

Animal Health Research Institute, Dokki,
Port -Said Laboratory for Food Hygiene

OCCURRENCE OF ESCHERICHIA COLI O157:H7 IN SOME DAIRY PRODUCTS AT PORT -SAID CITY MARKETS

(With 4 Tables)

By

AMANY M. SHALABY and MARCEL F. GALAB

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**تواجد الميكروب القولوني O157:H7 فى بعض منتجات الألبان المتداولة
فى أسواق مدينة بورسعيد**

أمانى محمود شلبى ، مارسيل فخرى جلاب

لا شك أن منتجات الألبان تلعب دورا هاما فى حياتنا. ويحتل الايس كريم شعبية كبيرة حيث يقبل عليه الكبار والصغار خاصة فى فصل الصيف. أما الزبادى فتعتبر من أكثر منتجات الألبان استهلاكها وذلك لقيمتها الغذائية والصحية. ولما كانت منتجات الألبان وسط جيد لنمو الكثير من الميكروبات، لذا فإنها تلعب دورا خطيرا فى نقل الكثير من الميكروبات للإنسان ومنها ميكروب الاشيرشيا كولاي O157 : H7 حيث يعتبر هذا الميكروب من اخطر الميكروبات الممرضة وذلك لأنه يمكن إحداث العدوى بجرعة هى الأقل بين مثيلاتها من الميكروبات وقد تؤدي إلى حدوث فشل كلوى وخاصة فى الأطفال لذلك تهدف هذه الدراسة إلى عزل وتقييم انتشار الميكروب القولونى O157 : H7 إلى جانب العترات الأخرى من الميكروب القولونى فى عينات الايس كريم والزبادى التى تباع فى السوبر ماركت، وقد تم تجميع خمسين عينة من كل منتج. وقد أسفرت التحاليل عن وجود ميكروب الاشيرشيا كولاي بنسب 58، 42% فى عينات الايس كريم والزبادى على التوالى. كذلك تم عزل وتصنيف العترة O157 : H7 من اجمالى عدد العينات الإيجابية لكل منتج. كما تم تصنيف عترات أخرى من العينات الإيجابية وهى O55, O26, O111, O119, O112 هذا وقد تم مناقشة خطورة وجود العترة O157:H7 الى جانب العترات الأخرى على الصحة العامة.

SUMMARY

A total of one hundred random samples, fifty from ice- cream and 50 from yoghurt were collected from Port-Said markets. Samples were examined to isolate and evaluate the prevalence rate of *E. coli* O157:H7 and other serotypes. *E. coli* was detected in ice-cream and yoghurt samples at a rate of 58 and 42 %, respectively. 9(18 %) and 5(10 %) out of 50 ice- cream and yoghurt samples, respectively were found to be contaminated with *E. coli* O157: H7. A total of 66 *E. coli* isolates

recovered from positive samples were identified to serogroups, O112(40.9%), O119(31.8%) O111(21.2%), O26(3 %) and O55(1.5%). The majority of *E. coli* serotypes recovered from the examined samples showed hemolytic activity. The public health significance of the isolated serogroups and consumer's safety were discussed.

Key words: Dairy products, ice-cream, yoghurt, *E. coli*

INTRODUCTION

Milk and dairy products are subjected to contamination with several types of microorganisms from different sources, such contaminants may render the milk and its products unsafe to use and expose the consumers to risk of infection. *E. coli* as a group of bacteria found in the intestines of human, animals and birds. Enterohaemorrhagic *Escherichia coli* (EHEC) was first identified as a human pathogen in 1982, when *E. coli* of serotype O157: H7 associated with two outbreaks of Haemorrhagic Colitis (HC) in the USA (Riley *et al.*, 1983). Although, there are several strains and serogroups of *E. coli* have been identified as HC, *E. coli* serotype O157: H7 is the predominant cause of HC associated disease in many countries around the world (Thomas *et al.*, 1993, Waters *et al.*, 1994 and Sharpe *et al.*, 1995).

Escherichia coli O157: H7 emerged as one of the most important foodborne bacterial pathogens within the last 20 years. The organism has a low infective dose as cfu/ml or/gm (Doyle *et al.*, 1997) and causes haemorrhagic colitis which is occasionally complicated by haemolytic uremic syndrome (Morrison *et al.*, 1986, Neil *et al.*, 1987 and Kelly *et al.*, 1990). Moreover, the bacterium can produce a variety of clinical symptoms including mild to sever bloody diarrhea and thrombotic thrombocytopenic Purpura haemorrhagica (Dean-Nystrom *et al.*, 1999, Todd and Dundas, 2001 and Meichtri *et al.*, 2004). The main virulence factor of EHEC bacteria is the production of verotoxins either verotoxins (VT1) and/or (VT2) so it's called Verocytotoxic *E. coli* (Kaper, and O'Brien, 1998 and Normanno *et al.*, 2004). *E. coli* O157: H7 can survive in the stomach at pH level of 3 for up to 5 hrs before passage to the intestinal tract (Price *et al.*, 2004). These characteristics provide a competitive edge for *E. coli* O157: H7 in ready-to-eat foods. *E. coli* O157: H7 can contaminate a variety of foods resulting in *E. coli* O157: H7 associated illness (Foley *et al.*, 2004). However many epidemiologic and microbiologic investigations on large epidemic outbreaks have implicated the consumption of raw milk (Benkerroum *et al.*, 2004) and

raw milk products including yoghurt and ice -cream (Cheng and Chou., 2001; Oksuz *et al.*, 2004). The organism may get access to milk and milk products through fecal contamination as well as post pasteurization contamination (Murinda *et al.*, 2002). In addition yoghurt and ice-cream are the most likely dairy products, which are preferred by adult and young. Ice- cream is a food commodity consumed widely during summer and is considered the major dairy product that dominates interest of large segments of population (Anuranjini *et al.*, 2008). On the other hand yoghurt is consumed by a wide cross section of people throughout the world, has an established market as a functional therapeutic food. It is also considered more digestible than ordinary milk, it is usually eaten for prevention and treatment of several disorders and particularly recommended for sick and convalescent people (Gilliland, 2000).

World Health Organization (1988) emphasized the importance of examining the behavior of pathogens during preparation of food products as a first step to control their growth .Therefore the aim of the present study was to determine the prevalence of *E. coli* O157 and other serotypes in ice- cream and yoghurt sold in Port-Said markets.

MATERIALS and METHODS

A total of one hundred random samples, fifty from each of ice-cream and yoghurt were collected from Port-Said markets. Ice-cream samples were thawed in their original containers in a refrigerator at 2-5°C. Twenty five gm of each of ice- cream and yoghurt samples were transferred to 225 ml of tryptone phosphate broth as a pre-enrichment fluid and thoroughly mixed then incubated for 4-6 hr. at 37°C. (Mehlman and Lovett, 1984). Two Mossel's enteric enrichment broth (E.E), tubes (10ml) were inoculated with 1ml from the pre-enrichment tryptone phosphate broth medium. One tube was incubated at 44°C for 24 hr, permit the growth of pathogenic *E. coli*, other than serovar O157:H7. The other tube was incubated at 37°C to permit the growth of *E. coli* O157 and other serovars which are unable to grow at higher (44°C) temperatures. (Mehlman and Romero, 1982) Then the two tubes were incubated for 24 hr. One loopful from each of the selective enrichment cultures incubated at 37°C & 44°C was inoculated onto MacConkey -Sorbitol agar (MACS) and Eosin Methylene Blue agar (EMB), then incubated at 37°C for 24hr. Suspected colonies: white and colorless with smoky center 1-2mm diameter, sorbitol negative colonies on MACS and metallic green colored smooth sides colonies on EMB, were picked up and subcultured onto nutrient agar plates and incubated

at 37°C for 24hr. The purified colonies were picked up and streaked onto slope nutrient agar for morphological and biochemical tests according to (Cruickshank *et al.*, 1975; Quinn *et al.*, 2002). The isolates were identified serologically by the slide agglutination test using diagnostic polyvalent and monovalent *E. coli* O157 antisera and H7 antisera. *Escherichia coli* antisera (Denka Seikenco., Ltd, Tokyo, Japan), following the manufacturer's specification.

Detection of haemolysin: (Beutin *et al.*, 1989).

Isolated *E. coli* were inoculated onto blood agar containing sheep blood 5% and incubated at 37°C for 24 hr. Positive haemolysin production was detected.

RESULTS

Table 1: Prevalence of *E. coli* in the examined samples (n=50 of each)

| Milk products | Positive samples | % of positive samples |
|---------------|------------------|-----------------------|
| Ice- cream | 29 | 58 |
| Yoghurt | 21 | 42 |

Table 2: Prevalence of *E. coli* O157:H7 among the examined samples (n=50 of each).

| Milk products | Positive samples for serovar O157:H7 | |
|---------------|--------------------------------------|----|
| | No | % |
| ice-cream | 9 | 18 |
| Yoghurt | 5 | 10 |

Table 3: Serovars of *E. coli* isolates (other than O157: H7) recovered from the examined milk products samples.

| Serovar | Total no. of isolates | O112 | | O119 | | O111 | | O26 | | O55 | |
|-----------|-----------------------|------|------|------|------|------|------|-----|-----|-----|-----|
| | | No | % | No | % | No | % | No | % | No | % |
| Ice-cream | 37 | 15 | 40.5 | 11 | 29.7 | 8 | 21.6 | 2 | 5.4 | 1 | 2.7 |
| Yoghurt | 29 | 12 | 41.4 | 10 | 34.5 | 6 | 20.7 | - | - | 1 | 3.4 |
| Total | 66 | 27 | 40.9 | 21 | 31.8 | 14 | 21.2 | 2 | 3 | 2 | 1.5 |

Table 4: Haemolytic activity of *E. coli* isolates recovered from the examined milk products samples.

| <i>E. coli</i> serovars | No. of isolates | Activity | |
|-------------------------|-----------------|----------|------|
| | | No. | % |
| O157:H7 | 17 | 17 | 100 |
| O112 | 27 | 27 | 100 |
| O119 | 21 | 21 | 100 |
| O111 | 14 | 12 | 85.7 |
| O26 | 2 | 2 | 100 |
| O55 | 2 | 2 | 100 |
| Total | 83 | 81 | 97.5 |

DISCUSSION

E. coli strains are important cause of diarrheal diseases in the world and remain one of the major public health problems of children and young infants (Levine *et al.*, 1986). Several well-documented outbreaks due to enterohaemorrhagic *E. coli* (EHEC) infection have been linked to consumption of milk and its products (Champman and Wright, 1993; Upton and Coia, 1994). Although more than 60 *E. coli* serotypes produce shiga-like toxins, (SLTS) serotype O157: H7, is the predominant pathogen in the EHEC group and the one associated most frequently with human infection worldwide (Karmali, 1989).

The present investigation was carried out to evaluate the prevalence of *E. coli* O157: H7 and other *E. coli* serotypes among selected types of milk products. The overall incidence of *E. coli* in ice-cream and yoghurt samples was recorded in Table 1; *E. coli* was recovered from ice-cream samples at a rate of 58%. El-Essawy and Riad (1990) and Anuranjini *et al.*, (2008) could detect *E. coli* in 8.16 and 21.1% respectively from the examined ice-cream samples. In comparative, higher isolation rate of *E. coli* in this study may be due to contamination during handling as well as in efficiency and bad sanitary conditions during storage (Anuranjini *et al.*, 2008). On the other hand, 42% of the yoghurt samples showed positive results. These results are nearly in agreement with that recorded by Moursy (1969) who detected *E. coli* in 55% of the examined yoghurt samples. While Moustafa *et al.* (1988) isolated 25(62.5%) *E. coli* strain out of the examined 40 yoghurt samples. This contamination rate of the examined yoghurt samples indicated unhygienic practices, the fact that the major route of *E. coli* transmission is through the consumption of contaminated food, water,

person-to-person and animal to person intact (Heuvelink *et al.*, 1995; Leyer *et al.*, 1995; Reilly 1998). Moreover, milk and milk products are good media for the growth of a number of pathogens. (Erskine *et al.*, 1988).

Results of biochemical and serological identifications of sorbitol negative *E. coli* isolates, revealed that 9 (18%) out of the 50 examined ice-cream samples were found to be contaminated with *E. coli* O157:H7 (Table 2). In this respect Wilson *et al.* (1997) isolated *E. coli* O157 from 18% of the examined ice-cream samples. On the other hand, Josefa *et al.*, (2005) recorded one case of outbreak due to consuming ice-cream in the United States .The fact that ice-cream is a good source for microbial growth due to its nutrient content ,neutral pH and long storage time even though, it is stored in a frozen state. (Kanbakna *et al.*, 2004). The results in Table 2 showed that 5(10%) out of the 50 examined yoghurt samples were found to be contaminated with *E. coli* O157. (Abdel -Hakiem *et al.*, 1988) could isolate *E. coli* O157: H7 from yoghurt samples. EL-Gaml, (2000) and Mohamed *et al.*, (2005) explained that yoghurt can serve as conveyors of *E. coli* O157: H7 infection despite of it is acidity because the pathogen have the ability to grow during processing and could survive for long period during cold storage. *E. coli* O157: H7 has the ability to tolerate acidic conditions of yoghurt (Lederberg, 2000) since milk fermentation usually produce anaerobic conditions within the fermented dairy products, it is thought that anaerobic growth of *E. coli* O157: H7in an acidic medium, like yoghurt, results in the development of acid tolerance (Cheng and Kaspar, 1998). This may be due to production of RPOs-regulated protein or arginine decarboxylase, which is also induced during anaerobic growth at acidic pH (Auger *et al.*, 1989; Lin *et al.*, 1996).

Regarding other serogroups of isolated *E. coli* as shown in Table 3 O112and O119 were the most prevalent serotypes recovered from the examined samples of ice-cream and yoghurt followed by O111.O26 which could be detected in ice-cream samples only. Two isolates of the serovar O55 were isolated from both ice-cream and yoghurt samples .Most of the isolated serotypes are usually associated with many cases of food borne outbreaks and multiple sporadic cases in different parts of the world. Anathan and Subramanian (1995) could isolate *E. coli* belonging to serotypes from cases of perisistent diarrhea in young children. Although *E. coli* O157was the main focus of attention, there is increasing evidence that verotoxigenic *E. coli* (VTEC) serotypes other than O157 are the cause of haemolytic uraemic syndromes (Goldwater

and Bettelheim, 1995). Furthermore, Saridakis *et al.* (1997) recorded that *E. coli* of serogroup O26 was commonly isolated from infants and calves affected with diarrhea and has been considered as one of the most important enteropathogenic *E. coli* (EPEC) and SLT producers.

Blood haemolysis is one of character of virulent *E. coli*. (Stephen *et al.*, 1985). Fourteen isolates identified serologically as *E. coli* O157: H7 were tested for haemolysis production using sheep blood agar. As shown in Table 4, all the tested isolates were haemolytic. Furthermore, the majority of *E. coli* isolates other than O157: H7 were isolated from the examined samples showed haemolytic activity. In this respect Adesiyun *et al.*, (1997) concluded that from *E. coli* isolates tested for haemolysis 13.8% were haemolytic and this could be used as phenotypic marker or virulence factors of serotypes. Meanwhile Gad EL-Said *et al.*, (2005) reported that 81.25% of isolated *E. coli* recovered from milk samples showed haemolytic activity. *E. coli* strains were found to be haemolytic is an indication contaminating of *E. coli* may result in problems for consumers. since haemolysin production has been associated with pathogenicity of *E. coli* strains.

The results of this study showed that, ice-cream and yoghurt can serve as conveyors of *E. coli* O157: H7 and some other serotypes (Cheng and Kaspar, 1998) of *E. coli* which pose a health hazard to consumers, therefore efficient heat treatment of raw milk and prevention of post-treatment contamination during processing ,storage and handling should be strictly adopted.

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