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**OCCURRENCE OF SOME ENTERIC PATHOGENS
AND THEIR INDICATORS IN SOME EGYPTIAN
RAW MILK PRODUCTS**
(With 2 Tables)

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تواجد بعض الميكروبات المعوية الممرضة والمدلات عليها في بعض منتجات
اللبن الخام المصرية

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نظرا للأهمية الكبرى لمجموعة الميكروبات المعوية الممرضة فقد أجريت هذه الدراسة لتحديد مدى تلوث بعض منتجات الألبان التقليدية المصرية بها وبالمدلات على وجودها. تم فحص ١٠٠ عينة (أ- جبن قريش طازج - ٣٠ عينة، ب- جبن قريش ناضج = ٢٥ عينة، ج- زبد فلاحى - ٣٠ عينة، د- قشدة خام = ١٥ عينة) للبحث عن تواجد ميكروبات ال *Salmonella* وال *E. coli* 0157 بالإضافة إلى مجاميع المدلات (ال *Enterobacteriaceae*، ال *coliforms*، ال *faecal coliforms*، ال *enterococci*). أظهرت النتائج تواجد ميكروب ال *Salmonella* في ٣% من العينات بواقع عينة إيجابية واحدة من أ (٣,٣٣%)، ب (٤%)، د (٦,٦٧%) بينما تم عزل ميكروب ال *E. coli* 0157 من ١٦ عينة (١٦% من مجمل العينات) بواقع عينتان من أ (٦,٦٧%)، ٦ عينات من ب (٢٤%)، ٥ عينات من ج (١٦,٦٧%)، ٣ عينات من د (٢٠%). بالنسبة لمجاميع المدلات فقد تواجدت مجموعة ال *Enterobacteriaceae* في ١٠٠%، ٩٠%، ١٠٠%، ١٠٠% من أ، ب، ج، د بمتوسطات ١٠٨، ١٠٨، ١٠٨، ١٠٨ ميكروب/ملى قدرها ٦,٣١، ٤,٤٤، ٣,٣٧، ٥,٧٧ على التوالي. مجموعتي ال *coliforms* وال *faecal coliforms* تواجدتا في ١٠٠% من أ، ٩٢% و ٧٦% من ب، ٩٦,٦٧% و ٨٦,٦٧% من ج، ١٠٠% من د. وكانت متوسطات ال *coliforms* ٦,٧٣، ٣,٤٦، ٤,٧٤، ٦,٦٣ في أ، ب، ج، د على الترتيب. بينما تراوحت متوسطات ال *faecal coliforms* بين ٥,٢٤، ٣,١١، ٣,٣٦، ٦,٠١ في العينات على نفس الترتيب. أما مجموعة ال *enterococci* فقد ثبت تواجدها في ١٠٠%، ٨٠%، ٩٣,٣٣%، ١٠٠% بمتوسطات ٥,٩٦، ٤,٤٧، ٥,٣١، ٥,٧١ على الترتيب. هذه النتائج تعكس إلى أي مدى تتعرض تلك المنتجات للتلوث بمختلف أنواع الميكروبات المعوية الممرضة وغير الممرضة وما يمثل ذلك من خطورة شديدة على الصحة العامة. هذا ومن الممكن الحد من تلك الخطورة باتباع الإرشادات الصحية وبستره اللبن قبيل تصنيع منتجاته المختلفة.

SUMMARY

The present study was conducted to evaluate the extent with which some domestic dairy products in Egypt may be exposed to contamination by enteric organisms and their indicators. 100 samples [i. fresh Kariesh cheese (n=30), ii. ripened Kariesh cheese (n=25), iii. Cooking butter (n=30) and iv. raw cream (n=15)] were used in the study. Each sample was microbiologically analysed for the presence of *Salmonella* spp. and *E. coli* 0157 serogroup as well as the count of *Enterobacteriaceae*, coliforms, faecal coliforms and enterococci group of organisms. The results showed the presence of *Salmonella* spp. in 3 samples (3%); one of which from i (3.33%), another from ii (4%) and the last from iv (6.67%). *E. coli* 0157 serogroup were isolated from 16 samples (16%); 2 from i (6.67%), 6 from ii (24%), 5 from iii (16.67%) and 3 from iv (20%). *Enterobacteriaceae* group were countable in 100%, 88%, 90% and 100% of i, ii, iii and iv with mean "log₁₀ CFU/g" values of 6.31, 3.44, 4.37 and 5.77, respectively. Coliforms and faecal coliforms were detectable in 100% of i, 92.76% of ii, 96.67% and 86.67% of iii and 100% of iv. The mean "log₁₀ MPN/g" values for coliforms were 6.73, 3.46, 4.74 and 6.63 in i, ii, iii and iv, respectively, while those for faecal coliforms were 5.24, 3.11, 3.36 and 6.01 in the examined samples, at the same respect. Enterococci were countable in 100%, 80%, 93.33% and 100% of i, ii, iii and iv with mean "log₁₀ CFU/g" values of 5.96, 4.47, 5.31 and 5.71, respectively. Such obtained results reflect to what extent these products are exposed to extreme contamination with enteric pathogens and their indicators constituting high degrees of public health risks to consumers. These risks can be preventable with application of certain hygienic measures and pasteurization of milk used in production of various dairy products.

Key words: *Enteric pathogens – Indicators – Kariesh cheese – Cooking butter – Raw cream.*

INTRODUCTION

The bacteria important in foods have been arbitrarily divided into several groups on the basis of similarities in certain characteristics. Enteric pathogens represent one of the most important bacterial groups in foods. Members belonging to this group can survive and multiply or

establish in the gastrointestinal tract of humans, food animals and birds. Under this term "enteric pathogens" several species of virulent microorganisms, such as pathogenic *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Escherichia*, *Vibrio*, *Listeria*, hepatitis A and others that can cause gastrointestinal infections, are involved (Ray, 1996). Enteric pathogens comprise a considerable proportion of the foodborne pathogens as a whole. Most cases of food poisoning are attributed to enteric pathogens. As stated before, they are originated primarily from the lower intestine of man and/or animals. Hence, the principle source of them is mainly their intestinal contents. A food contaminated directly or indirectly with faecal material may theoretically contain one or more of these pathogens and thus can be potentially hazardous to consumers. To implement regulatory requirements and to ensure the safety of consumers, it is necessary to know that a food is either free of some enteric pathogens, such as some *Salmonella* spp. and *E. coli* 0157:H7, or contains low levels of some other enteric pathogens, such as *Yersinia enterocolitica* and *Vibrio parahaemolyticus*. Owing to the relatively long time and high costs required for the isolation and confirmation of a pathogen from a food, it is not practical or economical to test the required number of product samples from each batch for all the pathogens or even those that are suspected of being present in a particular product. Alternatively, the food samples are examined for the number 'or level' of groups or species of bacteria that are of faecal origin, usually present in higher density than the pathogens, but considered to be nonpathogenic. Their presence is viewed as resulting from direct or indirect contamination of a food with faecal material and is an indication of the possible presence of enteric pathogen(s) in the food. These bacterial groups or species are termed "indicators of bacterial pathogens". There are certain microbial groups that are looked with special importance in this respect. They include *Enterobacteriaceae*, coliforms, faecal coliforms and enterococci.

Some of dairy products manufactured in Egypt that are commonly termed as "*FALLAIY*", such as Kariesh cheese, gravity-separated sour cream and Cooking butter, are made from raw milk under a poor hygienic status using primitive procedures. Under such circumstances, the finished products would be expected to harbour great number and different types of contaminants, particularly those belonging to enteric microorganisms. Therefore, the present study was outlined to explore the degree of truth arising from such expectation through microbiological examination of randomly collected samples of Kariesh cheese, Cooking

butter and raw cream. In order to secure such information, the product samples were examined for the presence of some enteric pathogens (*Salmonella* spp. and *E. coli* 0157 serogroup) as well as their indicators (*Enterobacteriaceae*, coliforms, faecal coliforms and enterococci).

MATERIAL and METHODS

Samples collection:

A total of 100 samples divided as follows:

- 30 fresh Kariesh cheese samples,
- 25 ripened Kariesh cheese samples,
- 30 Cooking butter samples and
- 15 raw cream samples

were collected randomly from special markets as well as farmers' houses making such products in the villages, rural and suburban areas belonging to Ismailia & Sharkeia Governorates. Collected samples were delivered to the laboratory to be examined without delay.

Isolation and identification of enteric pathogens

A) *Salmonella* spp. --Varnam and Evans (1991)

Pre-enrichment

25 g. of the prepared sample were thoroughly mixed with 225 ml warm (~ 40°C) buffered peptone water (BPW) using Seward Stomacher® (400 Lab System, England) and incubated at 37°C for 18-24 h.

Selective enrichment

About 10 ml inoculum from preenrichment broth culture was transferred to ~90 ml selenite broth (Leifson, 1936) before being incubated at 37°C for 24 h.

Selective plating

A loopful from the inoculated 24 h selenite broth was streaked on xylose lysine desoxycholate (XLD) agar plates (Taylor, 1965) in a way to produce separate colonies. Inoculated plates were incubated at 37°C for 24 h. Suspected colonics were picked up, spread on nutrient agar slants, incubated at 37°C for 24 h and then stored in refrigerator to be examined biochemically and serologically.

Biochemical and serological identification

Isolated strains suspected to be *Salmonella* were identified biochemically and then serologically using *Salmonella* Latex test (Oxoid, FT203).

B) *E. coli* 0157 serogroup. --Varnam and Evans (1991)

A loopful from the 18-24 h inoculated BPW was streaked on sorbitol MacConkey agar (SMA) and incubated at 35°C for 24 h. Suspected colonies were picked up, spread on nutrient agar slants, reincubated at 37°C for 24 h and stored in refrigerator to be examined biochemically and serologically.

Biochemical and serological identification

Isolated strains were identified through subjecting them to a series of appropriate biochemical tests as well as serotyping using latex co-agglutination test (Oxoid, DR620).

Detection and enumeration of enteric pathogens-indicators (APHA, 1992)

A) *Enterobacteriaceae* --APHA (1992)

After making ten-fold serial dilutions for each sample, one ml from each of the appropriate dilutions were thoroughly mixed with ~15 ml violet red bile dextrose agar (VRBD) at ~45°C. The plated medium was allowed to solidify and ~5 ml from the same medium were poured shortly thereafter as an overlay to avoid surface growth. Inoculated plates were incubated at 37°C for 24-48 h.

B) *Coliforms* --Harrigan (1998)

One ml from the appropriate dilutions were transferred separately into each of 3 MacConkey's broth tubes with Durhams' tubes and incubated at 37°C for 48 h. According to the number of positive tubes in each dilution, the coliform MPN/g sample was recorded using 3-tubes MPN index table.

C) *Faecal coliforms* --Harrigan (1998)

Loopfuls from each coliform positive tubes were inoculated into 45°C MacConkey broth tubes and incubated at that temperature in a thermostatically controlled water bath for 24 h. According to the number of positive tubes in each dilution, faecal coliforms MPN/g sample was recorded using 3-tubes MPN index table.

D) *Enterococci* --APHA (1992)

0.1 ml from the appropriate dilutions were spread evenly using surface plating technique on Kanamycin asculin azide (KAA) agar plates and incubated at 37°C for 24-48 h.

RESULTS and DISCUSSION

Firstly, it seems suitable to mention that the selected products in this study are relatives. Raw cream (the precursor of Cooking butter) is collected after overnighting freshly drawn milk in a warm place. The resultant skim milk is salted and drained off to form fresh Kariesh cheese. The latter may be used as such or after being ripened. Therefore, the microbial quality of these products depends mainly on the quality of raw milk from which they have been made and, consequently, the degree of hygiene and sanitation adopted in their production.

I. Occurrence of enteric pathogens

A) *Salmonella* spp.

Since the 1950s, foodborne salmonellosis has been the major cause of all foodborne diseases caused by bacteria and viruses, both in number of incidents (sporadic and outbreaks) and number of cases. There are over 2000 serovars of *Salmonella* potentially capable of causing salmonellosis in humans. Along with fecal-oral direct transmission, contaminated food and water can cause salmonellosis (Ray, 1996). It is obvious from the results presented in Table 1 that *Salmonella* spp. could be isolated from 3% of examined samples. The 3 positive samples were one out of the 30 fresh Kariesh cheese samples (3.33%), another out of the 25 ripened Kariesh cheese samples (4%) and the last one was out of the 15 raw cream sample (6.67%). Such finding represents a direct threat to consumers' health due to the high possibility with which such isolates could be potentially pathogenic. This is supported by the recently reported outbreaks of salmonellosis linked to raw-milk cheeses [Cerqueira *et al.* (1994), Djuretic *et al.* (1997), Altekruze *et al.* (1998); Ellis *et al.* (1998), Gody *et al.* (1999) and Villar *et al.* (1999)]. A more or less similar finding was reported by Garcia-Cruz *et al.* (1994), Tuladhar and Sharma (1997), Guarino *et al.* (1998), Sena *et al.* (1998), Pereira *et al.* (1999) and Scaramelli *et al.* (1999). Relatively higher incidences of *Salmonella* spp. were reported by Digrak *et al.* (1996), Florentino and Martins (1999) and Mendes *et al.* (1999) in the analyzed dairy products (almost cheeses). However, Lopez-Diaz *et al.* (1995), Santos *et al.* (1995), Sharma *et al.* (1995), Nichols *et al.* (1996), Mauro *et al.* (1998), Perez *et al.* (1998), Vivegnis *et al.* (1998) and Urarte *et al.* (1999) failed to detect any salmonellae from the examined cheese and butter varieties.

B) *E. coli* 0157 serogroup

Certain strains of *E. coli* have recently been implicated in some cases of haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). It has been shown that these strains produce a verocytotoxin (VT). The serotype most frequently isolated from IIC and HUS cases is 0157:H7. It is clear from the presented data in Table 1 that the incidences of *E. coli* 0157 (potential VT-producing strain) in fresh Kariesh cheese, ripened Kariesh cheese, Cooking butter and raw cream samples were 6.67%, 24%, 16.67% and 20%, respectively. As a whole, 16 out of the examined 100 samples (16%) contained the pathogen. Likely findings were obtained by Garcia-Cruz *et al.* (1994), Marino *et al.* (1997), Quinto and Cepeda (1997) and Altekruš *et al.* (1998). However, Lopez-Diaz *et al.* (1995), Aman *et al.* (1998) and Svoboda *et al.* (1998) couldn't isolate the pathogen from the analyzed cheese varieties. The higher incidence of the *E. coli* 0157 serogroup in the ripened Kariesh cheese in comparison to fresh one explains the ability of the pathogen to survive the adverse conditions that occur during cheese ripening. Reitsma and Henning (1996), Ramsaran *et al.* (1998) and El-Kosi *et al.* (1999) proved this phenomenon upon their tested cheeses.

II. Occurrence of enteric pathogens-indicators

A) *Enterobacteriaceae*

Because of the inability of some enteric pathogens (such as most *Salmonella* spp.) to ferment lactose, enumeration of all *Enterobacteriaceae* family instead of only enumerating coliforms or faecal coliforms in a food is advocated. As this family includes many genera and species that are enteric pathogens, enumeration of the whole group could be used as a good indicator of the level of sanitation, possible faecal contamination and possible presence of enteric pathogens (Hitchins *et al.*, 1992). It is clear from the findings presented in Table 2 that 100%, 88%, 90% and 100% of fresh Kariesh cheese, ripened Kariesh cheese, Cooking butter and raw cream samples were positive for *Enterobacteriaceae* group with mean ' \log_{10} CFU/g' counts of 6.31, 3.44, 4.37 and 5.77, respectively. Relatively similar rates of *Enterobacteriaceae* contamination were reported by Allam-Hanaa (1995), while somewhat lower values were obtained by Lopez-Diaz *et al.* (1995).

B) Coliforms and faecal coliforms

The counts of coliforms and faecal coliforms in the examined samples presented in Table 2 reveal that they are recovered from 100%

of fresh Kariesh cheese and raw cream samples with mean ' \log_{10} MPN/g' values of 6.73 and 6.63 for coliforms and 5.24 and 6.01 for faecal coliforms, respectively. Both groups were recovered from ripened Kariesh cheese and Cooking butter at a lesser rate. Coliforms were positive in 92% of ripened Kariesh cheese and 96.67% of Cooking butter samples, while faecal coliforms were positive in 76% and 86.67% of both samples, respectively. The mean ' \log_{10} MPN/g' counts were 3.46 and 4.74 for coliforms and 3.11 and 3.36 for faecal coliforms in both products, respectively. Likely findings were obtained by Abdel-Hakiem (1986), Abdel-Naser (1990), Nazem (1991), Cerqueira et al. (1994), Garcia-Cruz et al. (1994), Patir et al. (1995), Santos et al. (1995), Nichols et al. (1996), Kaldes (1997), Kameni et al. (1998), Florentino and Martins (1999), Mendes et al. (1999) and Pereira et al. (1999). The existence of coliforms and faecal coliforms in dairy products is suggestive of unsanitary conditions or practices during production, processing or storage (APHA, 1992).

Enterococci

Concerning enterococci, they are common in the intestine of man and animals, resistant to many unfavourable conditions and are considered to be opportunistic pathogens. They have been isolated from various types of foods suggesting their exposure to faecal pollution (Varnam and Evans, 1991). The enterococcus count is considered to be more reliable than the coliform count as an index of the sanitary quality of certain types of dairy products such as butter and yoghurt. This is because enterococci are better able than coliforms to survive the unfavourable microenvironment of salted butter as well as the low pH value of yoghurt and other types of fermented milks (Saraswat et al., 1965 & Salinas, 1984). Results in Table 2 declare to what extent the examined products had been exposed to contamination by enterococci. They recovered from 100%, 80%, 93.33% and 100% of fresh Kariesh cheese, ripened Kariesh cheese, Cooking butter and raw cream samples, with mean ' \log_{10} CFU/g' counts of 5.96, 4.47, 5.31 and 5.71, respectively. Abdel-Hakiem (1986), Nazem (1991), Lopez-Diaz et al. (1995), Patir et al. (1995), Santos et al. (1995), Kaldes (1997) Kameni et al. (1998) and Richard (2000) reported relatively similar rates of enterococci in their examined samples.

Upon observing the data presented in both tables collectively, one can recognize that ripened Kariesh cheese is the least contaminated product with various types of indicators. Simultaneously, it appears to be

the most risky product because of having the comparably higher incidences of enteric pathogens. Therefore, it is concluded that, the use of enteric pathogens-indicators as a sole parameter in determining the microbiological quality and, in turn, issuing the degree of safety of such products seems to be insufficient. Another conclusion derived from the study herein is the fairly high potential hazards and public health risks arising from the consumption of locally-produced dairy products. Such risks could have been prevented by pasteurization and application of strict hygienic measures in production of these products. The consumer should have a higher degree of awareness and education, so that he would not buy any suspicious food product. Such simple reaction, will lead to gradual reduction in the scale of production of likely products with the hope of their complete disappearance from our markets in the future.

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Table 1: Incidence of enteric pathogens in the examined samples.

Type Of Sample	Number of samples	<i>Salmonella</i> spp. Positive samples		<i>E. coli</i> 0157 Positive samples	
		NO	%	NO	%
Fresh Kariesh cheese	30	1	3.33	2	6.67
Ripened Kariesh cheese	25	1	4.00	6	24.00
Cooking butter	30	0	0.00	5	16.67
Raw cream	15	1	6.67	3	20.00
TOTAL	100	3	3.00	16	16.00

Table 2: Statistical analytical results of enteric pathogens-indicators recovered from the examined samples

Enteric pathogens-Indicators in examined samples	Positive samples		Log ₁₀ (CFU/g or MPN/g)		
	No	%	Minimum	Maximum	Average
Fresh Kariesh cheese					
<i>Enterobacteriaceae</i>	30	100.00	3.43	7.04	6.31
<i>Coliforms</i>	30	100.00	3.38	7.38	6.73
<i>Faecal Coliforms</i>	30	100.00	2.38	6.38	5.24
<i>Enterococci</i>	30	100.00	3.11	7.59	5.96
Ripened Kariesh cheese					
<i>Enterobacteriaceae</i>	22	88.00	1.60	4.26	3.44
<i>Coliforms</i>	23	92.00	1.38	4.38	3.46
<i>Faecal Coliforms</i>	19	76.00	1.38	4.38	3.11
<i>Enterococci</i>	20	80.00	2.00	5.20	4.47
Cooking butter					
<i>Enterobacteriaceae</i>	27	90.00	1.30	5.06	4.37
<i>Coliforms</i>	29	96.67	1.38	5.38	4.74
<i>Faecal Coliforms</i>	26	86.67	1.38	4.38	3.36
<i>Enterococci</i>	28	93.33	2.85	6.04	5.31
Raw cream					
<i>Enterobacteriaceae</i>	15	100.00	2.45	6.30	5.77
<i>Coliforms</i>	15	100.00	3.38	7.38	6.63
<i>Faecal Coliforms</i>	15	100.00	2.97	6.66	6.01
<i>Enterococci</i>	15	100.00	3.00	6.15	5.71