

INCIDENCE OF *E. COLI* O157:H7 IN SOME DAIRY PRODUCTS

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ABSTRACT

A total of 104 dairy product samples (33 kareish cheese, 38 yoghurt and 33 sour cream) were randomly purchased from different shops, markets and street vendors in Kafer El-Sheikh city for isolation and serological identification of E.coli serotype O157:H7. 24.2%, 21% and 42.2% of kareish cheese, yoghurt and sour cream respectively, contained E.coli. All the suspected strains were serologically identified for E.coli O157:H7, using E.coli O157:H7 kits. The organism could be detected in 3% of examined kareish cheese while could not be detected in yoghurt and sour cream examined samples. E.coli serotypes of O157:H7, O111:H4,, O26:H11, O114:H21,O86, O55:H7,, O125:H21,O126, O128:H2, and O124 were serologically identified by percentages of (12.5%, 0 and 0), (25%, 25% and 26.6%), (12.5%, 12.5 % and 13.3%), (12.5%,12.5% and 6.7%), (0 ,12.5 % and 0) , (0, 12.5 %and 6.7%) ,(0,0 and 20%), (12.5% ,0and 0), (12.5% , 25% and 20%) and (12.5%,0 and 6.7%) of the isolates of each examined kareish cheese, yoghurt and sour cream samples respectively.

Based on the obtained results in the current study, the fermented dairy products were a possible source of food poisoning caused by E.coliO157:H7.So, application of strict hygienic measures during processing of some dairy products and restriction of selling the dairy products to healthy authorized shops with high quality measures being very important.

Key words: *E.coli*, *E.coli* O157:H7, Dairy products, serological identification.

INTRODUCTION

Milk and dairy products are considered as the most perfect food for human especially for infants and mature human beings as they provide them with all nutrients required for rapid growth and prevent or reduce risks of many nutritional deficiency diseases as well (*Marshall et al., 2003*). On the other hand, dairy products have been shown to be an ideal media for growth and multiplication of microorganisms (*Wieneke et al., 1993*), improper handling and the use of improper storage temperatures are factors that contribute to the potential hazard of the dairy products (*Yusuf et al., 2013*).

E. coli is the main food poisoning microorganisms associated with raw milk products such as cheese, sour cream and yoghurt (*Verraes et al., 2015*) and was considered as an index of fecal contamination until 1950 but this perspective was changed with the discovery of pathogenic types that could cause deaths in both humans and animals (*Nataro and Kaper, 1998*).

E. coli O157:H7 is one of the foodborne pathogens of concern for the dairy industry. Raw dairy products have been identified as an important vehicle for the transmission of this pathogen (*Heuvelink et al., 1998*). *E. coli O157:H7* is responsible for bloody or non-bloody diarrhea, which may be complicated by hemorrhagic colitis and severe renal and neurological sequelae, including hemolytic uremic syndrome (HUS) (*Lynn et al., 2005*).

Therefore, the present study was undertaken to determine the prevalence of *E. coli O157:H7* and other *E. coli* in some Dairy products.

MATERIALS AND METHODS

Collection and preparation of samples:

A total of 104 dairy product samples (33 kareish cheese, 38 yoghurt and 33 sour cream) were randomly purchased from different shop, markets and street vendors in Kafer El-Sheikh City. The collected samples were transferred in ice box and examined immediately after arrival to the laboratory (Animal Health Research Institute, Kafer El-Sheikh branch).

Isolation of *E.coli* O157:H7 according to FDA, (1995):

Twenty five grams of prepared samples were added to 225ml of Enterohemorrhagic *E. Coli* enrichment broth (EEB), homogenized then incubated at 37°C for 24 hours. A loopful from the incubated broth was streaked onto Cefixime Tellurite Sorbitol Mac Conkey agar (CT-SMAC) plates and all plates were incubated at 37°C for 24 hours. Transparent, colorless with a weak pale brownish appearance (sorbitol negative) colonies were picked up on nutrient agar slopes as presumptive *E.coli* O157:H7. The purified colonies were subjected for further identification.

Biochemical identification of bacterial isolates:-

The isolated organisms were identified biochemically according to *Macfaddin, (2000)*.

Serological Identification of *E.coli* isolates:

According to *Kok et al., (1996)* the isolates were serologically identified by using rapid diagnostic *E.coli* antisera sets DENKA SEIKEN Co., Japan for diagnosis of *E.coli*.

RESULTS

Table (1): Incidence of *E. coli* O157:H7 in the examined dairy products samples:

| Type of samples | No. of examined samples | Suspected positive samples according to colonial characters | | Suspected positive samples according to biochemical identification | | Positive samples according to serological identification | |
|-----------------|-------------------------|---|--------------|--|--------------|--|-------------|
| | | No . | % | No. | % | No. | % |
| Kariesh cheese | 33 | 10 | 30.3 | 8 | 24.2 | 1 | 3 |
| Yoghurt | 38 | 12 | 31.5 | 8 | 21 | 0 | 0 |
| Sour cream | 33 | 15 | 45.4 | 14 | 42.4 | 0 | 0 |
| Total | 104 | 37 | 35.57 | 30 | 28.84 | 1 | 0.96 |

Table (2): Frequency distribution of *E.coli* serotypes other than *E.coli* O157:H7

| Types of <i>E.coli</i> | <i>E.coli</i> serotypes | Kariesh cheese isolates | | Yoghurt isolates | | Sour cream isolates* | |
|------------------------|-------------------------|-------------------------|------------|------------------|------------|----------------------|------------|
| | | No. | % ** | No. | % ** | No. | %** |
| EHEC | O157 : H7 | 1 | 12.5 | 0 | 0 | 0 | 0 |
| | O111 :H4 | 2 | 25 | 2 | 25 | 4 | 26.6 |
| | O26 : H11 | 1 | 12.5 | 1 | 12.5 | 2 | 13.3 |
| EPEC | O114 :H21 | 1 | 12.5 | 1 | 12.5 | 1 | 6.7 |
| | O86 | 0 | 0 | 1 | 12.5 | 0 | 0 |
| | O55 : H7 | 0 | 0 | 1 | 12.5 | 1 | 6.7 |
| ETEC | O125 :H21 | 0 | 0 | 0 | 0 | 3 | 20 |
| | O126 | 1 | 12.5 | 0 | 0 | 0 | 0 |
| | O128 :H2 | 1 | 12.5 | 2 | 25 | 3 | 20 |
| EIEC | O124 | 1 | 12.5 | 0 | 0 | 1 | 6.7 |
| Total | | 12 | 100 | 8 | 100 | 15 | 100 |

* One sample of sour cream represented by two isolates.

** Percent was calculated according to the number of isolates of each product.

DISCUSSION

E.coli is a common gram negative food borne enteric pathogen causing foodborne gastro-enteritis worldwide. From the results obtained in table (1) 30.3%, 31.5% and 45.4% of examined kariesh cheese, yoghurt and sour cream samples, respectively, showed the characters of *E.coli* O157:H7 on Cefixime Tellurite Sorbitol Mac Conkey agar (pale colonies) plates. After the biochemical identification of suspected isolates, 24.2%, 21% and 42.4% of examined kariesh cheese yoghurt and sour cream samples were presumed *E.coli* O157:H7. Serological identification of the suspected isolates, *E.coli* O157:H7 proved to be found in 3% of examined kariesh cheese samples, while it failed to be detected in any yoghurt and sour cream samples.

E.coli O157:H7 has been isolated from kariesh cheese in variable percentages ranged from 6.7% to 20% (*El-Awadi, 2004, El Sayed et al., 2011 and Ibrahim et al., 2015*) While, *Ibrahim and Sobeih, (2006); Tekinşen and Özdemir, (2006)* and *prencipe et al., (2010)* could not isolate the organism from any of the examined cheese samples. On the other hand, *Rahimi et al., (2011)* and *Ahmed and Shimamoto, (2014)* could not isolate the organism from yoghurt samples. While *Amany and Marcel, (2008)* and *Dehkordi et al., (2014)* isolated it with percentage 42% and 26%, respectively from yoghurt samples. The organism could be isolated in percentages varied from 6% to 20% in sour cream (*Elkosi, 2001, Eman, 2011 and Daood, 2013*).

E. coli O157:H7 has an optimum growth at pH of around 7 and is able to grow in a pH range between 4.5 and 9. Furthermore, some strains of *E. coli* O157:H7 are acid-resistant, being able to withstand pH values as low as 3. Thus, the lowest pH of cheese will not inactivate *E. coli* O157:H7 or other STEC (Shiga Toxin producing *E. coli*). Acid adaptation to the non-lethal pH in the cheese may promote greater survival of *E. coli* O157:H7 during passage through the acid environment of the stomach (*Jordan et al., 1999*), while in yoghurt *E. coli* O157:H7 growth is affected by pH and temperature of the storage. Decreasing of temperature to 4°C - 8 °C during yoghurt storage slightly affect the growth of *E. coli* O157:H7, while its low pH level affect the growth of nonpathogenic *E. coli* but *E. coli* O157:H7 was more resistant to the higher decrease in pH to level between 4 to 3.92 (*Bachroui et al., 2002*). *De Araújo and Giugliano, (2001)* attributed the presence of STEC in dairy products to the fact that milk carries a number of immune factors (principally IgA) and non-immune factors (lactoferrin and various free secretory components) that specifically hinder the adherence and subsequent proliferation of STEC on certain cell substrates. But the resistance exhibited by O157:H7 may be related to certain properties characteristic of this bacterium, not found in other STEC strains. O157:H7 has been reported to survive in refrigerated milk at temperatures ranging between 7 °C and 17 °C (*Heuvelink et al., 1998*), and may multiply even at 8 °C (*Massa et al., 1999*). Resistance to acid pH has been linked to the presence of certain genes, exclusive to the O157:H7 serotype, whose products ensure an extraordinary degree of acid tolerance (*Lin et al., 1996*). Also, the fat molecules may protect STEC during such heat treatment (*Erickson and Doyle, 2007*) allowing its survival in sour cream so the sour cream may cause health hazard as it have high fat content.

The result in table (2) showed that different *E.coli* serotypes as EHEC serotypes O157:H7, O111:H4 and O26:H11 were detected in percentage of 12.5%, 25% and 12.5% from the isolates obtained from kariesh cheese samples, while *E.coli* O157:H7 failed to be detected from yoghurt and sour cream. Other serotypes of *E.coli* (O111:H4 and O26:H11) could be isolated with percentages of 25% and 12.5% of yoghurt isolates and 26.6% and 13.3% of sour cream isolates. EPEC serotypes O114:H21 and O55:H7 were detected in kariesh cheese, yoghurt and sour cream isolates with percentages of (12.5% , 12.5% and 6.7%) and (0, 12.5% and 6.7%) respectively, while O86 was only detected in yoghurt samples with a percentage of 12.5%. ETEC serotypes O125:H21, O126 and O128:H2 could be detected in the examined kariesh cheese with percentages of 0, 12.5 and 12.5% respectively, while for sour cream isolates with percentages of 20, 0 and 20%. Concerning yoghurt isolates, O128:H2 was the only serotype of ETEC which detected by percentage of 25%. EIEC represented in O124 was detected in isolates of kariesh cheese and sour cream with percentage 12.5% and 6.7%.

The studies of *Nemr, (2005); Rey et al., (2006); Abou El-Makarem, (2009); Fadel and Ismail, (2009) and Madic et al., (2011)* investigated the distribution of other different serotypes of *E.coli* (O111:H4; O55:H7; O126; O125:H21; O26:H11; O114:H21; O128:H2 and O86) isolated from kariesh cheese. While, the study of *Dehkordi et al., (2014)* recorded the presence of *E.coli* O26:H11 in yoghurt with percentage of 12.5%.

E.coli strains were classified according to the presence of virulence factors to EHEC, EPEC, ETEC and EIEC **Holko et al., (2006)**. *Escherichia coli* cause a range of food illness as gastroenteritis and diarrhea but EHEC may also cause other type of severe illness as hemolytic uremic syndrome. One other hand, EPEC are a leading cause of infantile diarrhea in developing countries. While, Enterotoxin-producing *E.coli* (ETEC) are one of the main causative agents of travel diarrhea in adults **(Natro and Kaper, 1998)**.

Food-borne illnesses linked to non-O157 STEC, such as serotypes O26, O45, O111, O103, O145 and O121, have been increasing according to the data reported by Centers for Disease Control and Prevention (CDC) **(Hoefler et al., 2011)**.

Based on the obtained results in the current study, it was concluded that acidic fermented dairy products were a possible source of food poisoning caused by *E.coli*O157:H7 because this pathogen able to survive and grow in this products with its conditions of acidity and storage temperature.

In conclusion, high quality milk and proper condition of processing and storage should be considered in dairy product manufacturing as it is have a great role of the products contamination. So, restrict hygienic measures during processing of dairy products and restriction of its selling to healthy authorized shops with high quality measures being very important.

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مدى تواجد ميكروب الإيشيريشيا كولاي O157:H7 فى بعض منتجات الألبان

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تم تجميع 104 عينة من منتجات الألبان (33 من الجبن القريش، 38 من الزبادي و33 من القشدة الحامضة) جمعت عشوائيا من مختلف المتاجر والأسواق والباعة الجائلين في مدينة كفرالشيخ لعزل وتصنيف ميكروب الإيشيريشيا كولاي O157:H7 وقد تبين انه من خلال الفحص السيرولوجي تم عزل ميكروب الإيشيريشيا القولونية H7: O157 من 3% من عينات الجبن القريش بينما لم يتم عزل الميكروب من عينات الزبادى او القشدة الحامضة ومن خلال الفحص السيرولوجي تم عزل بعض الانواع الاخرى من الأنماط المصلية القولونية منها O157:H7 ، O111:H4 ، O26:H11 ، O114:H21 ، O86 ، O55:H7 ، O125:H21 ، O126 ، O128:H2 ، O124 بالنسب الآتية (12.5 ، 0 و 0) ، (25 ، 25 و 26.6) ، (12.5 ، 12.5 و 13.3) ، (12.5 ، 12.5 و 6.7) ، (0 ، 12.5 و 0) ، (0 ، 12.5 و 6.7) ، (0 ، 0 و 20) ، (0 ، 12.5 و 6.7) من العترات المعزولة من الجبن القريش ، الزبادى والقشدة الحامضة على التوالي.

واستنادا إلى النتائج التي تم الحصول عليها في الدراسة الحالية، تبين ان منتجات الألبان المتخمرة من الممكن أن تكون مصدرا للتسمم الغذائي الناجم عن الإيشيريشيا القولونية O157: H7.

لذلك يوصى باتخاذ الاحتياطات الصحية أثناء تصنيع و تخزين وتداول منتجات الألبان وكذلك

بيع منتجات الألبان في المحلات التجارية المصرح بها صحيا بمعايير الجودة العالية.