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INCIDENCE OF COLIFORMS IN MILK AND DAIRY PRODUCTS IN SOME EGYPTIAN MARKETS

By

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ABSTRACT

In order to determine the hygienic quality of milk and dairy products in some Egyptian markets, 150 samples, 30 each of : Raw milk,Kariesh cheese, Feta cheese, small scale yoghurt and large scale produced yoghurt, were randomly collected from different dairy shops and supermarkets in Giza, Cairo and Fayoum governorates, Egypt. Collected samples were examined microbiologically for the presence of coliforms group. Coliforms proved to be present in 100%, 86.7% and 96.7 % of examined samples of raw milk, Kariesh cheese and Small Scale yoghurt with a mean count of $13 \times 10^4 \pm 7.7 \times 10^4$, $90 \times 10^4 \pm 50.5 \times 10^4$ and $20 \times 10^7 \pm 7.5 \times 10^7$ MPN/ml. or g. respectively, while coliforms failed to be detected in fata cheese and large scale yoghurt samples.The identification of the isolated strains (78) revealed the presence of 6 different species of coliforms.The economic and public health significance, as well as, the preventive control measures of contamination of dairy products with coliforms were discussed.

Key Words:

Raw milk, Kariesh cheese, Feta cheese, yoghurt, Coliform, *E-coli.*

INTRODUCTION

Milk is a complex biological fluid and a nutrient dense food. Both milk and dairy products provide human diet with many nutrients as proteins, amino acids,fat,lactose,vitamins, calcium and phosphorus. They also provide good growth media for different microorganisms **(Saeed, 2016).**

The microbial quality of milk and dairy products is affected by many factors e.g. the initial flora of raw Milk, the applied hygienic measures during production, handling, processing and storage **(Van Kessel *et al.,* 2004 and Rajagopal *et al.,* 2005).**

Kareish cheese is a type of white soft cheese produced traditionally from naturally fermented raw milk. It is popularly consumed by poor and middle classes of Egyptians.

Its microbiological quality is directly affected by environmental hygienic conditions during manufacturing and retail **(Robinson and Tamime,2002)**. Due to the nature of raw milk cheeses; the presence of coliforms is not unexpected as coliforms are common in raw milk.

Feta cheese belongs to a family of Greek white brined cheeses **(Anifantakis, 1991).** It is made originally from sheep's milk or a mixture of sheep and goat's milk (goat's milk not exceed 30 %), with a slightly aromatic flavour due to the use of *Lactococcus lactis subspp. cremoris* and *lactis* or *Streptococcus thermophilus* and Lactobacillus delbrueckii *spp. bulgaricus* to ripen the milk at 30-32 ºC which lower the pH of the final product to 4.4 - 4.6, along with salt percentage that reaches up to 3%. It was traditionally made from unpasteurized milk **(Bintsis and Papademas,2002).** Nowadays the Egyptian type of feta cheese produced from pasteurized milk (mainly cow's milk).

Yogurt is produced from heat treated milk which is cooled before inoculation with starter culture and incubated at 44ºC for 3-4 hours, before being cooled storage. It is type of fermented dairy products which commonly consumed due to its high nutritional value and therapeutic benefits. Failure of heat-treatment, unhygienic production environment and post-processing conditions may lead to the contamination of the product with coliforms **(Mahmoud and Saleh, 2016).**

The existence of coliforms in milk and dairy products can be considered as a sign of insufficient hygienic conditions during production and further handling processes. Presence of coliforms possesses economic importance as it leads to inferior quality of the products; on the other hand, their presence may constitute biological hazards to the consumer **(Bülte, 2004 and Wernery, 2007).**

This study aimed to through light on the hygienic quality of raw milk and some dairy products exposed for sale in some Egyptian markets, through determining the incidence of contamination of such products with coliforms, identifying the isolated ones, as well as, suggesting the required control and preventive measures to produce high quality and safe dairy products.

MATERIAL AND METHODS

Collection of samples:

Hundred and fifty samples representing 30 each of (Raw milk, Kariesh cheese, Feta cheese, small scale yoghurt and large scale produced yoghurt) were collected from dairy shops and supermarkets in Giza, Cairo and fayoum governorates. Collected samples were transferred to the laboratory in an insulated ice-box with a minimum of delay to be immediately microbiologically examined.

Microbiological Examination:

-Preparation of samples: (APHA, 2004).

- Preparation of decimal dilutions: (APHA, 2004).

- Coliform Count (MPN/ml. or g.): (APHA 2001).

- Isolation and identification of coliforms: (Silva *et al,* 2013).

RESULTS

Table (1): Incidence and statistical analytical results of Coliforms count (MPN/ml. or gm.) in the examined positive samples.

Type of sample	No. of examined samples	positive samples		Coliform Counts (MPN/ml. or gm.)			
		No.	%	Min.	Max.	Mean	± S.E.M.
Raw milk	30	30	100	70	21×10^5	13×10^4	7.7×10^4
Kariesh cheese	30	26	86.7	110	11×10^6	90×10^4	50.5×10^4
Feta cheese	30	0	0	0	0	0	0
small scale yoghurt	30	29	97.7	0.3	11×10^8	20×10^7	7.5×10^7
large scale yoghurt	30	0	0	0	0	0	0
Total	150	85	56.7				

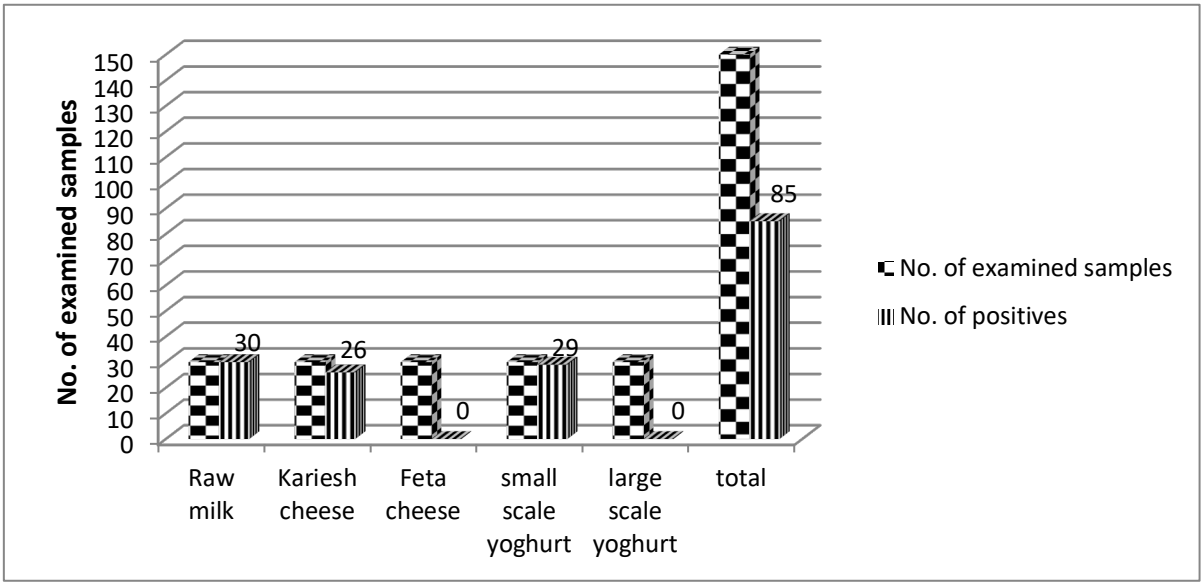

Fig. (1): Incidence of coliforms count (MPN/ml. or g.) in examined samples.

Table (2): Frequency distribution of examined samples based on their coliform count (MPN/ml.or g.).

Intervals	Raw Milk		Kariesh cheese		Feta cheese		small scale yoghurt		Large scale Yogurt	
	No.	%	No.	%	No.	%	No.	%	No.	%
< 10	0	0	4	13.3	30	100	3	10	30	100
10 - < 10^2	2	6.6	0	0	0	0	3	10	0	0
10^2 - 10^4	20	66.7	7	23.4	0	0	3	10	0	0
10^4 - 10^6	6	20	15	50	0	0	8	26.7	0	0
10^6 - 10^8	2	6.6	4	13.3	0	0	8	26.7	0	0
> 10^8	0	0	0	0	0	0	5	16.6	0	0
Total	30	100	30	100	30	100	30	100	30	100

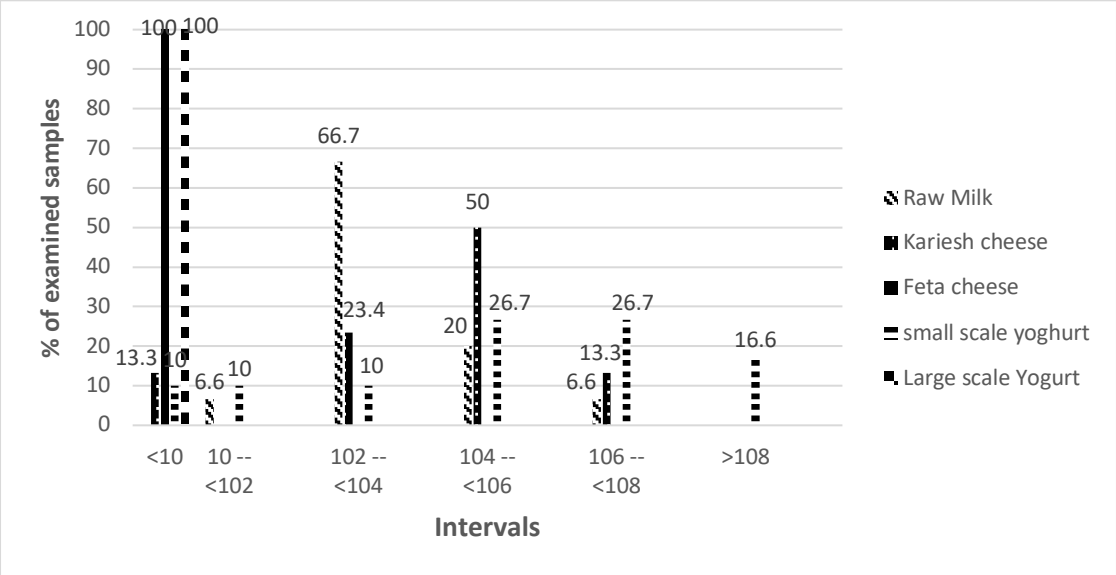

Fig. (2): Frequency distribution of examined samples based on their coliform count (MPN/ml. or g.).

Table (3): Frequency distribution of isolated coliform strains based on their identification: (No. of isolates 78), According to **Bergey's Manual of systematic bacteriology (2009):**

Isolated spp.	Raw milk		Kariesh cheese		Yoghurt (small scale)		Total	
	No.	%	No.	%	No.	%	No.	%
Escherichia coli	14	66.7	6	33.3	10	25.6	30	38.5
Klebsiella. spp.	5	23.8	1	5.5	14	35.9	20	25.6
Enterobacter intermedius	2	9.5	1	5.5	3	7.7	6	7.7
Citrobacter spp.	0	0	9	50	9	23.1	18	23.1
Serratia fonticola	0	0	0	0	1	2.6	1	1.3
Hafnia spp.	0	0	1	5.5	2	5.1	3	3.8
Total	21	100	18	100	39	100	78	100

DISCUSSION

Coliforms group are defined as gram negative, aerobic or facultative anaerobic non-spore forming rods, lactose fermenters with the production of acid and gas at 35ºC that normally inhabit the gastrointestinal tract of both human and animals **(Davidson *et al.,* 2004)**. They were formerly considered to represent "*Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter*" strains, differentiated originally by sucrose and dulcitol fermentation, indole production and gelatine liquefaction, Later on; **Parr (1983)** established the IMViC tests for more classification and differentiation of the group. **Leclerc *et al.,* (2001)** categorized them into 3 main groups: "thermophilic" group including *Escherichia coli* of faecal origin," thermophilic and ubiquitous" group that is found in natural environments e.g. soil, grass and silage, as members of *Klebsiella*, *Enterobacter* and *Citrobacter* and "psychrotrophic" group which are mainly environmental, e.g. some member of genera *Klebsiella*, *Enterobacter* and *Serratia.* Coliforms group is still a cornerstone in food and water testing in dairy industry giving somewhat an overall view on the hygienic conditions used during the process, Nevertheless, Coliform count is being traditionally used to be an indicator for possible fecal contamination, adopted during production, processing and handling of food which may contribute to its loss of quality, spoilage or may even create a potential health hazard **(Vanderzant and Splittstoesser, 2005).**

Regarding the results presented in (Tables 1, 2), coliforms could be detected in 100 % of the examined raw milk samples. The most samples (66.7 %) had coliforms count ranging from 10^2 to 10^4. The results were nearly similar to that reported by **(Ashenafi *et al.,* 2016) and Van Kessel *et al,* (2004)** who found that 96 % of samples collected from bulk milk in a study done in 2002, were contaminated by coliforms as well as many researches in the U.S. reported the coliforms level in raw milk vary greatly with a mean counts ranging from 31-2750 cfu/ml. **(Boor *et al.,* 1998; Jayarao and Wang, 1999; D'Amico *et al.,* 2008; Pantoja *et al.,* 2011 and Jackson *et al.,* 2012)**.

California Department of Food and Agriculture (2016) established a limit for coliforms in "Grade A" raw milk not exceeding 750 cfu/ml. and raw milk for sale shall not contain more than 10 coliform bacteria per ml, while the Egyptian standards don't mention any limits for coliform counts in raw milk, although the milk is still sold raw in Egypt.

The presence of Coliforms in dairy farm environment is perfectly understandable, since they

are normal inhabitants in raw milk. Some countries even set a permissible limit for their presence in milk; high counts may be attributed to many factors, e.g. using of contaminated water and equipment on dairy farms and faecal contaminated utensils during milking or prevalence of unsanitary practices along with failure in milking machine wash, up till milking of coliform mastitic cows that could facilitate the entrance of coliforms in bulk tank milk **(Jayarao and Wang, 1999; Hogan and Smith, 2003 and Pantoja *et al.,* 2011).**

Results given in (Tables 1, 3) showed that 86.7 % of Kariesh cheese samples were positive for the presence of coliforms, including *E.coli, Klebsiella pneumoniae subsp. Pneumoniae*, *Enterobacter intermedius* and *Citrobacter spp* in a percentage of 33.3 %, 5.5 %, 5.5 % and 50 % respectively. Lower results for total coliforms count were found by **(Baraheem *et al.,* 2007 and Hegab *et al.,* 2017),** while higher values were collected by **(Gamal *et al.,* 2015 and Hassan and Gomaa, 2016).**

The methodology of processing, handling and selling method of kariesh cheese afford many opportunities for contaminations of the product with many ubiquitous organisms among them are coliforms group. It is generally produced from raw milk often of poor quality and high microbial count, under bad hygienic conditions and finally it sold uncovered. Therefore, many Egyptian researches considered Kariesh cheese from the highly risk contaminated type of dairy product. **(Baraheem *et al.,* 2007; Gamal *et al.,*2015;Awad, 2016; Hassan and Gomaa, 2016 and Hegab *et al.,* 2017)**

Coliforms group failed to be detected in all examined feta cheese samples, as shown in (Tables 1, 2 and 3). The results could be due to the proper application of sanitary practices that followed during production till packaging of the product at large scale production in dairy plants. The results agreed with those reported by **(Bintsis and Papademas, 2002 and Osama *et al.,* 2014)** who attributed the absence of coliform organisms or their presence in very low counts in such product to the lowered pH and salt content after brining of cheese along with the proper application of sanitary practices applied during production till packaging of the final product**.**

Coliforms could be detected in 100 % of the examined samples of small scale produced yoghurt while failed to be examined in large scale produced ones, as mentioned in (Table 1). Our results match those given by **(Chimezie *et al.,* 2015; Bilgin and Binnur, 2016 and Awah *et al.,* 2016),** while lower ones detected by **(Sadek *et al.*, 2014).**

Yoghurt is an acidified type milk products; it contributes naturally to the lower findings of

coliforms in it, especially in large scale produced type where proper hygienic measures are supposedly being applied in a correct manner. Whereas, the much higher values that found in small scale produced yoghurt could be explained by the lack of sanitary practices or neglected hygienic measures supervision, along with the improper methods adopted throughout the process of production till the final packaging for consumption.

Results given in (Table 3) showed that, the most common coliform genera that have been isolated from raw milk samples include"*Escherichia coli, Klebsiella spp and Enterobacter spp"* in a percentage of 66.7%, 23.8%, and 9.5%, nearly similar findings were reported by **(Jayarao and Wang, 1999).**

The International Commission on Microbiological Specifications for Foods (ICMSF, 2002) suggested that *E.coli* possesses the attributes of a good indicator as a predictor of faecal contamination and possible presence of other enteric pathogens. The faecal types of coliforms group represented about 38.5% of total isolated coliform *spp.* in the examined samples. The highest percentages of *E.coli* found in that products which supposed to be produced in small scale production under bad hygienic conditions e.g. raw milk, kariesh cheese, and small scale produced yoghurt of (66.7%, 33.3%, 25.6%) respectively, higher findings were found by **Malt *et al.,* (2013)** who attributed the high results of faecal coliforms in yoghurt samples to post processing contamination as these organisms unable to survive the heat treatment applied during yoghurt manufacture.

E. coli has been emphasized by many authors as a cause of gastroenteritis, epidemic diarrhoea in infants, sporadic diarrhoea in children as well as in cases of food poisoning. *Klebsiella spp.* could be isolated from the examined raw milk, kariesh cheese and small scale produced yoghurt samples at % of 23.8%, 5.5% and 35.9 % of the identified isolates respectively (Table 3). *Klebsiella spp* are from the main causes of pneumonia among healthcare patients in hospitals receiving treatment through ventilators or intravenous catheters. The identified species of *Enterobacter intermedius* represented 7.7% of the total isolated strain from the positive examined products raw milk, kariesh cheese and small scale produced yoghurt. *Enterobacter intermedius* is considered one of major nosocomial opportunistic pathogens causing many infections compromising lower respiratory tract infections, intra-abdominal and ophthalmic infections, along with septic arthritis and osteomyelitis. **(Center for Disease control and prevention, 2017**).

Citrobacter spp. were isolated only from Kareish cheese and small scale produced yoghurt samples at 50 % and 23.1 % respectively. The organisms are well known to be the cause of pneumonia in immunocompromised individuals of nosocomial infection which could subsequently lead to a case of adenocarcinoma; such organism connects also with cases of urinary tract infection and bacteraemia **(Pennington *et al.*, 2016).**

Coliforms are hazard commonly considered as a component of food safety management systems such as HACCP system, ISO 22000:2005, the global standard for food BRC and FSSC. The presence of coliforms in food especially heat-processed foods is probably due to improper sanitation after heat treatment, contamination with faecal matter and their presence related to presence of enteric pathogens **(Ray, 2004).**

CONCLUSION

From the obtained results, it could be easily concluded that raw milk and dairy products are subjected to contamination with biological hazards due to neglected sanitary measures adopted during production, handling, transportation and marketing, especially kariesh and small scale produced yoghurt which depend mainly upon traditional manufacture methods which could pose favourable environment for bacterial contamination and multiplication. The unclean hands of workers, poor quality of milk used, unhygienic conditions of manufacturing, inferior quality of materials used, water supplied for washing utensils could be source of accelerating the bacterial contamination and the post manufacturing contamination of these products. Lack of proper cooling storage during marketing with ambient summer temperatures of Egypt are also factors that magnitude the problem.

So it seems necessary that concerned authorities should impose regulations and bacteriological standards especially for traditionally produced dairy products, taking active part in the control of milk production and handling.

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