Prevalence of *Escherichia albertii* and Other Escherichia species in Raw Milk and Some Dairy Products in Assiut City, Egypt

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Abstract: Aim: To determine the incidence of *Escherichia albertii* and other Escherichia species in raw milk and some dairy products in Assiut city, Egypt. **Materials and Methods:** A total of 120 random samples of raw milk, Damietta cheese, kareish cheese and cooking butter, (30 samples each) were collected from different localities in Assiut city, Egypt. Two media Hugh and Leifson agar (H&L) and Eosin Methylene Blue Agar (E.M.B.) were used for isolation of *Escherichia spp.* The strains were biochemically characterized. Latex agglutination test was performed on *Escherichia coli (E. coli)* isolates. Polymerase Chain Reaction (PCR) was performed on *Escherichia albertii (E. albertii)* strains that were biochemically identified. **Results:** The incidence of *Escherichia spp.* was 70% on H&L medium and 59.17% on E.M.B. medium. The strains were divided into 6 species; *E. coli, Escherichia (E. fergusonii), Escherichia vulneris (E. vulneris), Escherichia hermanni(E. hermanni), Escherichia blattae (E. blattae) & Escherichia albertii(E. albertii E.albertii* was isolated in an incidence of 0.83% on each medium. Three strains of *E.coli* were positive for *E. coli O157:H7* by Latex agglutination test. One of the tested *E. albertii* strains was confirmed by PCR. **Conclusion:** Considering the fact that *Escherichia species* contribute to the burden of food borne illness, and since its presence in milk & milk products could be attributed to their contamination during milking, handling or processing, more hygienic measures should be applied to improve the quality of the produced milk & milk products to ensure maximum safety to consumers.

1. Introduction

Many Escherichia are harmless commensals, however some species are human pathogens [1], and are known as the most common cause of the urinary tract infection [2]. While, *E. coli* is responsible for the vast majority of Escherichia related pathogenesis, other members of the genus have also been implicated in human diseases [3, 4].

Until recently, the genus *Escherichia* was composed of five species, including the type species *E. coli* and four less frequently encountered members: *E. blattae, E. hermannii, E. vulneris* and *E. fergusonii* [5]. Recently, a sixth species, *E. albertii* was described [6].

E. coli is the most completely characterized organism and one of the dominant indicator organisms for food and water quality testing [7]. There are two groups of pathogenic *E. coli.,* extra intestinal pathogenic *E. coli* represent *E. coli* associated with urinary tract infections and new borne meningitis, while the intestinal pathogenic *E. coli* are responsible for a range of diarrheal diseases [8].

E. coli O157:H7 can be responsible for bloody or non bloody diarrhea, which may be complicated by haemorrhagic colitis and severe renal and neurological sequelae, including haemolytic uraemic syndrome [9].

Estimates of foodborne-acquired *E. coli* O157:H7 cases result in 2,138 hospitalizations and 20 deaths annually [10]. In April, 2012, three children were hospitalized after consuming raw milk in *E. coli* outbreak in the USA, two were hospitalized with hemolytic uremic syndrome, HUS [11].

E. albertii was originally isolated and identified by John Albert and colleagues at the International Center for Diarrheal Diseases Research, Bangladesh (ICDDR,B) as *Hafnia alvei* [12]. However, subsequent phenotypic and genetic studies conducted by several international groups clearly indicated that these strains don't belong to the genus *Hafnia* [13]. Further molecular studies including 16S ribosomal DNA sequencing and DNA-DNA pairing studies have demonstrated that these strains belong to the genus *Escherichia*, and a new species, *E. albertii*, has been proposed [6]. However, Commercial systems have misidentified *Escherichia albertii* as *Yersinia ruckeri*, *Salmonella enterica serovar Enterica, Hafnia alvei* or *E. coli* [14].

E. albertii is probably a major enteric human pathogen [15]. It was reported to cause diarrheal disease in six children with accompanying symptoms of vomiting, mild dehydration, fever, and abdominal distention [16]. Furthermore, *E. albertii* could possibly contribute to the estimated 62,000,000 cases

of food-borne illnesses and 3,200 deaths in the United States that have an unknown etiological origin [17].

Escherichia species continue to be an important pathogen and there is an interest in Escherichia spp. particularly *E. albertii* and because of the involvement of milk and milk products in human Escherichia health risk. Therefore, the present study was designed to isolate Escherichia species from raw milk and some milk products sold under market conditions in Assiut city, Egypt.

2. Materials & Methods

A total of 120 random samples of raw milk, Damietta cheese, kareish cheese and Cooking butter, (30 samples each) were collected from different localities in Assiut city in clean, dry and sterile containers and transferred immediately to the laboratory to be examined.

The samples were prepared according to A.P.H.A. [18].

Enrichment procedures:

One milliliter of homogenized samples was aseptically inoculated into 10 ml of *Escherichia coli* broth (E.C. broth) with inverted Durham's tube [19]. The inoculated tubes were incubated at 37^0C for 24 hours.

Selective plating:

Incubated positive broth cultures (collection of gas) were streaked onto plates of Hugh and Leifson agar (H&L) and Eosin Methylene Blue Agar (E.M.B.). Streaked plates were incubated at 37^OC for 24 hours [20].

Identification of isolates:

Suspected colonies were identified by Gram stain, motility test, oxidase, catalase, pigment production, lysine decarboxylase, indole, lactose, D-sorbitol, L- rhamnose, raffinose fermentation and methyl red tests [6, 21].

Isolation of *E. coli O157:H7* Strains: [22]

A single typical well isolated lactose fermenting colony was cultured on sorbitol MacConkey agar. The plates were incubated aerobically at 37^OC for 24 hours. *E. coli O157:H7* produced colorless colonies on the medium because it did not ferment sorbitol. Most other *E. coli* strains ferment sorbitol producing pink colonies.

Serotyping of *E. coli* isolates by *E. coli O157* Latex Test (Oxoid, DR 120 M) [23]**:**

Test method as prescribed by the manufacture.

Identification of *E. albertii* (Multiplex PCR for *E. albertii*)

PCR assay based on nucleotide sequences of multiple conserved housekeeping genes to identify or confirm member of the *E. albertii/Shigella boydii* lineage. Two sets of primers were designed to include nucleotide polymorphisms unique to the new lineage, and amplify two different gene segments exclusively in the new lineage. A set of primers was designed to amplify a gene segment in the new lineage as well as in *E. coli* and *Shigella*, and serves as a control.

I- Protocol for isolation of genomic DNA from cultured cells:

This protocol is designed for rapid isolation of up to 25µg genomic DNA from up to 5×10^6 cultured cells.

Cell suspension from cells grown in Nutrient broth was prepared, 5×10^6 cells was pelleted by spinning at 1200 x g in a centrifuge tube. Cells were washed with PBS (Phosphate Buffer Saline), then cells were resuspended with 200µl cold (4^oC) PBS. Then 25 µl of Proteinase K (D3495) were added at 20 mg/ml solution, and then incubated at 65^oC for 5 minutes to affect complete lysis. Then 220 µl Buffer BL was added and incubated at 70^oC for 10 minutes. Then 220 µl absolute ethanol were added. A Hi Bind spin column was assembled in a 2 ml collection tube. The sample was transferred into the column. Then centrifuged at 8,000 x g for 1 min to bind DNA and discard the collection tube.

The column was placed into a second 2 ml tube and washed by pipetting 750 µl of wash buffer diluted with ethanol. Centrifuged at 8,000 x g for 1 min again. The column was washed with a second 750 µl of wash buffer, centrifuged as above. Then centrifuged at maximum speed (10,000 x g) for 2 min to dry the column. The column was placed into a sterile 1.5 ml microfuge tube and 200 µl of preheated (70^oC) Ellution Buffer was added. To elute DNA from the column; centrifuged at 8,000 x g for 1 min and repeated with a second 200 µl of Elution Buffer.

II- Multiplex PCR procedures:

PCR was carried out in 50 µl reaction volume containing 5 µl template DNA, 1 mM MgCl2, 1 mM of dNTP, 5 µl of x 10 PCR buffer (Quiagen), 1.25 unit of TaqDNApolymerase (Ampli Taq Gold Quiagen) and 20 pM of each primer. The sequences of the primers are given in the following table.

Name	Sequence	Product	Comment
clpX_28F	5' TGG CGT CGA GTT GGG CA 3'	384bp	Control
clpX_411R	5' TCC TGC TFC GGA TGT TTA CG 3'		
lysP_107F	5' GGG CGC TGC TTT CAT ATA TTC TT 3'	252bp	Specific
lysP_358R	5' TCC AGA TCC AAC CGC TAT CAG GA 3'		
mdh_50F	5' CTG GAA GGC GCA GAT GTG GTA CTG ATT 3'	115bp	Specific
mdh_164R	5' CTT GCT GAA CCA GAT TCT TCA CAA TAC CG 3'		

III- Amplification was according to Hyma and Whittam [24]**.**

The PCR cycles consisted of pre-heating at 94°C for 10 min, denaturation at 92°C for 1 min, annealing at 65°C for 1 min and extension at 72°C for 30 sec. the amplifications were performed for 25 cycles in a model T professional basic 070- 701 thermocycler, in the Molecular Biology Research and Genetic Engineering Center, Assiut University, Egypt, with a final extension step at 72°C for 5 min. The PCR products were visualized using a 2.5% agarose gel containing 0.5 µg of ethidium bromide/ml in relation to DNA mass ladder standard (1000-bp DNA ladder Quiagen).

3. Results and Discussion

Results given in Table 1 revealed that 27 out of 30 milk samples (90%) using H&L agar were positive for *Escherichia spp.* and 25 positive samples (83.33%) using E.M.B. agar. Contamination of milk during or after milking is probably of fecal origin; however improper washing and treatment of the udder with unsuitable disinfectant or contact of the milking pails with the floor may result in high level of contamination.

The minimum, maximum and the average values were $<10^2$, $1.5X10^7$ and $7.5X10^6$ cfu/ml on H&L agar and $<10^2$, $7X10^6$ and $3.5X10^6$ cfu/ml on E.M.B. agar, respectively.

E. coli was detected in a percentage of 80 and 73.33 using H&L agar and E.M.B. agar, respectively. These results were in agreement with those postulated by Campos et al. [25] Hassan and Elmalt [26], Altalhi and Hassan [27] in percentages of 79.2%, 76.0% & 72.7% respectively. Lower results were recorded by Nanu et al. [28] 31.6% and Kumar and Prasad [29] 13.3%. The difference between our results and other studies may be attributed to the sampling technique, sources and handling of samples, types of media used.

High incidences of *E. coli* in the examined raw milk samples indicated the neglected sanitary control during handling of milk and possibly fecal contamination. Given the anatomical location of a cow's udder and the presence of bovine feces in the barn where milking occurs, numerous hazard points exist during milking and handling of milk that can lead to fecal contamination, even when using recommended hygienic methods. Therefore, it is advisable to obligate strict hygienic measures during milk handling to prevent contamination and improve its quality, also proper heat treatment of milk to safeguard consumers against infection. *E. coli* exists in ever increasing number of strains due to mutation and high frequency recombination. Though people generally understand *E. coli* as harmless intestinal flora, they are opportunistic and some of the strains have been identified as the serious causal agents of various illnesses.

Comparing the obtained data in Tables 2 &3 with that of the Egyptian Standards [30] which stipulated that raw milk should be free from *E. coli* and samples with percentages of 80 and 73.33% using H&L agar and E.M.B. agar, respectively are not complying with these standards.

Other types of *Escherichia spp.* as shown in Tables 2 & 3, *E. fergusonii* and *E. hermannii* were isolated in percentages of 3.33% on H&L agar. The concerned organisms could be isolated in percentages of 3.33 and 6.67 % on E.M.B. agar, respectively from the examined milk samples. *E. vulneris* was isolated in a percentage of 3.33 on H&L agar, while could not be detected on E.M.B. agar. *E. albertii* and *E. blattae* could not be detected in the examined raw milk samples.

According to the data reported in Tables 6 & 7 the incidence of *Escherichia spp.* in Kareish cheese reached to 93.33% with minimum, maximum and average of $<10^2$, $3.2X10^7$ and $1.6X10^7$ cfu/g , respectively on H&L agar. 66.67% of the examined Kareish samples were contaminated with minimum, maximum and average of $<10^2$, $2.2X10^7$ and $1.1X10^7$ cfu/g on E.M.B. agar, respectively.

Regarding the results in Tables 2 & 3, the incidence of *E. coli* in the examined samples of Kareish cheese reached 83.33% on H&L agar and 60% on E.M.B agar, which failed to comply with the Egyptian Standards [31] which pointed out that the Kareish cheese must be free from *E. coli.* The obtained results were in harmony with the results recorded by Aboul-Kheir et al. [32] 80.95%. However, lower incidences were recorded by Hassan

and Elmalt [26], Fadel and Ismail [33] and Nazem et al. [34] representing 47.8%, 44.4% and 40%, respectively.

Data recorded in Tables 4, 5 illustrated that neither *E. fergusonii*, *E. vulneris* nor *E. albertii* were existed in kareish cheese samples. However, *E. hermannii* and *E. blattae* were isolated from one and 2 samples with percentages of 3.57 and 7.14% on H&L agar, respectively. While, on E.M.B. agar, *E. fergusonii*, E. *hermannii* and *E. albertii* could not be isolated, but, *E. vulneris* and *E. blattae* were recovered in a percentage of 5% for each. This may be due to the bad hygienic measures followed during manufacture, handling and distribution of Kareish cheese in Assiut City. Hence, obligate strict hygienic measures should be applied to improve its quality and to avoid public health hazards.

Results obtained in Tables 1, 6 & 7 revealed that 40% of Damietta cheese samples were contaminated by *Escherichia spp.* with minimum, maximum and averages of $<10^2$, $3x10^6$ and $1.5X10^6$ cfu/g using H&L agar. While on E.M.B. agar, 30% of samples were contaminated with minimum, maximum and average of $<10^2$, $7X10^4$ and $3.5X10^4$ cfu/g, respectively. Isolation of *Escherichia spp.* from the examined Damietta cheese samples may be due to the use of unpasteurized milk for production of cheese or contamination after pasteurization during production and handling of cheese. Furthermore, the way of selling some Damietta cheese (not properly packed) could be a cause of contamination of cheese with *Escherichia* after production.

E. coli was recovered from 5 samples of Damietta cheese with a percentage of 16.67% as shown in Tables 2 & 3, which are not complying with the Egyptian Standards [31]. Higher incidence (30%) was reported by El Sayed et al. [35]. However, Thabet [36] could not detect *E. coli* in any of the examined Damietta cheese samples.

For other *Escherichia species,* as regarding in Tables 2 & 3, *E. fergusonii* , *E. vulneris* and *E. albertii* were recovered from one Damietta cheese sample in a percentage of 3.33% for each of them , while, *E. hermannii* could be isolated in a relatively high incidence (13.33%) on H&L agar. The results on E.M.B. agar, *E. albertii* was isolated from one sample with a percentage of 3.33%, *E. hermannii* in 3 samples with a percentage of 10% and none of *E. fergusonii, E. vulneris* were detected. Moreover, *E. blattae* could not be recovered on both media used.

The incidence of *Escherichia spp.* was higher in Kareish cheese (93.33%, 66.67%) than in Damietta cheese (40%, 30%) on both media used (Table 1). This could be attributed to the fact that kareish cheese is usually made from raw milk, the primitive way of

processing, handling and methods of selling this particular type of cheese.

Results given in Table 1 gave an idea about the positive cooking butter samples with *Escherichia spp.* which were 17 out of 30 examined samples with a percentage of 56.67% on both H&L agar and E.M.B agar used. The minimum, maximum and average counts were $<10^2$, $1.8x10^5$ and $9x10^4$ cfu/g on H&L agar and couldn't be detected on E.M.B. agar plates as illustrated in Tables 6 & 7. *E. coli* was detected in 13 (43.33%) and 12 (40%) on H&L and E.M.B agar, respectively (Tables 2 & 3). So they are not complying with the Egyptian Standards [30] which pointed that cooking butter must be free from it. Higher incidence (64.4%) was reported by Karagozlu and Ergonul [37]. Lower results were recorded by Meshref [38] and Kumar and Prasad [29], 31.7% & 13.3%. There was no recognition neither for *E. fergusonii* nor *E. albertii* on both media. Although *E. blattae* was noticed in a percentage of 3.33% on E.M.B agar only. *E. hermannii* was represented by 6.67, 3.33% on H&L agar and E.M.B. agar, respectively. The incidence of *E. vulneris* was 6.67 and 10% on H&L and E.M.B. agar, respectively.

In the present study two media were used which are Hugh and Leifson agar (H&L) and Eosine Methylene Blue agar (E.M.B). Results given in Table 1, 2 & 3 revealed an obvious comparison between both media. *Escherichia spp.* were isolated from 84 samples (70 %) on H&L agar but for E.M.B agar those were 71 positive samples (59.17 %).

E. coli on H&L agar revealed 67 positive samples out of 120 examined samples with a percentage of 55.83 % but for E.M.B agar those was 57 positive samples with a percentage of 47.5 %. Moreover, 2 and 1 positive samples of *E. fergusonii* in percentages of 1.67 % and 0.83 % on H&L agar and E.M.B agar, respectively. For *E. hermannii* samples on H&L agar revealed 8 positive samples with a percentage of 6.67 % but for E.M.B agar those were 6 positive samples with a percentage of 5 %. However, *E. vulneris, E. blattae* and *E. albertii* were isolated with the same percentage for both media. So, we claim that H&L agar was better than E.M.B. agar for most of *Escherichia spp.*

In this study, latex agglutination test was used as a serological test for confirming *E. coli O157:H7*. By first isolating the colonies on Sorbitol MacConkey agar which is perceived as the "gold standard" for isolation of *E. coli O157:H7* [39] because it differentiated colonies by naked eye producing colorless colonies for *E. coli O157:H7*and pink colored colonies for other types of *E. coli* . The sensitivity and specificity of latex test (Oxoid) kit are 100 and 100%, respectively [23].

Regarding to Table 8, it is obvious that results of latex agglutination test for *E. coli O157:H7*, 3 positive samples (2 from kareish cheese and 1 from Damietta cheese samples) with 1 negative sample (from milk samples). Nearly similar findings were recorded by Abd El-Atty and Meshref [40] who detected *E. coli* O157:H7 in one sample of kareish cheese, while could not detect it in milk samples. Also, Hill et al. [41] couldn't detect it in milk samples. *E. coli O157:H7* was present in one sample of Damietta cheese. Experiments *in vitro* have shown that *E. coli* O157:H7 develop an adaptive tolerance response (ATR) to acid environments and become acid-tolerant [42].

Cattle are the principal source of *E. coli O157:H7* infection, as it can live in the intestine of healthy cattle, their manure is also an important source for contamination. Contamination with as few as 10 *E. coli* O157*:H7* bacteria might be sufficient to cause human infection [43]. Thus, to adequately control the microbial risks, pasteurization is strongly recommend, which substantially decreases or eliminates pathogens and effectively prevents disease transmission [44]. Furthermore, it could be recommended that storage of Damietta cheese for more than one month may assure the safety of the cheese from *E. coli* O157 H7 [45].

Researches on *E. albertii*, revealed that it is a new pathogenic microorganism with not enough information. But it contributes greatly to other diarrheagenic bacteria. According to Hyma and Whittam [24] and Hyma et al. [46] the used multiplex PCR primers were the same because it is the same lab. in Michigan with the same primers used.

One of the isolated *E. albertii* was confirmed with PCR. It was from damietta cheese samples which could be attributed to the acid resistance which is perceived in *E. albertii* to be an important property of enteric pathogen enabling them to survive passage through stomach acidity. But the negative sample may be due to genetic variation between strains isolated from our country than others all over the world. So we suggest making a genetic sequencing for the isolated microorganism and creating specific primers for it locally.

Table 1. Incidence of *Escherichia spp.* in the examined samples using two different media

| Examined samples | No. of examined samples | Positive samples on | | | |
| | | H&L | | E.M.B. | |
		No.	%	No.	%
Raw milk	30	27	90	25	83.33
Kareish cheese	30	28	93.33	20	66.67
Damietta cheese	30	12	40	9	30
Cooking butter	30	17	56.67	17	56.67
Total	120	84	70	71	59.17

Table 2. Incidence of *Escherichia spp.* in the examined samples using H&L agar.

| Examined samples (30 each) | E. coli | | E. fergusonii | | E. vulneris | | E. hermanni | | E. blattae | | E. albertii | |
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Raw milk	24	80	1	3.33	1	3.33	1	3.33	–	–	–	–
Kareish cheese	25	83.33	–	–	–	–	1	3.33	2	6.67	–	–
Damietta cheese	5	16.67	1	3.33	1	3.33	4	13.33	–	–	1	3.33
Cooking butter	13	43.33	–	–	2	6.67	2	6.67	–	–	–	–
Total (120)	67	55.83	2	1.67	4	3.33	8	6.67	2	1.67	1	0.83

Table 3. Incidence of *Escherichia spp.* in the examined samples using E.M.B. agar.

| Examined samples (30 each) | E. coli | | E. fergusonii | | E. vulneris | | E. hermanni | | E. blattae | | E. albertii | |
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Raw milk	22	73.33	1	3.33	–	–	2	6.67	–	–	–	–
Kareish cheese	18	60	–	–	1	3.33	–	–	1	3.33	–	
Damietta cheese	5	16.67	–	–	–	–	3	10	–	–	1	3.33
Cooking butter	12	40	–	–	3	10	1	3.33	1	3.33	–	–
Total (120)	57	47.5	1	0.83	4	3.33	6	5	2	1.67	1	0.83

Table 4. Frequency distribution of *Escherichia spp.* in the examined samples using H&L agar

Examined samples	Positive samples	E. coli		E. fergusonii		E. vulneris		E. hermanni		E. blattae		E. albertii	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Raw milk	27	24	88.89	1	3.70	1	3.70	1	3.70	–	–	–	–
Kareish cheese	28	25	89.29	–	–	–	–	1	3.57	2	7.14	–	–
Damietta cheese	12	5	41.67	1	8.33	1	8.33	4	33.33	–	–	1	8.33
Cooking butter	17	13	76.47	–	–	2	11.76	2	11.76	–	–	–	–

Table 5. Frequency distribution of *Escherichia spp.* in the examined samples using E.M.B. agar.

Examined samples	Positive samples	E. coli		E. fergusonii		E. vulneris		E. hermanni		E. blattae		E. albertii	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Raw milk	25	22	84.62	1	3.85	–	–	2	7.69	–	–	–	–
Kareish cheese	20	18	90	–	–	1	5	–	–	1	5	–	–
Damietta cheese	9	5	55.56	–	–	–	–	3	33.33	–	–	1	11.11
Cooking butter	17	12	70.59	–	–	3	17.65	1	5.88	1	5.88	–	–

Table 6. Statistical analytical results of *Escherichia spp.* in the examined samples using H&L agar.

Examined samples (30 each)	Positive samples		Counts/ml		
	No.	%	Min.	Max.	Average
Raw milk	27	90	$<10^{2*}$	1.5×10^7	7.5×10^6
Kareish cheese	28	93.33	$<10^{2*}$	3.2×10^7	1.6×10^7
Damietta cheese	12	40	$<10^{2*}$	3×10^6	1.5×10^6
Cooking butter	17	56.67	$<10^{2*}$	1.8×10^5	9×10^4

* Colonies could not be detected on the plates.

Table 7. Statistical analytical results of *Escherichia spp.* in the examined samples using E.M.B. agar.

Examined samples (30 each)	Positive samples		Counts/ml		
	No.	%	Min.	Max.	Average
Raw milk	25	83.33	$<10^{2*}$	7×10^6	3.5×10^6
Kareish cheese	20	66.67	$<10^{2*}$	2.2×10^7	1.1×10^7
Damietta cheese	9	30	$<10^{2*}$	7×10^4	3.5×10^4
Cooking butter	17	56.67	$<10^{2*}$	1.5×10^4	7.5×10^3

* Colonies could not be detected on the plates.

Table 8. Incidence of total *E. coli* strains and *E. coli O157:H7* in the examined samples using Latex agglutination test.

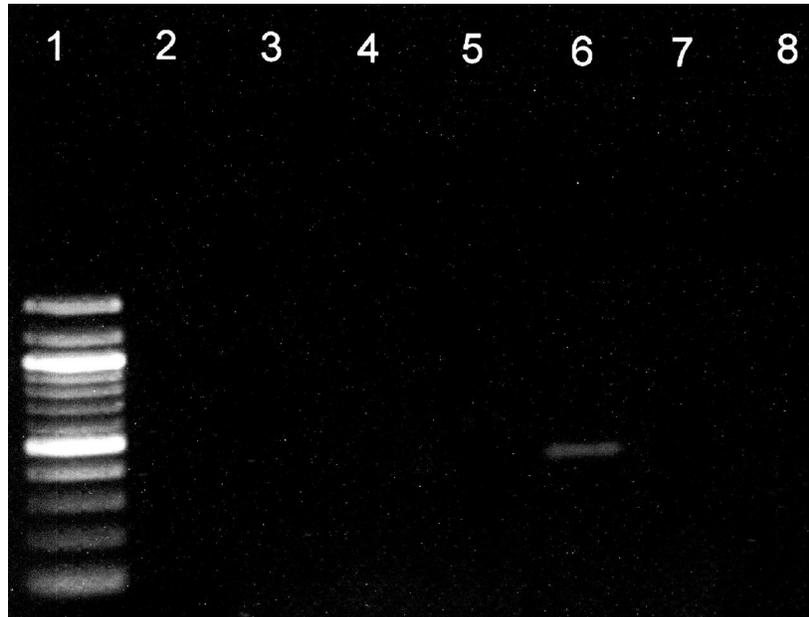| Samples	No. of examined samples	Positive samples					
		No.	%	E. coli strains		E. coli O157:H7	
				No.	%	No.	%
Raw milk	24	1	4.17	1	100	0	0
Kareish cheese	25	2	8	0	0	2	100
Damietta cheese	5	1	20	0	0	1	100

Photo 1. Results of PCR testing for *E. albertii*

Conclusion:

Raw milk and milk products available to the consumers in markets of Assiut city are contaminated with Escherichia species. *E. coli O157:H7* was detected in Kareish and Damietta cheese and *E. albertii* was confirmed to be isolated from Damietta cheese. Therefore, application of good manufacturing practices (GMPs) by food manufacturers and implementation of HACCP system are required to ensure the freedom of milk & milk products from these pathogens. In addition, pasteurization of milk is required before consumption and product making.

Competing interests: Authors declare that they have no competing interests.

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