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ORIGINAL ARTICLE

Prevalence of *Escherichia coli* in Milk and Some Dairy Products in Beni-Suef Governorate, Egypt

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Abstract
The aim of the study was directed to investigate the prevalence of Escherichia coli in raw milk, cream, large and small scale yoghurt, Kareish cheese, and large and small scale ice cream by conventional bacteriological methods as well as detection of the wzy gene (O-antigen polymerase gene) using polymerase chain reaction for further identification of recovered isolates. A total of 200 random samples including 40 raw milk, 40 fresh cream, 20 of each large and small scale yoghurt, 40 Kareish cheese and 20 of each large and small scale ice cream were obtained from different localities in Beni-Suef governorate, Egypt. The present study could provide useful information for the prevalence of *E. coli* in the examined samples: 75%, 62.5%, 0%, 25%, 80%, 10% and 25% respectively. *E. coli* isolate were serologically identified as: O26, O111:H4, O121, O125:H21, O169, O126:H7 and O158 in raw milk, O6:H16, O55:H7, O119:H6, O125:H6 and O146:H21 in fresh cream, O55:H7, O125:H21 in small scale yoghurt and O18, O55:H7, O114:H21, O158, O125:H21and O153:H45 in Kareish cheese. Interestingly PCR successfully amplified the wzy gene (O-antigen polymerase gene) in all *E. coli* isolates which is associated with LPS biosynthesis and bacterial pathogenicity and increases the ability of *E. coli* to withstand the anti-bacterial defense mechanism.

Keywords Dairy products, *E. coli*, milk, PCR, serology, wzy gene.

1. Introduction
The value of raw milk as repeatedly assumed by many people favors its consumption by huge numbers of persons who believe that raw milk and its products have a good nutritive value over the pasteurized one. Recently, consumption of dairy products has increased rapidly all over the globe, as a result of rising income levels and economic growth **(FAO 2013).**

Based on high nutritive value of milk, many bacteria including spoilage and pathogenic bacteria are known to grow and propagate in it after gaining entrance into the milk through many ways, as colonization of teat canal or infected udder, milkers, unclean milk utensils and unclean water **(Gruezmacher and Bradly 1999)**. Hygienic milk production is very essential to provide safe and high-quality products. During different handling procedures, milk and dairy products may be contaminated by variable types of organisms from several sources impairing its utility and render the products unfit for human consumption beside public health significance.
Recovery and counting of *E. coli* in milk is considered as a reliable indicator of fecal contamination together with possible presence of other enteropathogenic and/or toxigenic bacteria with

public health significance. Most *E. coli* strains are saprophytic, but some are known to be pathogenic bacteria, causing variable intestinal and extra intestinal disease problems in man **(Kaper et al 2004).** Therefore, it is considered as an actual challenge as it is highly infectious in both humans, and animals causing serious acute illness and serious long-term complications.

The pathogenic groups include 6 main types of diarrheagenic *E. coli* that have been associated with food borne disease. The first; enterotoxigenic *E. coli* (ETEC) which is responsible for watery diarrhea which is commonly known as traveler's diarrhea (affecting human especially during visiting warmer countries) as consequence to production of heat labile toxin (Cholera like toxin) and heat stables toxin (diarrheal toxin). The second; enteropathogenic *E. coli* (EPEC) which is associated with infantile diarrhea which is watery and mucoid without blood as a result of production of the characteristic attaching and effacing lesions in the brush border microvillus membrane. The third; enterohaem-orrhagic *E. coli* which may result in bloody diarrhea, Hemorrhagic Colitis, "Hemolytic Uremic Syndrom" and thrombic thrombocytopenic Purpura. The fourth; entero-aggregative *E. coli* (EaggEC) which is responsible for persistent watery diarrhea, especially in children, that lasts more than 14 days. They align themselves in parallel rows on either tissue cells or glass. This aggregation has been described as 'stacked brick-like'. They produce a heat-labile toxin, antigenically related to hemolysis but not hemolytic, and a plasmid-encoded heat-stable toxin (EAST 1) unrelated to the heat-stable enterotoxin of ETEC. It is thought that EAggEC adhere to the intestinal mucosa and elaborate the enterotoxins and cytotoxins, which result in secretory diarrhea and mucosal damage. The fifth, enteroinvasive *E. coli* (EIEC) which may result in fever and profuse watery diarrhea containing mucous and streaks of blood. The last; diffusely adherent *E. coli* (DAEC) has been associated with diarrhea in some studies **(Forsythe 2000).**

Food quality assurance programs assure on production of good quality milk with low bacterial count and low somatic cell, which conveys better quality products with longer shelf life **(Pamela et al 2008)**. High *E. coli* count in milk and milk products represents a public health hazard to the consumers and necessitates the need for improving the application of hygienic standards. In this regard, this study was conducted to investigate the prevalence of *E. coli* in raw milk and some dairy products in Beni-Suef governorate.

2. Materials and methods

2.1. Collection of samples

Two hundred samples including 40 raw milk, 40 fresh cream, 20 of each large and small scale yoghurt, 40 Kareish cheese and 20 of each large and small scale ice cream were collected randomly from dairy shops, markets, supermarkets and farmers houses from different localities in Beni-Suef governorate, Egypt. All samples were aseptically transferred to the laboratory in an insulated ice box (4 - 6 °C) within 1–2 h of collection and analyzed immediately upon arrival.

2.2. Preparation of Samples:

Raw milk, fresh cream and yoghurt samples were thoroughly mixed before preparation of serial dilutions. cheese samples were homogenized with 2% sterilized sodium citrate solution in a Colworth stomacher at 45°C for 2 min, while ice cream samples were melted in a thermostatically controlled water bath at a temperature of up to 40 °C for not more than 15 min and mixed well. Serial dilutions of all samples were made in sterile 0.1% peptone water.

2.3. Enumeration of *Escherichia coli* (APHA, 1992):

One tenth of ml of the previously prepared serial dilutions was inoculated into tryptone bile-x-glucouronide agar medium (TBX) and incubated at 44ºC for 18-24h using the spreading method (ISO 2001). Positive reactions were indicated by blue green colonies and sometimes white colonies. Plates showing 30-300 colonies were counted and reported as colony forming unit (CFU) for each ml or g of the sample.

2.4. Biochemical examination of *E. coli* isolates:

Biochemical identification of suspected colonies was carried out according to the methods recommended by APHA (1992) employing the following tests: Indole production, Methyl red test, Voges-Proskauer test and Citrate utilization test.

2.5. Serological identification of *E. coli* isolates:

Identified *E. coli* strains were serotyped using slide agglutination test as described by **Edwards and Ewing (1972),** using polyvalent and monovalent diagnostic *E. coli* antisera (Denka Seiken Co., Ltd. Tokyo, Japan)

2.6. Molecular characterization of *E. coli* using Polymerase chain reaction:

E. coli isolates recovered from raw milk and dairy product samples were subjected for further identification using PCR. Extraction of DNA from the isolates was done following the manufacturer's recommendations using G-spin™ Total DNA Extraction Kit (iNtRON Biotechnology, Korea) according to the technique described by **(Dan Li et al. 1998).** PCR amplification using primer sequences for identification of *E. coli* (*wzy*) gene: (**Colom et al. 2003).** F:AGAGATCCGTCTTTTATTTGTTCGC R:GTTCTGGATACCTAACGCAATACCC

3. Results

Table (1): Statistical analytical results of *E. coli* in raw milk, cream, yoghurt, ice cream and Kareish cheese samples (CFU/ml or gm):

Product	No. of examined samples	Positive samples		Minimum	Maximum	Mean ± SEM
		No	%			
Milk	40	30	75	2×10^2	2×10^7	$8 \times 10^5 \pm 7 \times 10^5$
Cream	40	25	62.5	1×10^3	2.4×10^7	$2 \times 10^6 \pm 1 \times 10^6$
Large scale yoghurt	20	0	0	-	-	-
Small scale yoghurt	20	5	25	5×10^2	2×10^4	$7 \times 10^3 \pm 4 \times 10^3$
Large scale ice cream	20	2	10	14×10^3	1×10^5	$6 \times 10^4 \pm 4 \times 10^4$
Small scale ice cream	20	5	25	2.3×10^2	9×10^5	$2 \times 10^5 \pm 1.8 \times 10^5$
Kareish cheese	40	32	80	5×10^4	6×10^8	$6 \times 10^7 \pm 2 \times 10^7$

Table (2): Serological identification of *E. coli* strains isolated from raw milk samples:

Serodiagnosis *E. coli*	Strain characterization	No	%
O26	EHEC	4	13.3
O111:H4	EHEC	2	6.7
O121	EHEC	3	10
O125:H21	ETEC	5	16.7
O169	ETEC	7	23.3
O126:H7	EPEC	6	20
O158	EPEC	3	10

Table (3): Serological identification of *E. coli* strains isolated from cream samples:

Serodiagnosis *E. coli*	Strain Characterization	No	%
O6:H16	ETEC	4	16
O55:H7	EPEC	7	28
O119:H6	EPEC	3	12
O125:H6	EPEC	6	24
O146:H21	EPEC	5	20

Table (4): Serological identification of *E. coli* strains isolated from small scale yoghurt samples:

Serodiagnosis *E. coli*	Strain Characterization	No	%
O55:H7	EPEC	3	60
O125:H21	ETEC	2	40

Table (5): Serological identification of *E. coli* strains isolated from ice cream samples:

Product	Serodiagnosis *E. coli*	Strain Characterization	No	%
Large scale ice cream	O146:H21	EPEC	2	100
Small scale ice cream	O18	EHEC	3	60
	O159	ETEC	2	40

Table (6): Serological identification of *E. coli* strains isolated from Kareish cheese samples:

Serodiagnosis *E. coli*	Strain Characterization	NO	%
O18	EHEC	8	25
O55:H7	EPEC	7	21.8
O114:H21	EPEC	3	9.4
O158	EPEC	6	18.8
O125:H21	ETEC	3	9.4
O153:H45	ETEC	5	15.6

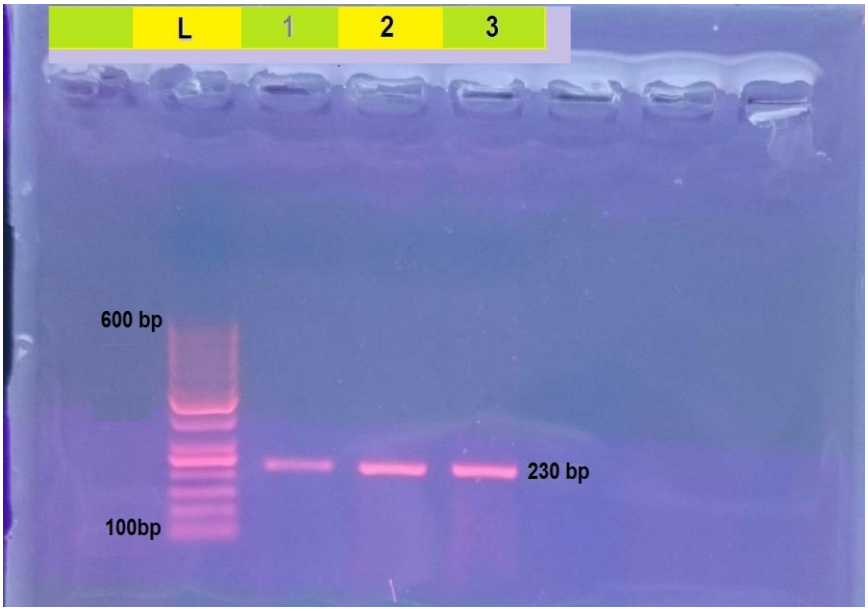

Fig. 1. PCR amplification of *wzy* gene (230 bp) of the isolated *E. coli* strains. Lane L: Ladder. Lanes 1-3 *E. coli* positive isolates.

4. Discussion

E. coli normally inhabits the human and animal gastrointestinal tracts. Hence, its isolation from milk and dairy products is considered as an indicator of direct or indirect fecal pollution. *E. coli* has been repeatedly incriminated in cases of cystitis, pyletitis, pyelonephritis, peritonitis and septicemia as well as food borne outbreaks as reported by **Farahat (1999).** The public health significance of *E. coli* is based on its implication in gastrointestinal illness as epidemic diarrhea in children and cases of food poisoning. *E. coli* bacteria are cited for their ability to grow well in a variety of substrates and utilize a number of carbohydrates and some other organic compounds, therefore, presence of high *E. coli* counts in milk and its products render them of inferior quality being unmarketable during storage or even unfit for human consumption causing economic losses.

It is evident from **(Table 1)** that the prevalence of *E. coli* was 75% in the examined raw milk samples. These results are nearly agreeing with those recorded previously by **Ibrahim et al. (2015).** Higher prevalence was reported by **Oueslati (2011).** Meanwhile, lower prevalence was reported by **Zeinhom (2011).**

The results shown in **(Table 1)** revealed that the prevalence of *E. coli* was 62.5% in the examined cream samples. These results are approximately coinciding with those recorded by **Al-Hadethi et al. (1992).** While lower prevalence was reported by **El-Gendi (2012).**

The results displayed in **(Table 1)** explained that the prevalence of *E. coli* was 0% in the examined large scale yoghurt samples. Similar results were reported by **Moustafa (2004).** In addition, the results shown in **(Table 1)** revealed that the incidence of *E. coli* was 25% in the analyzed small scale yoghurt samples. Higher results were mentioned by **Kandil et al. (2018),** while lower results were recorded by **Mahmoud (1993).**

The results shown in **(Table 1)** clarify that the prevalence of *E. coli* was 10% in the examined large scale ice cream samples. Higher results were detected by **Kandil et al. (2018),** while lower results were recorded by **Sayed and Sayed (2003).**

The results illustrated in **(Table 1)** showed that the prevalence of *E. coli* was 25% in the analyzed small scale ice cream samples. These findings agreed with those recorded previously by **Anuranjini et al. (2008).** Higher results were detected by **Jadav and Rout (2014),** while lower results were mentioned by **Chaleshtori et al. (2017).**

The results tabulated in (Table 1) depicted that the incidence of *E. coli* was 80% in the examined kariesh cheese samples. These results are nearly agreed with those recorded by **Khudir et al. (2013).** Higher results were reported by **Kandil et al. (2018).** While lower results were recorded by **Zeinhom (2011)** and **Nosir (2014).**

Concerning the *E. coli* count, the results found in **(Table 1)** showed that the count of *E. coli* in the tested milk samples ranged from 2×10^2 to 2×10^7 CFU/ml with a mean count of $8.0\times10^5\pm7\times10^5$cfu/ml. Lower results for count were detected by **Gurler (2013).** The results shown in (Table 1) revealed that the count of *E. coli* in the examined cream samples ranged from 1×10^3 to 2.4×10^7 CFU/gm with a mean count of $2\times10^6\pm1\times10^6$ cfu/gm. Lower results for count were mentioned by **Meshref (2013).**

The results tabulated in **(Table 1)** reported that the count of *E. coli* in the examined large scale yoghurt samples was 0%, where *E. coli* could not be detected in any examined sample. Similar results were reported by **Moustafa (2004).**

The results shown in **(Table 1)** revealed that the count of *E. coli* in the tested small scale yoghurt samples ranged from 5×10^2 to 2×10^4 cfu/gm with a mean count of $7\times10^3\pm4\times10^3$ cfu/gm. Higher results were mentioned by **El –Essawy (2000).** On the other hand, lower results were detected by **Morsy (2016).** The results shown in (Table 1) explained that the count of *E. coli* in the tested large scale ice cream samples ranged from 14×10^3 to 1×10^5 cfu/gm with a mean count of $6\times10^4\pm4\times10^4$ cfu/gm. Lower results were mentioned by **Hassan (2003).**

The results illustrated in **(Table 1)** showed that the count of *E. coli* in the analyzed small scale ice cream samples ranged from 2.3×10^2 to 9×10^5 cfu/gm with a mean count of $2\times10^5\pm1.8\times10^5$ cfu/gm. Higher results were postulated by **Sobeih et al. (2002),** while lower results were detected by **Caglayanlar et al. (2009).**

The results shown in (Table 1) explained that the count of *E. coli* in the tested kareish cheese samples ranged from 5×10^4 to 6×10^8 cfu/gm with a mean count of $6\times10^7\pm2x10^7$ cfu/gm. Lower results were mentioned by **Hassan and Afify (2007).**

Although most strains of *E. coli* are harmless, several are known to produce toxins that can cause diseases. The pathogenic groups include enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli*, enteroaggregative *E. coli* (EaggEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC).

It is worth to mention that serological identification of *E. coli* strains revealed: O26 (13.3%), O111: H4

(6.7%), O121 (10%), O125: H21 (16.7%), O169 (23.3%), O126: H7 (20%) and O158 (10%) for raw milk. Also Serological identification of isolated *E. coli* carried out by **El-Nahas et al. (2015)** isolated *E. coli* revealed O114: H21, O11: H4, O26, O127: H6. The isolated strains of *E. coli* were O6: H16 (16%), O55:H7 (28%), O119:H6 (12%), Moreover, O125:H6 (24%) and O146: H21 (20%) for fresh cream. Also, Serological identification of isolated *E. coli* achieved by **El-Nahas et al. (2015)** revealed O114:H21, O111: H4, O26, O127: H6, O119: H6, O55: H7 and O124.

Concerning the small-scale yoghurt, the isolated strains of *E. coli* were O55:H7 (60%) and O125: H21 (40%). Serotyping of the isolated strains of *E. coli* from small scale yoghurt samples achieved by Ahmed (2016) revealed O157, O78, O103, O118, O124, O145 and O164.

The serologically identified *E. coli* revealed O146:H21 (100%) for large scale ice cream and O18 (60%), O159 (40%) for small scale ice cream. Serotyping of the isolated strains from ice cream samples carried out by **Dalal (2012)** showed O86, O55, and O11. Moreover, serotyping of the isolated strains of *E. coli* were O18 (25%), O55: H7 (21.8%), O114:H21 (9.4%), O158 (18.8%), O125:H21 (9.4%) and O153:H45 (15.6%) for Kareish cheese. Also, Serological identification of isolated *E. coli* done by Soukayana (2012) detected *E. coli* O86, O55, O111.

In this study, using the polymerase chain reaction (PCR) on some isolates of *E. coli*, the results revealed that all isolates harbor *wzy* gene. The *wzy* gene is one of virulence genes of *E. coli*, which plays an important role in the synthesis of lipopolysaccharide (LPS) of *E. coli*. LPS are essential in processes critical for bacterial pathogenicity and environmental adaptations **(Zuo et al. 2019).** Moreover, expression of a functional *wzy* gene in *E. coli* strain **Nissle (1917)** increases its ability to withstand the antibacterial defense mechanism of the blood serum of the human **(Grozdanov et al. 2002),** thus *wzy* gene can increase the virulence and the pathogenicity of *E. coli* and consequently, *E. coli* can easily cause diseases for man representing health hazard on human health.

5. Conclusion

The majority of examined raw milk, fresh cream and kareish cheese samples collected from Beni-Suef governorate were contaminated with *E. coli*. The presence of *E. coli* is considered as an index of fecal pollution and probable presence of associated enteric pathogens, which may be accompanied with food poisoning. Interestingly, PCR confirmed that all *E. coli* isolates recovered in this study harbor *wzy* gene (O-antigen polymerase gene) which is associated with LPS biosynthesis and bacterial pathogenicity and increases the ability of *E. coli* to withstand the anti-bacterial defense mechanism. It is highly recommended to enhance milk quality. Raw milk should be produced from healthy animals, proper cleaning of animal's udder and teats before milking, proper cleaning and sanitizing of all dairy utensils and equipment as well as educational packages for handlers and workers.

6. Conflict of interest

No conflicts of interest.

7. References

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