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Escherichia coli O157 IN DAIRY PRODUCTS FROM RETAILERS AND SMALL DAIRY SHOPS

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ABSTRACT

Twenty-five samples of each of raw milk, UHT milks, dried milk, yoghurt, Kariesh cheese, Fetta cheese and Domiatti cheese, were collected from small shops, retailer venders and factories made in Giza, Beni-Suef Governorates markets, and tested for the presence of *E.coli* O157:H7. Raw milk, Kariesh cheese, Fetta cheese and yoghurt obtained from small shops and retailer venders showed high rate of contamination. Forty-four percent of raw milk samples were found to be contaminated. Kariesh cheese from factories or small shops were also contaminated. Its contamination reached up to 72%. Fetta cheese samples from small shops contained up to 24% contamination. Even 12% of yoghurt samples being obtained from small shops were found to be contaminated with12%. On the other hand, UHT milk and dried milk samples were completely free of the contamination.

Keywords: *E.coli* O157:H7, cheese, Dairy, Pathogenic, UHT milk, ice cream, yoghurt, Hemorrhagic colitis, EHEC.

INTRODUCTION

E.coli O157:H7 has been associated with several diseases such as hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. Hemolytic uremic syndrome (HUS) results from Shiga toxins (Stxs; Stx1, Stx2) produced by the bacteria in the intestine which act systematically on sensitive cells in kidneys, brain and other organs. These toxins are among the most potent bacterial toxins, it acts on cell ribosomes disrupting protein synthesis causing cell death. Hemorrhagic colitis caused by this pathogen is characterized by severe abdominal cramps with bloody stool. Cattles and possibly small-domesticated ruminants constitute a primary animal reservoir of this pathogen. The pathogen infects chickens injuring their liver and kidney. Most outbreaks of hemorrhagic colitis have associated with consumption of undercooked ground meat or raw milk. Shiga toxin-producing Escherichia coli (STEC) has long survival periods in effluent and environment (soil, plants, crops, grass, and water).

E.coli O157:H7 serotypes differ widely in their ability to cause human disease, colonize animal carriers and their survival in the environment (Ferens and Hovde 2011). Almost all EHEC O157 display negative fermentation of D-Sorbitol with > 99% of the sorbitol negative H7 strains are of the O157 serotype, do not produce β-glucuronidase and resistant to several wide-spectrum antibiotic and antibacterial agents. There are *E.coli* O157:H7 and non-O157 (026, 103, 0145, 0111 …..). Probability of detection

of *E.coli* O157:H7 is highly dependent upon the specific approaches to sampling and culturing (Reuben and Owuna 2013; Daood 2007).

E.coli O157:H7 was discovered as human pathogen in 1982, and now it ranks as the fourth most costly foodborne disease. It was estimated 73480 illness due to the pathogen infection occur each year in USA leading to 2168 hospitalization causing 61 deaths. It can cause the disease at a very low dose of 5 – 50 cells, with 3 – 8 days incubation period. Rate of death is 0-2% and up to 16 – 35% in elderly. It has a wide range of growth temperature between 10 – 46˚C and some strains grow at 8˚C and 6.5˚C, tolerate freezing temperature of - 18˚C for several weeks (Massa *et al*. 1999).

Milk is contaminated through mastitic milk (which is rare), fecal route, directly or indirectly and through post contamination of processed milk. The pathogen has ability to biofilm on equipment surfaces. Human contamination occurs through the contact with animals, or their environment, person-to-person contact, contaminated dairy products and ready-to-eat foods.

Presence and behavior of the pathogen in milk and dairy products were thoroughly investigated in different countries. Raw milk of cow, Buffalos, goats, sheep and camel show percentage of contamination. Percent of contamination varies according to sanitation conditions that prevails on the farm. Rates vary from 1.4% and up to 80% in literature (Daood 2007; Seker and Yardimci 2008; Rahimi *et al*. 2012; Abd Al-Azeem *et al*. 2013; Mahanti *et al*. 2014). Percentage of 30.9 of raw milk, 77.4% of hard cheese, 18% of sweet cheese and 9% cream cheese were infected in Syria (Daood 2007). Skim milk appears to be favorable for the survival of *E. coli* (Usajewicz and Beata 2006; Gyawalia *et al.* 2013). In hard cheese making, lactic acid culture which supposed to act antagonistically against STEC by the production of acid, cooking step, removal of whey and long period of ripening steps which should act synergistically against the presence of the pathogen, still the pathogen existed in Cheddar cheese for 270 days at 7˚C. It was found in Camembert and Fetta cheeses at 2˚C. The pathogen tolerates 8.5% NaCl and a_w of 0.945. Moreover, the pathogen acid adaptation might increase its survival in some acidic foods. Therefore, the practice of using 60 days ripening for making raw milk cheese safe is not valid anymore (Maher *et al.* 2001).

The information of the presence of STEC in Egypt is scarce though, the unsanitary conditions that prevail throughout milk production, distribution and processing cycle. Abdul-Raof *et al.*, in 1996 reported 6% infection of milk samples tested.

This research was undertaken to fill up this gap and test for the presences of STEC in number of dairy products sold in small shops, retailer venders or factories in Giza and Beni-Suef Governorates.

MATERIALS AND METHODS

Samples were collected and handled with sterile gloves and placed in sterile containers, stored frozen until analysis. Analysis was accomplished within few hours from their collection. Twenty-five samples of each fresh milk,

UHT milk, dried, Kariesh cheese, Fetta cheese, Domiatti cheese and yoghourt (samples of yoghourt and cheeses were of factories made or from retailer venders).

For the determination of E. Coli O157:H7, twenty-five milliliters of milk or 25 g of dairy product were added to 225 ml of enrichment solution of modified trypton soy broth (m TSB) containing (20 g) of Novobiocin plus 1.12 g/l of bile salt. Flasks were shaken for 24 h at 37˚C. Turbid cultures were considered presumptive positive. Confirmation was made by inoculating 100 ml of the broth onto tellurite (2.5 mg/l) – Cefixime (0.05 mg/l) sorbitol Macconky agar plates supplemented with 200 μg/ml ampicillin (CT-SMAC). Plates were inoculated at 37˚C / 24h. Colorless or neutral gray with smoky center (1-2 mm) colonies were selected to be positive identification.

Gram staining, oxidase, indole, methyl red (MR), Voges-Proskauer (VP), Citrate, Urease, hydrogen sulphate (H_2S), lysine, decarboxylase, glucose, sucrose, lactose, salicin, adonitol, inositol, cellobiose, arabinose, trehalose, mannitol, rhamnose, xylose, raffinose, and dulcitol tests were performed to identify selected colonies.

For the Confirmation of the obtained result, the *E. Coli* O157 Latex agglutination test (Oxoid, DR620M) was performed.

Statistical analysis were run in triplicates. Results were statistically analyzed by means of the Chi-square test of independence to determine the relationship between the type of the dairy product and the presence of *E. Coli* O157 Statistical analysis was performed by running the SPSS 20 (IBM Corp., Copyright© 2011) package on a personal computer.

RESULTS AND DISCUSSION

The enriched process encouraged the growth of STEC and inhibited unwanted ones. Identification started with inoculating (CT-SMAC) agar, proper identified colonies were counted, their biochemical properties were identified, and their final identification was carried out with Latex agglutination test.

Table (1) shows the biochemical characteristics of *E. Coli* O157:H7. These results disclosed the proper characteristics of STEC. Therefore, the latex agglutination test with anti-serum against the flagella antigen H7 to be sure of the positive results.

Raw milk showed high percentages of STEC contamination (Table 2). A range between 24-44% of samples were contaminated. These results impose a very dangerous health problem because this milk is consumed by many consumers as liquid milk, and small shops process it into number of dairy products. Retailed raw milk is produced in small farms where milking occurs within the barn where animals manure, which is the major source of STEC, is within milk atmosphere. Small farmers are not aware of the importance of animal health, udder cleaning, can washing and milk cooling on milk contamination. It is clear that raw milk in many countries is the main contaminated with STEC, since literature reported rates of raw milk contamination from 1.4 up to 80% (Daood 2007; Seker and Yardimci 2008).

Table (1) Biochemical characteristics of Escherichia coli O157:H7

Test		Result
Gram stain		–
Oxidase		–
Indole		+
MR		+
VP		-
Citrate		-
Urease		-
TSI agar	Glucose	+
	Sucrose	+
	Lactose	+
	H_2S	-
Lysine decarboxylase		+
Salicin		-
Adonitol		-
Inositol		-
Cellobiose		-
Trehalose		+
Arabinose		+
Mannitol		+
Rhamnose		+
Xylose		+
Raffinose		+
Dulcitol		+
Enterohaemolysin		+

UHT milk samples were free from STEC as supposed to be, this product is heated at 130-150 ˚C for 2-4 S and aseptically packaged.

Dried milk samples were free of STEC, because the two brands tested were processed and packaged abroad. This means that processors took all precautions to avoid contamination, particularly during handling and packaging of the powder. This step is considered the most critical step for powder contamination.

Table (2): Presence of *E. coli* O157:H7 in Milk.

Sample	Place	Source	Positive Samples	
			Number	%
Raw Milk	Beni-Suef	Retailer	11	(44%)
	Giza	Retailer	6	(24%)
UHT (Two Trade Names)	Trade Name (x)	Supermarket	0	(Zero %)
	Trade Name (y)	Supermarket	0	(Zero %)
Dried Milk (Two Trade Names)	Trade Name (x)	Supermarket	0	(Zero %)
	Trade Name (y)	Supermarket	0	(Zero %)

Total tested samples of each product were 25.

Table (3): Presence of *E. coli* O157:H7 in Egyptian Soft Cheeses And Yoghurt.

Sample	Place	Source	Positive Samples	
			Number	%
Kariesh Cheese	Beni-Suef	Home-made	18	(72%)
		Factory	5	(20%)
	Giza	Home-made	12	(48%)
		Factory	2	(8%)
Fetta Cheese	Beni-Suef	Retailer	4	(16%)
		Factory	0	(Zero %)
	Giza	Retailer	6	(24%)
		Factory	0	(Zero %)
Domiati Cheese	Beni-Suef	Factory	0	(Zero %)
Yoghurt	Beni-Suef	Factory	0	(Zero %)
		Baladi	1	4%
	Giza	Baladi	3	12%

Total tested samples of each product were 25.

Kariesh cheese samples were highly contaminated whether processed in small shops or factories. A range of 48-72% of small shops and 8-20% of factories' samples contained STEC as shown in Table 3. This is a disturbing result, since Kariesh cheese is popular, particularly for its low price. This means that number of people are at risk. The presence of factories contaminated samples lead to grief situation since these factories supposed to use proper use practice in their process. This implies that the Egyptian consumers might not trust even the large companies.

Fetta cheese samples of small shops had a contamination rate of 16-24% as shown in Table 3. On the other hand, samples of factory Fetta cheese were free of STEC. Fetta made in small shops exposed to contamination because the lack of proper sanitation, moreover, these shops probably use number of improper and inexpensive ingredients.

The difference in results between factories Kariesh and Fetta could be due to methods of processing. Kariesh cheese is made from skim milk mostly without heat treatment. They depend on whole milk pasteurization before skimming which is not enough. Fetta cheese in dairy plants is made from pasteurized milk concentrate and usually is loaded with different types of preservatives. Moreover, factories nowadays use milk powder instead of liquid milk. Samples of factory Domiati cheese were free of STEC. The high salt content of the cheese probably helped in this result.

Yoghurt samples had a contamination rates of 4-12% compared to the zero contamination of factory samples. STEC tolerates high acidity and cold temperature; therefore, the pathogen would grow and increase in numbers during storage.

Literature also pointed out different contamination rates in fermented milk (Farrokh *et al.* 2013; Reuben and Owuna 2013) and soft cheese such as sweet chees and cream cheese (Daood, 2007).

The statistical analysis revealed that there is a significant relationship ($P < 0.05$) between the type of dairy products and the presence of E. *Coli*

O157. Such results confirm what was previously pointed out that the differences between these dairy products depend mainly on the raw milk and steps of processing in relation to potential sources of contamination. Actually, there is no awareness in Egypt of the existence of this pathogen and their health hazards. In addition, the government did not educate the processors as well as consumers of the presence of the pathogen.

If we realize that the STEC can grow at a pH as 3-5 and a very low dose of 5-50 cells with 3-8 days incubation period is enough to cause the disease, then Egypt is facing a big problem. The scope of the problem is not defined because of the lack of data concerning the presence and distribution and percentages of the presence of STEC in Egypt. However, our results proved the presence of the pathogen in the Egyptian dairy products.

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بكتريا الـ O157 *Escherichia coli* في منتجات الألبان من المحلات الصغيرة والباعة الجائلين

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خمسة وعشرون عينة من كل من اللبن الخام والزبادي واللبن المعقم UHT واللبن المجفف والجبن القريش والجبن الفيتا والجبن الدمياطي، بعضها تم تجميعها من الباعة الجائلين، المحلات الصغيرة وبعضها مصنع في المصانع، تم تجميع العينات من كل من محافظة الجيزة وبني سويف ثم تم اختبار تلوثهم واحتوائهم على بكتريا *Escherichia coli* O157. أوضحت النتائج أن عينات اللبن الخام والجبن القريش والجبن الفيتا والزبادي التي تم الحصول عليهم من المحلات الصغيرة والباعة الجائلين ملوثة ببكتريا *E. coli* O157 وبدرجة كبيرة. حيث وجد أن ٤٤% من عينات اللبن الخام كانت ملوثة بالميكروب. وكذلك أظهرت النتائج ان ٧٢% من عينات الجبن القريش المتحصل علية من المصانع أو المحلات الصغيرة كانت ملوثة بالميكروب أيضا. كما أن ٢٤% من عينات الجبن الفيتا المتحصل عليها من المحلات الصغيرة كان ملوثة بالبكتريا. وكذلك ١٢% من عينات الزبادي أوضحت النتائج أنها ملوثة بالميكروب. في حين أن عينات اللبن المعقم والمجفف أظهرت نتائج التحليل أنها خالية من بكتريا *Escherichia coli* O157.