Assessment Of Milk, Eggs And Some Of Their Products For Occurrence Of Coliforms

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ABSTRACT

This survey study was conducted along a period of more than 5 years (from April 2004 to September 2009) to estimate the prevalence of coliforms in milk, eggs and their products. Therefore, a total of 690 samples (450 of milk and milk products in 6 categories and 240 of eggs and egg products in 3 categories) were collected randomly from different markets, supermarkets, groceries, retailers, confectionary shops, bakeries, poultry farms and farmers distributed in Assiut city, Egypt. The 450 milk and milk products samples were represented as 30 samples of each of raw market milk, plain yoghurt, small scale ice cream, cooking butter, cheese (10 types) "Kareish, Domiati, Tallaga, Bramily, Fayomi, processed, Cheddar, Gouda, Roquefort and Edam" in addition to locally manufactured dried milk-based baby food. Moreover, the 240 eggs and egg products samples were represented as 30 samples of each of table eggs "poultry farm eggs, Baladi hen eggs and duck eggs", egg-based sour products "commercial mayonnaise" and egg-based dessert products "cream caramel, cake, Jatooh and biscuit". To assess their bacteriological quality, all the collected samples were examined for the incidence and counts of coliforms, fecal coliforms and *E. coli* using MPN technique. The most important obtained results were the occurrence of coliforms in all the examined raw market milk and Kareish cheese samples, in addition to, the occurrence of coliforms and fecal coliforms in 86.67 and 60% with an average count of 3.01×10^2 and 98.35 cfu/g in the examined Jatooh samples, respectively. The obtained results were compared with the international and Egyptian standards. The suggestive hygienic control measures for controlling the incidence of these bacteria were discussed.

INTRODUCTION

It is generally accepted that milk and eggs are valuable and indispensable foods. There is no need to talk about the nutritive value of milk. Also, eggs are considered as a complete perfect food, rich in many nutrients and economically accessible *(1)*. Eggs are one of the few foods that are used among popular dishes consumed by people at home, restaurants and convenience stores in their natural states with no artificial additives. Eggs are usually consumed either as table eggs or as egg products. In 2004, more than 30% out of 76 billion eggs were used in the form of egg products, as they provided certain desirable function attribute *(2)*.

In spite of the high nutritive value of milk and eggs, they are responsible for several outbreaks and considered vehicles for transmission of certain human pathogens *(3)*. Eggs are as rapidly perishable as milk, yet the fragile shell, if undamaged and dry will usually keep the eggs edible for reasonable time. Generally, fresh eggs are devoid of bacteria, unless the ovary of laying hens is infected with pathogens (congenitally) *(4)* and egg shells also could be contaminated by hen's fecal matter and nest's lining or during washing of eggs, handling and packaging *(5)*. Microorganisms either on egg shells or egg contents can contaminate egg products and upon appropriate conditions, they proliferate resulting in illness or food poisoning to consumers.

Coliforms are intestinal and non-intestinal inhabitant, so, their presence in food gives an index of poor sanitation and the presence of enteric pathogens *(6)*. Their occurrence indicates the unhygienic condition during production, processing, handling and distribution. Coliforms are considered one of the contaminants causing spoilage.

The presence of coliforms in milk and milk products reflect the inadequate sanitation during production and handling of raw materials *(7)*. Coliforms count is traditional indicator of possible fecal contamination, microbial quality and wholesomeness and reflect the hygienic standards adopted in the dairy processing *(8)*. The presence of numbers of coliforms in cheese indicates that opportunity for proliferation might have occurred, which could also have allowed multiplication of Salmonella, Shigella, Staphylococci or other organisms possibly introduced due to poor sanitation *(9)*. High coliforms count render eggs of inferior quality and become unmarketable during storage or even unfit for human consumption leading to public health hazards and economic losses *(10)*.

As *E. coli* constitutes a part of the intestinal normal flora of human and some animals, the microbiological criteria involving *E. coli* are commonly used as an indicator of fecal contamination and process failure and/or post-process contamination by equipment, personnel or raw materials are possible causes for the presence of *E. coli* in heat-processed food. *E. coli* is commonly used in the microbiological evaluation of foodstuffs as indicators of poor hygiene and poor handling practices *(11)*. The presence of *E. coli* above certain threshold is indicative that the foodstuff is either unsatisfactory or unacceptable with potentially hazardous *(12)*.

Infection with *E. coli* is emerging as an important public health problem due the gravity of the disease and its increasing trend *(13)*. Moreover, from the public health point of view enteropathogenic serotypes of *E. coli* has been implicated in human gastroenteritis, epidemic diarrhea in infants, summer diarrhea in children as well as many cases of food poisoning *(14)*.

According to the aforementioned facts about the significance of coliforms in foods, this survey study was designed and run to assess and evaluate a wide range of consumed foods (milk & milk products and eggs & egg products) for incidence and counts of coliforms, fecal coliforms and *E. coli* as indicator organisms of choice in examining foodstuffs.

MATERIAL AND METHODS

1. Samples

A total of 690 samples, 450 samples of milk and milk products (in 6 categories) and 240 samples of eggs and egg products (in 3 categories), were collected randomly from different markets, supermarkets, groceries, retailers, confectionary shops, bakeries, poultry farms and farmers distributed in Assiut city, Egypt. Each sample was obtained in its retail container as sold to the public and directed to the laboratory under strict hygienic measures with a minimum of delay to be examined bacteriologically.

2. Preparation of samples

Each product was represented by 30 samples . All the examined samples were opened aseptically and 10 g of each sample were aseptically weighed and added to 90 ml of 0.1% sterile peptone water and homogenized for 2 min to obtain a dilution of 10^{-1} *(15)*, then decimal dilutions were made and followed by bacteriological examination.

3. Bacteriological examination

3.1. Coliforms count (MPN/g) *(16)*

3.1.1. The presumptive test: One ml of each previously prepared 10^{-1}, 10^{-2} and 10^{-3} dilutions was inoculated into 3 replicate test tubes of sterile Lauryl Sulphate broth (LS) with inverted Durham's tubes. The inoculated LS tubes were incubated at 35±0.5°C for 48±2 h. The tubes showing gas in the Durham's tubes (positive tubes) within 48±2 h were submitted to confirmatory test for coliforms group.

3.1.2. The confirmatory test: The positive LS tubes were subcultured into corresponding sterile Brilliant Green Lactose Bile 2% broth (BGLB) with inverted Durham's tubes. Inoculated tubes were incubated at 35±0.5°C for 48±2 h. BGLB tubes showing gas production were recorded and considered positive for coliforms. The numbers of

coliforms/g were calculated from MPN Tables for 3 tubes dilutions.

3.2. Fecal coliforms count (MPN/g) *(16)*: The positive BGLB tubes showing gas production were subcultured into corresponding sterile EC broth with inverted Durham's tubes. Inoculated EC broth tubes were incubated at 45.5±0.5°C for 48±2 h. Positive tubes showing gas production in their Durham's tubes were recorded and considered positive for fecal coliforms. The numbers of fecal coliforms/g were calculated using MPN tables for 3 tubes dilution.

3.3. *E. coli* count (MPN/g) *(16)*: The positive EC broth tubes denoted by gas production within 48±2 h were subcultured by streaking onto sterile Eosin Methylene Blue (EMB) plates. Inoculated EMB plates were incubated at 35°C for 24±2 h. The typical nucleated (dark center) colonies with metallic sheen were considered to be *E. coli*. Positive EMB plates for *E. coli* were recorded and the numbers of *E. coli*/g were calculated from MPN Tables for 3 tubes dilution.

3.4. Biochemical reactions for identification of *E. coli* by IMViC tests: *E. coli* is positive for indole production *(17)* and methyl red *(17)* tests and negative for Voges-Proskauer *(18)* and citrate utilization *(17)* tests.

RESULTS

The bacteriological examination using MPN technique assessed coliforms (Table 1) and fecal coliforms (Table 2) for the 450 samples of milk and milk products which revealed that 52 samples (11.56%) contained *E. coli* (Table 3). While, the assessment of coliforms (Table 4) and fecal coliforms (Table 5) for the 240 samples of eggs and egg products revealed 2 samples (0.83%) contained *E. coli* (Table 6).

Table 1. Incidence of coliforms in the examined milk and milk products.

Category	Products	Samples (n = 30)		Count (MPN/g)		
		Positive	%	Min.	Max.	Average
Milk	Raw market milk	30	100	23	1.11×10^3	1×10^3
Fermented milk	Plain yoghurt	14	46.67	<3*	1.11×10^3	2×10^2
Ice cream	Small scale ice cream	28	93.33	<3	1.11×10^3	5.83×10^2
Butter	Cooking butter	22	73.33	<3	1.11×10^3	6.43×10^2
Cheese	Kareish cheese	30	100	3	1.11×10^3	8.77×10^2
	Domiati cheese	7	23.33	<3	1.11×10^3	46.25
	Tallaga cheese	12	40	<3	1.1×10^3	2.13×10^2
	Bramily cheese	10	33.33	<3	39	6.84
	Fayomi cheese	1	3.33	<3	6.2	0.21
	Processed cheese	5	16.67	<3	1.11×10^3	53.07
	Cheddar cheese	1	3.33	<3	64	2.13
	Gouda cheese	1	3.33	<3	53	1.77
	Roquefort cheese	1	3.33	<3	6.1	0.2
	Edam cheese	7	23.33	<3	1.6×10^2	10.74
Baby food	Dried milk-based baby food	2	6.67	<3	7.3	0.45

*<3 mean negative LS broth tubes otherwise BGLB broth tubes.

Table 2. Incidence of fecal coliforms in the examined milk and milk products.

Category	Products	Samples (n = 30)		Count (MPN/g)		
		Positive	%	Min.	Max.	Average
Milk	Raw market milk	18	60	<3*	1.11×10^{3}	5.16×10^{2}
Fermented milk	Plain yoghurt	11	36.67	<3	1.11×10^{3}	1.95×10^{2}
Ice cream	Small scale ice cream	17	56.67	<3	1.11×10^{3}	2.57×10^{2}
Butter	Cooking butter	22	73.33	<3	1.11×10^{3}	5.41×10^{2}
Cheese	Kareish cheese	25	83.33	<3	1.11×10^{3}	7.81×10^{2}
	Domiati cheese	2	6.67	<3	93	4
	Tallaga cheese	5	16.67	<3	1.1×10^{3}	46.9
	Bramily cheese	5	16.67	<3	39	3.04
	Fayomi cheese	1	3.33	<3	3	0.1
	Processed cheese	2	6.67	<3	9.1	0.42
	Cheddar cheese	0	0	<3	<3	<3
	Gouda cheese	1	3.33	<3	7.2	0.24
	Roquefort cheese	0	0	<3	<3	<3
	Edam cheese	4	13.33	<3	12	1.02
Baby food	Dried milk-based baby food	0	0	<3	<3	<3

*<3 mean negative EC broth tubes.

Table 3. Incidence of *E. coli* in the examined milk and milk products.

Category	Products	Samples (n = 30)		Count (MPN/g)		
		Positive	%	Min.	Max.	Average
Milk	Raw market milk	1	3.33	<3*	9.1	0.3
Fermented milk	Plain yoghurt	6	20	<3	1.11×10^{3}	56.18
Ice cream	Small scale ice cream	2	6.67	<3	1.11×10^{3}	37.37
Butter	Cooking butter	19	63.33	<3	1.11×10^{3}	3.3×10^{2}
Cheese	Kareish cheese	10	33.33	<3	1.11×10^{3}	2.59×10^{2}
	Domiati cheese	1	3.33	<3	93	3.1
	Tallaga cheese	4	13.33	<3	2.1×10^{2}	8.21
	Bramily cheese	3	10	<3	39	2.07
	Fayomi cheese	0	0	<3	<3	<3
	Processed cheese	1	3.33	<3	3.6	0.12
	Cheddar cheese	0	0	<3	<3	<3
	Gouda cheese	1	3.33	<3	7.2	0.24
	Roquefort cheese	0	0	<3	<3	<3
	Edam cheese	4	13.33	<3	12	1.02
Baby food	Dried milk-based baby food	0	0	<3	<3	<3

*<3 mean negative EMB plates.

Table 4. Incidence of coliforms in the examined eggs and egg products.

Category	Products	Samples (n = 30)		Count (MPN/g)		
		Positive	%	Min.	Max.	Average
Table eggs	Poultry farm eggs	11	36.67	<3*	75	6.89
	Baladi hen eggs	15	50	<3	1.1×10^3	53.28
	Duck eggs	10	33.33	<3	1.1×10^3	81.06
Egg-based sour products	Commercial mayonnaise	0	0	<3	<3	<3
Egg-based dessert products	Cream caramel	2	6.67	<3	23	1.53
	Cake	4	13.33	<3	23	2.47
	Jatooh	26	86.67	<3	1.11×10^3	3.01×10^2
	Biscuit	7	23.33	<3	43	4.12

*<3 mean negative LS broth tubes otherwise BGLB broth tubes.

Table 5. Incidence of fecal coliforms in the examined eggs and egg products.

Category	Products	Samples (n = 30)		Count (MPN/g)		
		Positive	%	Min.	Max.	Average
Table eggs	Poultry farm eggs	3	10	<3*	64	2.43
	Baladi hen eggs	7	23.33	<3	75	6.07
	Duck eggs	3	10	<3	64	3.01
Egg-based sour products	Commercial mayonnaise	0	0	<3	<3	<3
Egg-based dessert products	Cream caramel	0	0	<3	<3	<3
	Cake	0	0	<3	<3	<3
	Jatooh	18	60	<3	1.1×10^3	98.35
	Biscuit	0	0	<3	<3	<3

*<3 mean negative EC broth tubes.

Table 6. Incidence of *E. coli* in the examined eggs and egg products.

Category	Products	Samples (n = 30)		Count (MPN/g)		
		Positive	%	Min.	Max.	Average
Table eggs	Poultry farm eggs	1	3.33	<3*	6	0.2
	Baladi hen eggs	0	0	<3	<3	<3
	Duck eggs	1	3.33	<3	7.3	0.24
Egg-based sour products	Commercial mayonnaise	0	0	<3	<3	<3
Egg-based dessert products	Cream caramel	0	0	<3	<3	<3
	Cake	0	0	<3	<3	<3
	Jatooh	0	0	<3	<3	<3
	Biscuit	0	0	<3	<3	<3

*<3 mean negative EMB plates.

DISCUSSION

There is no doubt that assessment of milk and milk products and eggs and their products for coliforms is highly significant for their hygienic evaluation. However, coliforms and fecal coliforms still continue to be considered as indicator organisms of choice in examining foods as their absence indicated the product is of a good microbiological quality. Therefore, the authors aimed to investigate coliforms organisms including fecal coliforms and *E. coli* than most other groups of bacteria owing to their importance as indicator organisms in routine analysis to ascertain the

microbiological quality *(19)*. In recent years much attention had been paid towards *E. coli*, due to its importance as an organism of true fecal origin with possible existence of associated enteric pathogens. Otherwise, *E. coli* was the end goal of coliforms examination through MPN technique.

It was obvious from Table 1 that all the examined raw market milk samples (100%) contained coliforms, while coliforms could be detected in 46.67, 93.33 and 73.33% in the examined plain yoghurt, small scale ice cream and cooking butter, respectively (Table 1). It was expected to find high incidences of coliforms in these products as their manufacture was made from raw milk without further heat treatment *(20)*.

Among the examined cheese types, the relatively high coliforms including fecal coliforms and *E. coli* counts were recorded in Kareish cheese (Tables 1-3) due to its exposure to contamination during its production and handling, as the Kareish cheese is mostly made from raw milk and usually sold fresh, besides to the organisms could be grown readily in the product *(20)*. The high count of coliforms gave an indication about fecal contamination, un-personal hygiene and unsanitary conditions during processing and handling of cheese *(8)* as well as possible existence of other enteric pathogens *(21)*.

Egyptian Standards *(22)* stated that cheeses must be free from *E. coli*. According to these standards, all the examined cheeses were unsatisfactory except Fayomi, Cheddar and Roquefort because they were free from *E. coli* (Table 3). Gilbert et al. *(23)* represented guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale including cheese. The microbiological quality for criterion *E. coli* count was satisfactory as <20 cfu/g, acceptable as 20-$<10^2$ cfu/g, unsatisfactory as $\geq 10^2$ cfu/g. According to these guidelines, all the examined cheeses were satisfactory except the Kareish one was unsatisfactory (Table 3). Moreover, Food Standards *(24)* declared the microbiological limit for cheese regarding the incidence of *E. coli* to be acceptable at 10 cfu/g, while if exceeded more than 10^2 cfu/g in one or more samples would cause rejection of the lot. By comparing the obtained results (Table 3) with Food Standards *(24)*, all the examined cheese samples are acceptable except the Kareish one.

As the proper pasteurization could kill *E. coli* but its presence in the products gave an indication about post-pasteurization contamination. The high incidence and count of *E. coli* in the examined samples revealed that the sanitation was improper. Spiliman and Schmidt-Lorhnz *(25)* concluded that the most effective measures for keeping the coliforms count low in cheese were efficient pasteurization of cheese milk and avoidance of recontamination during subsequent processing.

Comparing the results obtained of coliforms in the examined dried milk-based baby food samples (Table 1), it could be concluded that the average coliforms count was in agreement with microbiological standards for dry milk products (10 cfu/g) *(26)* and also with the published microbiological criteria (<100 cfu/g) *(27)*.

Presence of coliforms either in eggs or egg products accounts a significant indicator for pinpointing the unhygienic conditions during production, processing, handling and distribution. The results recorded in Table 4 proved that most of the Gatooh samples (86.67%) had coliforms. The fecal coliforms as shown in Table 5 could not be recovered from all the examined egg-based dessert products except Gatooh that revealed fecal coliforms with considerable percentage (60%). These data proved that most of these products except Gatooh had non significant numbers of coliforms and all of them failed to recover *E. coli* (Table 6).

Unfortunately, the presence of coliforms and fecal coliforms in Gatooh samples was attributed to either post-processing contamination or from contaminated egg shells during breaking and blending egg contents, as well as the unpasteurized cream layer added after processing *(28)*. However, it was worthwhile to state that the contamination of

Gatooh samples by coliforms beyond certain level should be considered of a public health hazard as they might cause dreadful diarrheal disease *(29)*. Besides, the existence of fecal coliforms may be a real index of fecal pollution and possible existence of associated pathogens. According to the obtained data, the authors recommend the need of improving the quality of Gatooh through the use of pasteurized ingredients especially the cream layer added to the product after its preparation.

The obtained data presented in Table 4 regarding the freedom of all the examined mayonnaise samples from coliforms was in agreement with Sayed and Abdel-Haleem *(30)*. The absence of coliforms in the examined mayonnaise samples was associated with failure to detect fecal coliforms (Table 5) and *E. coli* (Table 6) could be considered as an index of satisfactory sanitation.

Among the examined eggs samples, duck eggs contained relatively higher average count of coliforms (Table 4), and that might be attributed to duck eggs contain a rather high percent of contamination as they lay their eggs nearer to damp places (ponds) with high moisture content. They used to pick up flies and other infective materials, their egg shells were thinner than that of hens' eggs and finally the antibacterial activity of the albumen deteriorates rapidly on storage *(31)*. Seviour *et al.* *(32)* recorded that Enterobacteriaceae mainly *E. coli*, constituted the main part of flora in wild fowl and duck more than that of chicken eggs.

The obtained results of poultry farm eggs (Tables 4- 6) showed good quality according to Speck *(18)* who stated that fresh hens' eggs mostly contained microorganisms <10 cfu/g and seldom 10^2 cfu/g. Also, Speck *(18)* found that the coliforms count ranged from 10^3-10^5 cfu/g of fresh liquid eggs. Morton *(33)* reported that *E. coli* was isolated from gastrointestinal outbreak following the consumption of fresh shell-intact table eggs.

Finally, for production of high quality milk and milk products and eggs as well as egg products, good manufacturing practices and implementation of HACCP program in food manufacturing and food preparation should be done to improve the quality and control the pathogenic microorganisms.

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الملخص العربي

تقييم اللبن والبيض وبعض منتجاتهما لوجود الميكروبات القولونيه

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لقد أجريت هذه الدراسة المسحية على مدار أكثر من ٥ سنوات (من أبريل ٢٠٠٤ حتى سبتمبر ٢٠٠٩) وذلك لمعرفة مدى تلوث اللبن والبيض وبعض منتجاتهما بالميكروبات القولونيه حيث تم جمع اجمالي عدد ٦٩٠ عينة (٤٥٠ عينة من الألبان ومنتجاتها في صورة ٦ أصناف و٢٤٠ عينة من البيض ومنتجاته في صورة ٣ أصناف) جمعت عشوائيا من مختلف الأسواق والسوبر ماركت ومحلات البقالة والباعة ومحلات الحلويات والمخابز ومزارع الدواجن والفلاحين الموزعة بمدينة أسيوط، مصر. حيث كانت ٤٥٠ عينة من الألبان ومنتجاتها ممثلة بمعدل ٣٠ عينة من كل من لبن الأسواق الخام والزبادي البلدي والأيس كريم المنتج على المستوى الصغير والزبدة الفلاحي والجبن (عشرة أنواع) "القريش والدمياطي والثلاجة والبراميلي والفيومي والمطبوخ والشيدر والجودا والريكفورت والإديم" بالإضافة إلى أغذية الأطفال المصنعة محليا والمطعمة باللبن الجاف، علاوة على ذلك كانت ٢٤٠ عينة من البيض ومنتجاته ممثلة بمعدل ٣٠ عينة من كل من بيض المائدة "بيض مزارع الدواجن والبيض البلدي وبيض البط" ومنتجات البيض الحامضية "المايونيز التجاري" ومنتجات الحلاوي المحتوية على البيض "الكريم كراميل والكيك والجاتوه والبسكويت". ولقياس الجودة البكتريولوجية، فقد تم فحص كل العينات لوجود وأعداد الميكروبات القولونيه والقولونيه البرازية وبكتريا الإيشيريشيا كولاي القولونيه بإستخدام MPN technique. وقد أسفرت أهم النتائج عن تواجد الميكروبات القولونيه في جميع عينات لبن الأسواق الخام والجبن القريش بالإضافة إلى تواجد الميكروبات القولونيه والقولونيه البرازية بنسب ٨٦,٦٧ و٦٠% بمتوسط قدره ٣,٠١×١٠[٢] و٩٨,٣٥ للجرام في عينات الجاتوه على التوالي. وقد تمت مقارنة النتائج المتحصل عليها بالمواصفات القياسية العالمية والمصرية والاشتراطات الصحية الواجب اتباعها لمنع تلوث اللبن والبيض ومنتجاتهما بتلك الميكروبات.