصة إذا كان الطعام مـــن مصـــدر حيواني. بالإضافة إلى ذلك قد أمكن عزل الميكروبات المعوية الآتيـــة: الكلبيســـيلا اوكســـى توسي، و كلبيسيلا نومونيا، انتيروباكتر جرجينـــا، انتيروبـــاكتر اجلومـــيرانز، انتيروبـــاكتر ايروجينس، سيتروباكتر دايفرسز ، سيدوموناس سيبيشيا علاوة على اليرسينيا انتيروكولينكـــا بالنسبة الآتية: ١٠، ٣، ٥، ١، ١، ٦، ٢، ١ % على التوالي. و بمقارنـــة قـــدرة الأوســـاط

السائلة و الصلبة على عزل هذه الميكروبات وجد أن أفضلها هي استخدام XLD مع R.V.
والتي كانت معنوية عند مستوى ٠,٥، أو أقل. هذا و قد تحت مناقشة الشروط الصحية الواجب
اتخاذها لإنتاج أيس كريم ذو جودة غذائية عالية تتفق و المواصفات الصحية و خـــالي مـــن
الميكروبات الممرضة التي تؤثر على صحة المستهلك.

SUMMARY

One hundred random samples of ice cream were collected from different street vendors in Assiut city and examined for the prevalence of Salmonella species and other Enterobacteriaceae using two selective enrichment broths and two selective media. Salmonella spp. could be detected in 4% of the examined samples with frequency distribution of 12.12% of the total isolated strains. *S. ohio* and *S. typhimurium* were isolated in percentages of 3 and 1%, respectively. The most frequently encountered enterobacteriaceae isolates were *Klebsiella oxytoca* (10%), *Klebsiella pneumoniae* (3%), *Enterobacter gergoviae* (5%), *Enterobacter agglomerans* and *Enterobacter aerogenes* (1%), *Citrobacter diversus* (6%), *Pseudomonas cepacia* (2%) and *Yersinia enterocolitica* (1%). Combination of Rappaport-Vassiliadis enrichment broth and Xylose Lysine Desoxycholate agar (XLD) as a selective medium was superior when compared with the other used media. The public health importance of the isolated species as well as suggestive measures to improve the quality of such product were discussed.

Key words: *Occurrence – Enterobacteriaceae - ice cream solmonella species.*

INTRODUCTION

Ice cream is considered to be one of the most popular frozen milk products consumed widely in our country and all over the world. It is highly appreciable not only by adults and children, but also by sick and convalescent persons suffering from irritation and infection of mouth and throat (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 (Bryan, 1983). These

additives may contribute to food poisoning outbreaks as salmonellosis or gastroenteritis in human beings or other bacterial problems either by adding microflora or by creating conditions favourable for the growth and survival of bacteria (Sharma & Joshi, 1992, Eley, 1996 and Kumari et al., 1996).

Among the pathogens causing foodborne diseases, Salmonella serovars account for numerous cases. *Salmonella typhimurium, S. enteritidis* and *S. typhi* are the major Salmonella serovars causing salmonellosis and human gastrointestinal disease (Millemann et al., 1995). They are ubiquitous in nature and continue to be a major concern for the dairy industry because these bacteria have been implicated in recent outbreaks of human salmonellosis. Furthermore Salmonellosis is one of the most commonly occurring zoonotic disease in most countries . The symptoms of this illness usually appear 12 to 36 hs after eating contaminated food and characterized by sudden onset of abdominal pain, diarrhoea, nausea, vomiting, chills and fever. Dehydration, headache and prostration also may occur. Moreover, the severity and duration of illness depend on the type of Salmonella species, the amount of food consumed and the susceptibility of the individual (El-Gazzar and Marth, 1992). Also, salmonellosis cause considerable morbidity, mortality and economic burdens and are severe in the very young people, the elderly and the immunocompromised. Current trends indicate that the incidence of human salmonellosis is increasing and remains one of the most commonly occurring foodborne pathogens both nationally and internationally (Bean and Griffin, 1990). Numbers of outbreaks and individual cases are most frequently associated with food products of animal origin including poultry, eggs, raw eggs-based foods and meat (Cohen and Tauxe, 1986, FAO, 1989, Rice et al. 1997, Bailey, 1998, Fedorka-Cray et al., 1998 and Seo et al., 1999).

In addition, ice cream has been incriminated as a transmittor of other pathogenic microorganisms. *Aerobacter, Klebsiella, Micrococcus, E. coli, Serratia, Proteus, Pseudomonas, Providencia, Yersinia* and *Citrobacter species* were the most predominant enterobacteriaceae previously isolated by El-Bassiony and Abou-Khier (1979), Saad (1983), El-Bassiony et al. (1985), Riad (1988), Saleh (1989), Mahmoud (1993), 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).

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Unfortunately, the sanitary conditions of street vendors ice cream is still underway due to the absence of adequate hygiene and regular microbiological control as well as lacking of standards and specifications for methods of manufacturing and examination. When ice cream is produced at home it can easily become a vehicle for many foodborne pathogens. *Salmonella typhimurium* is of special importance due to its ability to cause major dairy-related outbreaks in the United States and elsewhere (Ryser, 1998). These observations prompted us to assess the prevalence of pathogenic organisms, particularly *Salmonella species* and other *Enterobacteriaceae* in ice cream sold in Assiut city by street vendors.

MATERIAL and METHODS

Collection of samples:

One hundred samples of ice cream were collected at random from street vendors in Assiut city during summer, between the months of May and September. Samples were obtained in their containers as sold to the public and transferred promptly to the laboratory in ice-box where they were prepared and examined for the presence of *Salmonellae* and other *Enterobacteriaceae species*.

Preparation of samples:

Samples of ice cream were left to be melted in a thermostatically controlled water bath at 44°C for not more than 15 minutes. Each sample was thoroughly mixed before being examined (A.P.H.A. 1985).

Microbiological examination:

The standard procedures for *Salmonella* and *Enterobacteriaceae* isolation were done according to the ISO Standard 1993.

A weight of 10 g of ice cream was added to 90 ml lactose broth (Oxoid CM 137) in a sterile stomacher bag to be homogenized in a Stomacher (Lab. Blender 400 Seward) for 2 min, and then allowed to stand for 1 h at room temperature, securely sealed. Samples were incubated for 24h at 37°C, Then 10 ml portions of the pre-enrichment media were transferred to both 10 ml of selenite F broth (Difco B 275) and 10 ml Rappaport-Vassiliadis R10 broth which were incubated for 24 h at 37°C and 43°C, respectively. Loopful of each selective broth was streaked onto plates of XLD (Xylose Lysine Desoxycholate agar, Oxoid CM 469) and BGA (Brilliant Green agar, Difco B 285). After incubation for 24h at 37°C, presumptive colonies of *Salmonellae* or *Enterobacteriaceae* species were transferred to TSI (Triple Sugar Iron

agar, Oxoid 277) and LIA (Lysine Iron agar. Oxoid CM 381) slants. Then the suspected strains were identified by the API 20 E system (BioMerieux, France). The serological typing of isolates presumed to be salmonella were carried out.

Statistical analysis:

Data were expressed as the mean ± S.D. for the relationship between the used selective enrichment broth and media and the isolated strains. Analysis of variance (ANOVA) for all treatments was done and differences between them were analyzed by least significant difference (LSD) using PC-stat computer program. Results were considered significant at P<0.05 or less.

RESULTS

Obtained results were recorded in Tables 1-3

Table 1: *Incidence of Salmonellae and other Enterobacteriaceae species in the examined ice cream samples.*

| Isolates	Positive samples	
	No./100	%
Salmonella species		
Sal. Ohio	3	3
Sal. Typhimurium	1	1
Klebsiella species		
Kleb. Oxytoca	10	10
Kleb.pneumoniae	3	3
Enterobacter species		
Enter. Gergoviae	5	5
Enter. Agglomerans	1	1
Enter. Aerogenes	1	1
Citrobacter species		
Citro.diversus	6	6
Pseudomonas species		
Pseud.cepacia	2	2
Yersinia species		
Yer.enterocolitica	1	1

Table 2: *Frequency distribution of Salmonellae and other Enterobacteriaceae species isolated from ice cream samples.*

Isolates	Isolated strains	
	No.	%
Salmonella species		
Sal. Ohio	3	9.09
Sal. Typhimurium	1	3.03
Klebsiella species		
Kleb. Oxytoca	10	30.30
Kleb.pneumoniae	3	9.09
Enterobacter species		
Enter. Gergoviae	5	15.15
Enter. Agglomerans	1	3.03
Enter. Aerogenes	1	3.03
Citrobacter species		
Citro.diversus	6	18.18
Pseudomonas species		
Pseud.cepacia	2	6.07
Yersinia species		
Yer.enterocolitica	1	3.03
Total	33	100.00

Table 3 & Fig. 1: *The statistical relationship between the used selective enrichment broth and media and the isolated strains.*

The used selective broth and media	Mean ± S.D.	P<
- Rappaport-Vassiliadis broth with XLD	2.17 ± 0.75^a	0.05
- Selenite F broth with XLD	2.0 ± 0.89^b	
- Selenite F broth with BGA	1.83 ± 0.41^{ab}	
- Rappaport-Vassiliadis broth with BGA	1.17 ± 0.41^a	

Means in the same row with the same superscript letters are not significantly different.

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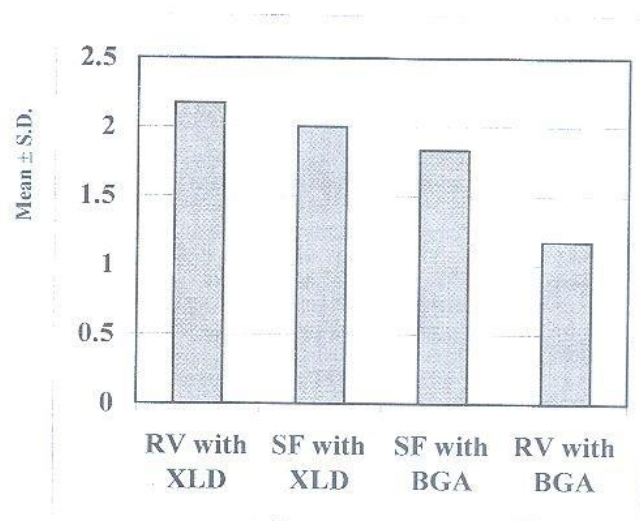
RV with XLD= Rappaport-Vassilidis broth with XLD
SF with XLD= Selenite F broth with XLD
SF with BGA= Selenite F broth with BGA
RV with BGA= Rappaport-Vassilidis broth with BGA

DISCUSSION

In recent years, the examination of dairy products for the presence of *Salmonellae* and other *Enterobacteriaceae species* has been recommended which gives indication upon the hygienic conditions of production.

Results in Table 1 reveal that the incidence of *Salmonella species* in the examined ice cream samples was found to be 4 % with frequency distribution of 12.12% of total isolates as shown in Table 2. Salmonella isolates were differentiated serologically into *S. ohio* (3%) and *S. typhimurium* (1%). These results substantiate those obtained by Sharma and Joshi (1992) as they could isolate *Salmonella species* from ice cream samples in a percentage of 3.92%, whereas *S. typhimurium* was isolated from one sample. *S. typhimurium* was recovered from stool specimens of 4 patients in Georgia, where home made ice cream had been served at party as reported by Bryan (1981) and Centers for Disease Control (1981) and Ryser (1998). Moreover, the Communicable Disease Surveillance Centre (London, England) received reports of three family outbreaks of *Salmonella enteritides* PT4 associated with consumption of homemade ice cream from fresh eggs, 7 people were affected. It has been also reported that the organism, which probably found in the eggs, was isolated from ice cream in all three outbreaks (Anonymous, 1991).

Excretion of *S. typhimurium* from cow's udder has been reported by Giles and King (1987). Such milk if consumed raw or used for ice cream manufacturing may cause food poisoning. The presence of Salmonella in ice cream also could be attributed to the use of contaminated water and other ingredients during their preparation (Sharma and Joshi, 1992).

Conversely, Ahmed (1980), Yanng et al. (1991), Cotton and White (1992), Mahmoud (1993) and Abdel-Haleem (1995) recorded that *Salmonella* organisms failed to be detected in the examined ice cream samples. The absence of this organism, in spite of the unhygienic practices employed in milk or ice cream production, could be attributed to its escape detection in case of small number or due to its inhibition by other fast growing organisms (Sharma and Joshi, 1992). Methodology may also have influenced recovery of Salmonellae. However, use of different culture media can dramatically affect the outcome of results (Fedorka-Cray et al., 1995 and 1998).

According to the results presented in Table 1, it is evident that different *Enterobacteriaceae* organisms could be isolated in variant

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percentages. These organisms (29 isolates) were identified as *Klebsiella oxytoca* (10%), *Klebsiella pneumoniae* (3%), *Enterobacter gergoviae* (5%), *Enterobacter agglomerans* (1%), *Enterobacter aerogenes* (1%), *Citrobacter diversus* (6%), *Pseudomonas cepacia* (2%) and *Yersinia enteroclitica* (1%). The frequency distributions of these microorganisms were 30.30, 9.09, 15.15, 3.03, 3.03, 18.18, 6.07 and 3.03% respectively as recorded in Table 2. Similar isolates could be recovered from ice cream at variable incidences by several investigators (El-Bassiony and Aboul-Khier 1979, El-Bassiony et al., 1985, Riad 1988, Saleh, 1989; Mahmoud 1993; Abdel-Haleem 1995; Kumari et al., 1996 and Mansour et al., 2000). The public health importance of *Enterobacteriaceae* it has been well documented that these organisms implicated in various human infections including enteritis, various respiratory diseases, urinary tract infections, infections of bones, joints, nails, skin and wounds as well as some cases of food poisoning outbreaks (Eley, 1996).

Pseudomonas species could be isolated from ice cream samples examined by Grover et al. (1993), Shanker et al. (1994), and Mansour et al. (2000) in higher percentages. Concerning *Yersinia enterocolitica*, higher incidence were recorded also by various investigators ranged from 4.5% to 22% (Delmas and Vidon 1985, Boer et al., 1986, Moustafa, 1990, El-Kholy, 1992 and Pritchard et al., 1995). The presence of these organisms in finished product is most likely due to post pasteurization contamination. Furthermore it follows from the foregoing that 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.

The obtained results summarized in Table 3 verify that the combination of Rappaport-Vassiliadis enrichment broth and XLD medium was found to be significant (P< 0.05) procedures in terms of percentages of positive samples. These findings are in agreement with the results of previous studies on the superiority of motility enrichment for Salmonella detection from naturally contaminated products (De Smedt and Bolderdijk, 1987).

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

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cream are necessary to prevent its contamination and safeguard consumers against infections.

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