Incidence of *E. coli* in raw milk and its products

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A B S T R A C T

The aim of this study was to investigate the incidence of *E. coli* in raw milk, kariesh cheese and cream. In addition, serological identification of the isolated strains of pathogenic *E. coli* was performed. A total of 120 random samples of raw milk, kariesh cheese and cream (40 each) were collected from different localities in El Menofiya Governorate. Samples were analyzed for total bacterial count (TBC), total coliforms and *E. coli*. The incidences of *E. coli* in the examined samples were 55%, 50%and 47.5%, respectively. Serological identification of isolated pathogenic strains of *E .coli* were: O114:H21, O111:H4, O26 & O127:H6 in raw milk, and were O114:H21, O111:H4, O26, O127:H6, O119:H6 & O55:H7 for kariesh cheese and were O114:H21, O111:H4, O26, O127:H6, O119:H6, O55:H7 and O124 for cream. High microbial counts of the examined samples may constitute a public health hazard to the consumers and emphasizes the need for improved hygienic standards.

Key words: milk, milk products, *E. coli*.

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1. INTRODUCTION

Milk and dairy products are vital sources of nutrition for many people. Milk is universally known as first class of food either for adults or children due to its contributions of high quality animal protein. Milk is highly nutritious medium. Therefore, many bacteria including spoilage and pathogenic bacteria, can grow and propagate in it. Generally, bacteria occur in milk through several ways; colonization of the teat canal or infected udder (clinical and subclinical mastitis), milker (manual as well as automated), extraneous dirt, milk utensils and unclean processing water (Gruezmacher and Bradly,1999; Hayes et al., 2001). Raw milk is also used to produce cheese and other homemade foods. However, several outbreaks from cheese related food borne diseases have been reported (Johnson, 1990) Total bacterial count indicates on farm general hygienic condition, herd health status, milking equipment sanitation and milk storage temperatures (Hayes et al., 2001). Most bacteria in raw milk, (either pathogenic or non pathogenic)can grow and multiply in milk resulting in objectionable changes that rendering it of inferior quality or even unfit for human consumption (Asamenew et al., 2012). *E. coli* is one of the main in habitants of the intestinal tract of most mammalian species, including humans and animals, most *E.coli* are harmless but some are known to be pathogenic bacteria , causing sever intestinal and extra intestinal diseases in man (Kaper*et* al.,2004). Recovery and counting of *E. coli* is used as reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and /or toxigenic *E. coli,* which constitute a public health hazard. Food quality assurance programs focus on producing milk with low somatic cell and bacterial count, resulting in better quality

products with longer shelf life (Pamela et al., 2008).

2. MATERIAL AND METHODS

2.1. Samples:

A total of 120 random samples of raw milk, kareish cheese and cream (40 samples of each) were collected from dairy shops and supermarkets and street vendors in El Menofiya Governorates. All collected samples were aseptically transferred without delay in an insulated ice box to the laboratory and then subjected to microbiological and serological examination. All samples were prepared according to (APHA, 1992).

2.2. Bacteriological examination:

2.2.1. Preparation of serial dilution (APHA, 1992):

25 ml each of the examined samples were add to 225 ml of sterile buffered peptone water and thoroughly mixed to make a dilution 1/10. From which 10 fold serial dilution were prepared. One ml of the clear homogenate was mixed carefully with 9ml of buffered peptone water (% 0.1).

2.2.2. Determination of total bacterial count (TBC) (APHA, 1992).

One ml of the previously prepared serial dilutions was inoculated into peteridishes ,to which melted and cooled to 45c standard plate count was added by pouring method , inoculated plates after thoroughly mixed were left to be solidified before being incubated at 35c° for 48 hours. Plates showing 30-300 colonies were counted and reported as total colony count per ml of sample.

2.2.3. Coliforms content (MPN/ml or g) (APHA, 1992).

One ml from the appropriate dilution were transferred separately into each of 3 MacConkey broths with Durham's tubes, and incubated at 37c° for 48 hrs, the tubs showing acid (yellow color) and gas collected in the inverted Durham's tube were considered positive result. The most probably number (MPN) of coliforms\ ml was determined by using standard table (APHA, 1992).

2.2.4. Isolation and Identification of Coliforms (APHA, 1984).

Isolation of coliforms was achieved by streaking a loopful from each positive MacConkey broth tube on Eosin Methylene blue ager plates, and then incubated for 24 hrs at 37c°. Suspected colonies were transferred to nutrient agar slants and incubated at 37c° for 24 hours. Identification of Coliforms: Morphological examination microscopically and Motility tests (Cruickshank et al, 1980). (BAM, 2001).

2.2.5. Biochemical tests and Serological identification of isolated E. coli (Kok et al .1996).

3. RESULTS

The incidence of pathogenic *E. coli* in raw milk were 55%, 50% and 47.5%, respectively and the incidence of coliforms were 100%, 75% and 95%, respectively. The high incidence of coliforms in raw milk, kareish cheese and cream indicates the bad quality and neglected sanitary measure of these products. Detection of *E. coli* in milk often reflects faecal contamination, although environment coliforms have also been detected in milk. The results tabulated in (table 2) the mean count of *E. coli* was $3.0\times 10^4 \pm 1.3\times 10^4$ in raw milk, for kariesh cheese was $1.2\times 10^3 \pm 0.3\times 10^3$ and for cream was $2.1 \times 10^5 \pm 1.1 \times 10^4$. *E. coli* established to be among the etiological agents causing enteritis and several extra gastrointestinal diseases, as well as the organism recognized as pathogens for humans and animals. Milk can be easily contaminated by infected food handlers who practice poor personal hygiene or by water containing human discharge. Therefore, farmers must be educated in safe handling techniques and proper personal hygienic practice; water

must be safe and practically free from pathogens. The incidence of pathogenic *E. coli* in raw milk were 55%, 50% and 47.5%, respectively and the incidence of coliforms were 100%, 75% and 95%, respectively. The high incidence of coliforms in raw milk, kareish cheese and cream indicates the bad quality and neglected sanitary measure of these products. Detection of *E. coli* in milk often reflects faecal contamination, although environment coliforms have also been detected in milk. Although most strains of *E. coli* are harmless several are known to produce toxins that can cause diarrhea, the pathogenic groups includes Enterotoxigenic *E.coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enterohaemoaghic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAggEC) and diffusely adherent *E. coli* (DAEC).

Table (1): Incidence of coliforms and pathogenic *E. coli* of examined samples (raw milk, kariesh cheese and cream) (n=40).

Type of samples	Coliforms		*E.coli*	
	No. +ve samples	%	No.+ve samples	%
Raw milk	40	100	22	55
Kareish cheese	30	75	20	50
Cream	38	95	19	47.5

Table (2): *E. coli* count in examined samples (raw milk, kariesh cheese and cream cfu/ml or g).

Type of samples	Min	Max	Mean ± SD
Raw milk	4× 10	2.9×10^5	$3.0\times 10^4 \pm 1.3\times 10^4$
Kariesh cheese	2× 10	1.5×10^4	$1.2\times 10^3 \pm 0.3\times 10^3$
Cream	3× 10	9.2×10^5	$2.1\times 10^5 \pm 1.1\times 10^4$

Table (3): Serological identification of *E. coli* Strains isolated from raw milk samples (n=40).

Serodiagnosis*E.coli*	Strains' Characterization	No. of +ve	%
O114:H21	EPEC	3	13.64
O111:H4	EHEC	8	36.36
O26	EHEC	5	22.73
O127:H6	ETEC	6	27.27

Table (4): Serological identification of *E. coli* Strains isolated from kareish cheese samples (n=40).

Serodiagnosis *E.coli*	Strains Characterization	No. of +ve	%
O114:H21	EPEC	5	25
O111:H4	EHEC	2	10
O26	EHEC	7	35
O127:H6	ETEC	3	15

OII9:H6	EPEC	2	10
O55:H7	EPEC	1	5

Table (5): Serological identification of *E. coli* Strains isolated from cream samples (n=40).

Serodiagnosis*E.coli*	Strains Charachterization	No. of +ve	%
O114:H21	EPEC	2	10.53
O111:H4	EHEC	3	15.79
O26	EHEC	4	21.05
O127:H6	ETEC	3	15.79
OII9:H6	EPEC	2	10.53
O55:H7	EPEC	1	5.26
O124	ETEC	4	21.05

4. DISCUSSION

In our study, the incidence of pathogenic *E. coli* in raw milk, kariesh cheese and cream were 55%, 50% and 47.5% respectively. Similar results were reported by Arafa (2013) & Bahout and Mustafa (2006). Higher results were reported by Meshref and Hassan (2009) and AbdEl-Hameid (2013). Lower results reported by Tasci (2011), Hassan et al., (2008) and El-Gendi (2012). The incidence of coliforms was 100% in raw milk, 75% in kareish cheese and 95% in cream. These results of coliforms in raw milk was similar to El Prince *et al.,* (2010), Lower results reported by Abd El-Latif et al., (2012). Our results of coliforms in kariesh cheese stated an incidence of 75% (Table 1). Similar results were reported by Abo Zeed (2014). Higher results were reported by Abd El-Latif et al., (2012), Lower result was reported by Abd-Elmaaboud (2014). The incidence of coliforms in cream was 95% (Table 1). Nearly similar results were reported by El Bagoury (1988). Higher results were reported by Abd El-Hameid (2013). While lower results were reported by El-Bakery (2004). -*E. coli* count. The results tabulated in (Table 2) stated the incidence of *E. coli* in the examined samples for raw milk, the mean count of *E.coli* was $3.0\times 10^4 \pm 1.3\times 10^4$cfu/ml. Nearly similar result was recorded by Arafa (2013). However, lower results were recorded by Tasci (2011). For kariesh cheese samples, the mean count of *E. coli* was $1.2 \times 10^3 \pm 0.3\times 10^3$cfu/g (Table 2). Similar results were recorded by El-Syed et al., (2011). Higher results were recorded by Meshref and Hassan (2009). The mean count of *E. coli* in cream was $2.1\times 10^4 \pm 1.1\times 10^4$ cfu/g (Table 2). Similar results to the results reported by Arafa (2013). Serological identification of isolated *E. coli.* In our study, the isolated strains of pathogenic *E. coli* isolated from examined raw milk samples were illustrated as O_{114}: H_{21}, O_{111}:H_4, O_{26}, and O_{127}:H_6. Serological identification of isolated *E. coli* also carried out by AbdEl-Maabud (2014) isolated *E. coli* O26 and O128 from raw milk samples. The isolated strains of *E. coli* from Kareish cheese, the strains were *E. coli* O_{114}:H_{21}, O_{111}:H_4 O26, O127:H6 O119:H6 and O55. Serological identification of *E. coli* was carried out by Abd El-Maaboud (2014) isolated *E. coli* O26 and O124 from Kareish cheese samples.The isolated strains of *E. coli*from cream, the strains were *E. coli* O114:H21, O111:H4, O26, O127:H6, O127:H6, O119:H6, O55:H7 and O124.Sabet (2003) isolated *E. coli* O55from cream samples.

5. CONCLUSION

Information given by the results obtained allowed conclude that the majority of

examined raw milk, kariesh cheese and cream collected from El-Menofiya Governorate were contaminated with different degrees with pathogenic and non pathogenic organisms give an indication of poor sanitary measures adopted during milking, manufacturing, handling and distribution of milk products. The following suggestion must be put in consideration to improve milk quality; raw milk should be produced from healthy animals, proper cleaning of animals udder and teats before milking, washing and sanitization of all dairy utensils and equipment and refrigeration of milk, to about 4c and milk products.

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