

MICROBIOLOGICAL QUALITY OF AND INCIDENCE PATHOGENIC BACTERIA IN SOME TYPES OF CHEESE

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ABSTRACT

A total of 125 samples represent various kinds of cheese: Karish, Egyptian Cheddar, Imported Cheddar, Romey cheese and Goat milk cheese were tested for coliform count, faecal coliform, *E coli*, enterococci, *Listeria* spp., *Listeria monocytogenes* and *Salmonella*. Coliform count average in Karish, Egyptian Cheddar, Romey and Goat milk cheese were 4100 cfu/g, 360 cfu/g, 290 cfu/g and 3800 cfu/g, respectively. Averages of faecal coliform count detected in Karish, Romey, Goat milk cheese were 240 cfu/g, 500 cfu/g and 400 cfu/g, respectively. While *E coli* detected only in 2 kinds of cheese, Karish and Goat milk cheese. On the other hand the average of enterococci count were 4500 cfu/g, 610 cfu/g, 560 cfu/g and 700 cfu/g in Karish, Egyptian Cheddar, Romey and Goat milk cheese, respectively. At the same time coliform, faecal coliform, *E coli* and enterococci not detected in Imported Cheddar. The percentage of isolation of pathogenic, *Listeria* spp. and *Listeria monocytogenes* were detected in 40.0 to 60.0% and 12.0 to 60.0% of all types of cheese tested, while *Salmonella* spp. detected only in 16% of Karish cheese samples.

Keywords: Karish cheese, Romey cheese, Enterococci, *Listeria*, Coliform, *Salmomella*,

INTRODUCTION

Cheese is considered as an important source of food for human consumption because it has a high content of protein and some minerals which are essential in human diet, so the hygienic status is very important to be evaluated to avoid its hazards. Microbiological tests on finished cheeses have an important role in quality control, but these tests can not ensure the microbiological safety of the cheese (Rambourg, 1996).

A large number of listeriosis outbreaks linked to the consumption of dairy products as occurred in the last 20 years in different countries (Bell and Kyriakides, 1998)

Despite of the efficiency of pasteurization on bacterial cell destruction, production of cheese through the processing steps are considered the main source of contamination by *Listeria monocytogenes*, in particular to some cheese which made from raw milk in some countries. Mexican style fresh cheese produced in California, soft and semi soft cheese in Canada, French domestic soft cheese, European cheese, Switzerland soft mold, smear ripened cheese and soft cheese from different origin in England, Anari cheese and white ripened cheese were positive for the incidence of *Listeria monocytogenes* reported by James *et al.* (1985), Ryser and Marth (1991), Pini and Gilbert (1988), Mclauchlin *et al.* (1990), Greenwood *et al.* (1991) and Gohi. *et al.* (1995).

Also, Maria Christina *et al.* (1998) said that some cheese samples were contaminated with *L. monocytogenes*, *L. innocua*, *L. grayi* and *L. welshimeri*.

However, the incidence with different Listeriosis in raw milk was, in generally, dependent on the quality of spoiled silage, fecal material, cleanness of animal and milking procedure (unclean teats, tools and equipment) used for milking processes (Sanaa *et al.*, 1993).

The incidence of coliform had been detected in commercial cheese in USA by Khayat *et al.* (1987). Also, Tawfek *et al.*, 1988; Abo-El-Khier *et al.*, 1986 and Khayat *et al.*, 1987 detected coliform and faecal coliform in Karish cheese and Ras cheese in Egypt.

The incidence of *Staphylococcus aureus* was detected in Cottage cheese and Ras cheese (Mohmoud *et al.*, 1985).

The objective of this study is to assess the incidence and identification of some pathogenic bacteria i.e. *L. monocytogenes*, coliform, faecal coliform, enterococci and *Salmonella* spp. in five types of cheese (Karish, Egyptian Cheddar, Imported Cheddar, Romey and Goat milk cheese).

MATERIALS AND METHODS

125 representative samples of the five types of cheese Karish, Egyptian Cheddar, Imported Cheddar, Romey and Goat milk cheese (cheese made from milk of Goat) as more popular cheese in Cairo market, twenty five samples from each. Tested cheeses were examined microbiologically to determine their hygienic and quality status.

Preparation of tested samples, initial suspension and decimal dilution according to ISO 68873 (2001). Ten gram of cheese weighted into a sterile stomacher bag and 90 ml diluent (buffered peptone water) was added, blending for 1-2 min., then decimal dilution to 10^{-7} in buffer peptone was made to perform enumeration of coliform, faecal coliform, *E. coli* and enterococci.

The coliform group enumeration was done by pour plate method according to ISO 4832 (1991) on crystal violet neutral red bile lactose (VRBL) and incubated at 37°C for 48 hr.

Also, enumeration of *Escherichia coli* was done by surface plate method according to NMKL No 125 (1998) modified on VRB-Mug and incubated at 44.5°C for 24 hr.

The *Escherichia* spp. enumeration was done by surface plate method according to NMKL No 68 (1992) modified on KF *Streptococcus* agar and incubated at 37°C for 48 hr. Suspected colony confirmed by using API 20 E.

On the other hand, *Salmonella* spp. were tested according to ISO 6579 (1999), Pre-enrichment. 25g sample were performed in 225 ml buffer peptone water and incubated at 37°C for 16-20 hr., then transferred 1.0 ml and 0.1 ml into 10.0 ml in two selective enrichment broth selenite cystine broth and Rappart vassiliadis broth (RV) as well as incubated at 37°C and 41.5°C for 24 hr, respectively. One loop from each selective enrichment was streaked on Hektole enteric agar, XLD and phenol red Brilliant green agar at

37°C for 24hr. Suspected colonies were subjected to biochemical identification on lysine decarboxylase, triple sugar iron agar and urea agar at 37°C for 24hr. Positive colony subjected to confirmation by using API 20 E system and serology antibody reaction.

Listeria were tested according to ISO 11290-1 (1996). 25g of sample were added to 225 ml Half Frazier broth and incubated at 30°C for 24 hr. 0.1 ml from primary enrichment was transferred to 10.0 ml of Frazier broth (secondary enrichment) and incubated at 37°C for 48hr. Two loops from second enrichment were streaked on Oxford and Palcam agar at 37°C for 24 hr. Suspected colony subjected to confirmation with API *Listeria*.

RESULTS AND DISCUSSION

FDA (1995) and Marshall (2001) recorded that, microorganisms are important to dairy products. The proper selection and balance of starter cultures are critical for the manufacture of fermented products of desirable rheological, textures, and flavor characteristics. Undesirable microorganisms are responsible for the spoilage of dairy products, and pathogens introduced into milk and milk products by unsatisfactory milk production practice, failures in processing systems, or unsanitary practices are of primary concern. The important of the desirable and undesirable microorganisms in milk and milk products as resulted in the development of methods to enumerate them and in the establishment of standards to reflect the safety or quality of milk and milk products.

Results obtained from analysis of 125 samples of five types of cheese (Karish, Egyptian Cheddar, Important Cheddar, Romey and Goat milk cheese) are shown in Tables (1 and 2).

Data recorded in Table (1) revealed that, the coliform, enterococci and faecal coliform were detected in 80%, 84% and 56% of Karish cheese ranged from (100-2100 cfu/g), (110-3200 cfu/g) and (100-500 cfu/g), respectively. While the *E coli* not detected in 48% of cheese samples. The prevalence of either *Salmonella* spp., *Listeria* spp or *Listeria monocytogenes* in Karish cheese were 16.0%, 40.0 % and 12.0%. respectively. The results were accompanied with data obtained by McCoy (1961), who stated that, the presence of intestinal inhabitants in dairy product should be considered to indicate a lack of cleanliness. Also, Khayat *et al.* (1987) stated that 54% of 256 commercial cheese samples from USA, contained 10^2 - 10^7 cfu coliforms/g.

Tawfek *et al.* (1998) reported that, all Domietta, Karish and Ras cheese samples had *E coli* with an average of 3.6×10^5 , 3.9×10^5 and 0.17×10^5 cfu/g, respectively.

Cousin, (1982) and other reported that fresh cheese, such as cottage cheese and others high moisture cheeses, may be subject to spoilage by gram-negative psychro-trophic bacteria (*Pseudomonas*, *Flavobacterium*, or *Alcaligenes*) coliforms, yeast and molds that enter as post-pasteurization contaminates.

Table (1): Incidence of different microorganisms (cfu/g) in various kinds of cheese collected from Egyptian markets

Kinds of cheese and No. of samples	Total coliform cfu/g			Faecal coliform			<i>E coli</i>			Enterococci		
	N.D	Mean	Range	N.D	Mean	Range	N.D	Mean	Range	N.D	Mean	Range
Karish (25)	5	4100	100-21000	11	240	100-500	12	190	50-500	4	4500	110-50000
Egyptian Cheddar (25)	12	360	50-900	25	-	-	25	-	-	16	610	120-3200
Imported cheese (25)	25	-	-	25	-	-	25	-	-	25	-	-
Romey cheese (25)	13	290	100-1500	23	500	500	25	-	-	10	560	100-1400
Goat milk cheese (25)	14	3800	100-25000	23	1400	1300-1500	23	1500	1500-1500	3	4700	300-17000

* N.D. : Not detected < 10 cfu /g

* cfu colony forming unit. cfu/g

Olson and Mocquot (1980) stated that unclean udders and teats samples explained by Jervis (1988) and Mikolajcik (1980) who reported that a slow starter culture (due to bacteriophage, antibiotics, etc.) can allow growth of bacteria related to food borne illnesses such as *Staphylococcus*, *Salmonella*, *Listeria* and enteropathogenic *E coli*, which enter with raw milk or as post-pasteurization contaminants.

While, in case of Egyptian Cheddar cheese (Table 1), results indicated that, the enterococci count was ranging from 120-3200 cfu/g, (36%). In the same time the colony counts of coliform was ranged from 50 to 900 cfu/g, while *E. coli* and faecal coliform not detected (<10 cfu/g) in all samples. *Listeria* spp and *Listeria monocytogenes* were detected 60% and 24%, respectively. On the other hand *Salmonella* not detected in all tested samples of Egyptian Cheddar.

Kosikowski (1982) mentioned that most hard-ripened cheeses are not subjected to Gram negative spoilage though coliform contamination has been associated with the gassy defect in cheese making (for example, Cheddar). Also, Parks and Ingham (1993) detected coliforms and enterococci in some Swiss cheese samples with an average of >1000 cfu/g. While, Mikolajick (1980) mentioned that most (not all) enteropathogenic strains of *E coli* are inactivated at pH <5.0, although in low-acid, semi soft, surface-ripened cheese, faecal coliform are commonly found.

Rosenow and Marth (1987) reported that *Listeria* is capable of surviving in cheddar, camembert because of the low pH of most cheese.

Also data obtained from Imported Cheddar cheese was summarized in (Table 1). The bacterial counts of coliform, faecal coliform, *E. coli* and enterococci are not detected (<10 cfu/g) in all tested samples. While the prevalence of pathogenic microorganisms either *Listeria* spp. or *Listeria monocytogenes* were 40% and 28% from tested sample, respectively, while *Salmonella* spp. not detected in all samples. This result indicates that good hygienic of raw milk. Kwee *et al.* (1986), reported that psychrotrophic bacteria, coliform bacteria are reduced to very low level during preheating and their presence in milk products indicates contamination from equipment or environment during or after manufacture. The presence of *Listeria* come agree with Rosenow and Marth (1987). The growth and survival of *Listeria* spp. may be due the storage temperature, where the growth rate was increased with increasing storage temperature. Papageoriou *et al.* (1996) and Gahan *et al.* (1996) found that pH 3.5-5.5 enhanced the survival of *L. monocytogenes* in Cottage cheese and Cheddar cheese.

Romey cheese has high coliform and enterococci count ranged from 100-1500 cfu/g, (48%) and 100-1400 cfu/g, (60%) of tested samples, respectively. While faecal coliform was only detected in 2 samples only (500 cfu/g), and *E coli* not detected (< 10 cfu/g) in all samples. On the other hand, the *Listeria* spp and *Listeria monocytogenes* were detected in 44% and 60% of cheese samples, respectively. However, *Salmonella* was not detected in all samples. Bryan (1993) stated that microbial competition, reduced water activity, organic acids, and a low pH generally limit the growth of pathogens in cheese. Also, Donnelly (1988) mentioned that post-heating contamination or

the use of contaminated raw milk can be a source of *L. monocytogenes* as was implicated in an out break involving a low – acid Mexican-style cheese.

Table (2): Prevalence of pathogenic microorganisms in different kinds of cheese collected from Egypt market.

Type of cheese	Salmonella spp		Listeria spp		Listeria monocytogenes	
	Detected sample	%	Detected sample	%	Detected sample	%
Karish	4	16	10	40	3	12
Egyptian Cheddar cheese	-	-	15	60	6	24
Important Cheddar cheese	-	-	10	40	7	28
Romey cheese	-	-	11	44	15	60
Goat milk cheese	-	-	17	68	11	44

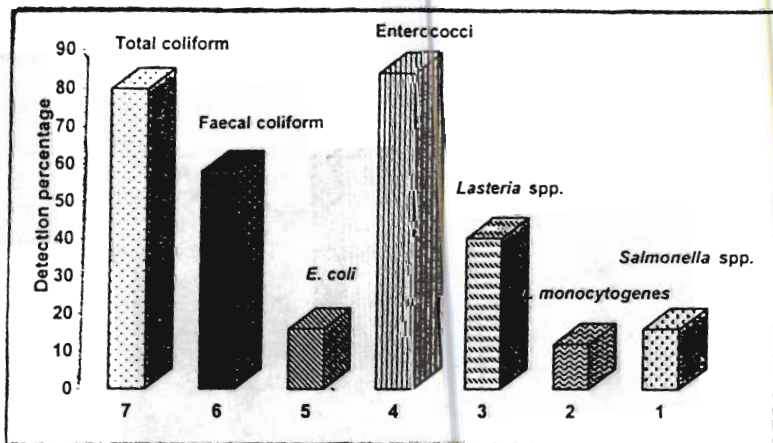
Total number of samples = 25 samples for each

Finally, Goat milk cheese showed that enterococci and coliform were detected in 88% and 44% of the samples ranged from (300 –17000 cfu/g and 100-25000 cfu/g), respectively. While faecal coliform and *E coli* detected in 8% of sample in range (1300-1500 cfu/g) and (1500 cfu/g), respectively. *Listeria* spp and *L. monocytogenes* were detected in 68% and in 44% of tested samples. However, *Salmonella* was not detected in all samples. Bryan (1993) recorded that post pasteurization contamination with *L. monocytogenes* of major concern to the dairy industry since these organisms grow at refrigerator temperatures. Olsen and Macquot (1980) mentioned that large numbers of other moduric bacteria *Micrococcus*, *Microbacterium*, *Streptococcus*, *Lactobacillus*, *Enterococcus*, *Bacillus* spp. are associated with persistent poor cleaning of milking machines, pipelines, bulk storage tanks, and transfer hoses while higher numbers of gram negative bacteria or lactococci may occur from occasional neglect.

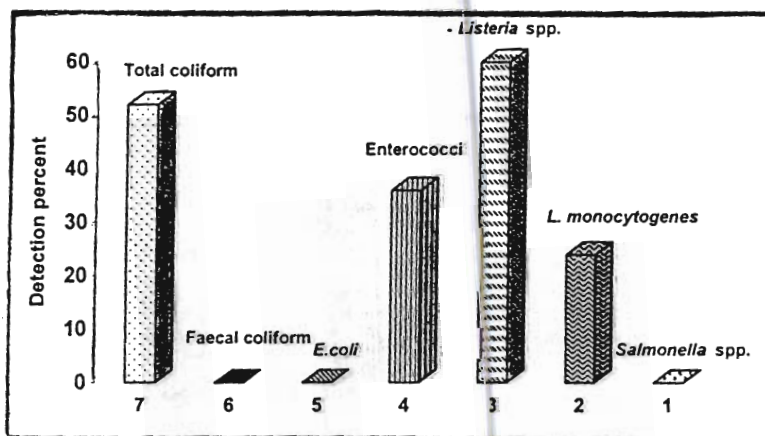
U.S. Government National Archives and Records Administration (1998) mentioned that microbiological standards for manufactured milk products were coliform 10 cfu/g, psychotropic 100 cfu/g and 10 cfu/g yeast and mold.

Cheese have a low pH (less than 5.0) prevent the growth and survival of pathogenic bacteria, both of salt and temperature were controlled the growth and survival of pathogenic microorganisms in dairy products, cheese in generally highly susceptible to environmental contamination during processing and extensively handled as well as stored for longer period without being covered.

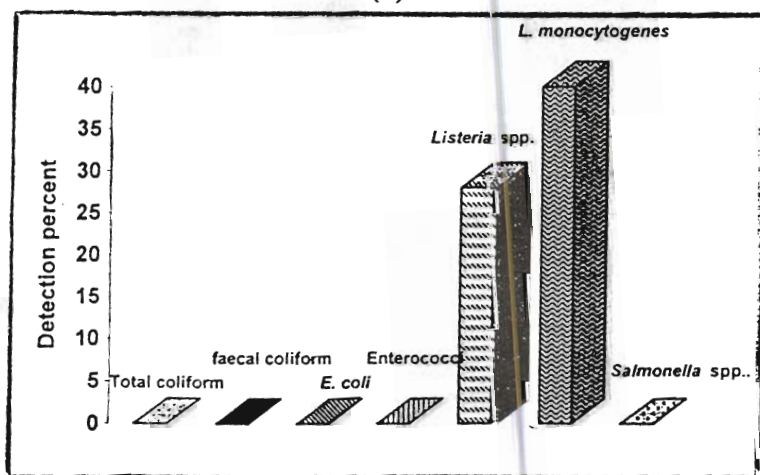
The general impression held from the obtained results of this study that, much attention should given to Karish, Egyptian cheddar and Romey cheeses because these types of cheese were more consumed in Egypt. Also, the responsible food security are requested the necessity to use a sanitary conditions in the production, storage and handling of such dairy product.

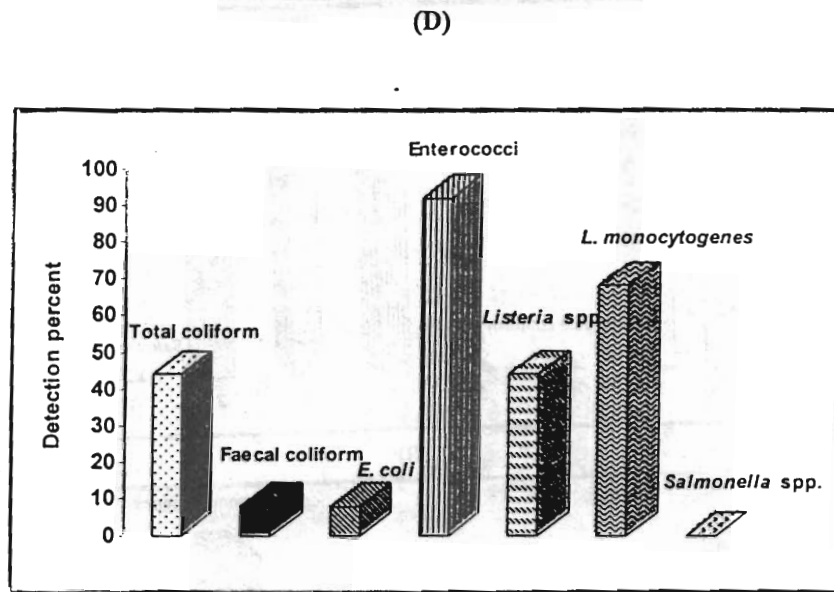
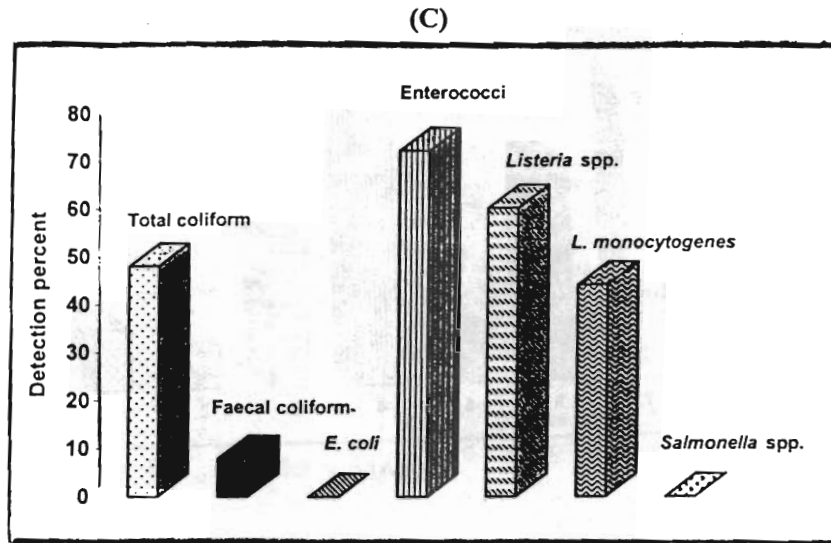


(A)



(B)





(E)

Fig. (1): Detection percentage of different bacterial count estimated in various cheese types collected from Egyptian markets.

- (A): Karish cheese (B): Egyptian Cheddar cheese
 (C): Important Cheddar cheese (D): Romey cheese
 (E): Goat milk cheese

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تقدير جودة وجود الميكروبات المرضية في بعض أنواع الجبن
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تم اختبار عدد ١٢٥ عينة جبن تمثل مختلف أنواع الجبن مثل الجبن القريش - الشيدر المصري - الشيدر المستورد - الرومي - الجبن المصنع من لبن الأغنام من حيث تقدير أعداد كل من الكوليفورم - الكوليفورم المرضية - أنتيروكولاي - اللاستريا - اللاستريا مونوسيتوجينيس ، السالمونيلا ، قد بلغ متوسط أعداد الكوليفورم في كل من الجبن القريش - الشيدر المصري - الرومي - الجبن المصنع من لبن الأغنام حوالي ٤١٠٠ مجموعة ، ٣٦٠ مجموعة ، ٢٩٠ مجموعة ، ٣٨٠٠ مجموعة تقريبا .
بينما بلغ متوسط أعداد الكوليفورم المرضية والمقدرة في كل من الجبن القريش - الشيدر المصري - الرومي - الجبن المصنع من لبن الأغنام حوالي ٢٤٠ مجموعة ، ٤٠٠ مجموعة ، ٥٦٠ مجموعة ، ٧٠٠ مجموعة . بينما في الجبن الشيدر المستوردة فلم يثبت وجود كل من مجموعة الكوليفورم ، الكوليفورم المرضية - الأشيريشيا كولي - الأنتيروكولي .
كذلك بلغت نسبة الميكروبات المرضية مثل الليستريا ، اللسيريا مونوسيتوجينيس الى قيم تتراوح ما بين ٤٠,٠ % الى ٦٨,٠ % ، ١٢,٠ % الى ٦٠,٠ % في كافة أنواع الجبن المختبرة . بينما بلغت أعداد السالمونيلا الى حوالي ١٦ % فقط في عينات الجبن القريش .