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Prevalence of some pathogenic microorganisms in factories Domiati, Feta cheeses and UHT milk in relation to public health sold under market conditions in Cairo

Osama.M.Sharaf[1]*, Gamal, A. Ibrahim[1], Nabil F. Tawfek[1], Baher.A.M. Effat[1], Kawther El Shafei[1], Hala M. F. El-Din[1] and Moussa M.A. Salem[1,2]

[1]Dairy Science Department (Dairy Microbiol. & Chem. Labs.), National Research Center, Dokki, Cairo, Egypt.

[2] Department of Biology, Faculty of Sciences and Arts, Khulais, King Abdulaziz University, Jeddah, Saudi Arabia.

***Corres.author: sharafosama@hotmail.com**

Abstract: A total of 100 samples of the factories dairy products (45 samples of Domiati, 25 samples of Feta cheeses and 30 samples of sterilized milk) were collected from Cairo governorate markets and microbiologically examined. Coliform group and *Escherichia coli* as fecal indicator contamination were implicated in 18 and 7% of Domiati cheese samples, and were not found in any Feta cheese and sterilized milk samples. *Staphylococcus aureus*, aerobic sporeformers, yeasts and molds were detected in 4.5%, 40%, 22.5% and 4.5% of Domiati cheese samples respectively. When researchers examine the samples received by the laboratory, they reported the following observations: weak textures (softness) of two Feta cheese samples as well as colored colonies (color defects) appeared on surface of three Domiati cheese samples. Cheese can be considered a good medium for bacterial growth due to their nutrient content and long storage duration. The results and observations indicate the unhygienic conditions prevailing during distribution or sale cheeses. It is recommended to use good manufacturing practices as well as distribution and retail storage practices for ensuring microbiological safety of cheese

Key words: Domiati cheese, Feta cheese, UHT milk, pathogenic bacteria, yeast, molds, *Escherichia coli* , *Staphylococcus aureus.*

Introduction

The quality of milk is determined by aspects of composition and hygiene. Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms[1.] The microbiological quality of milk and dairy products is influenced by the initial flora of raw milk, the processing conditions, and post heat treatment contamination. Among all microorganisms Escherichia coli is frequently contaminating organism, and is reliable indicator of fecal pollution generally in unsanitary conditions of water, food, milk and other dairy products [2]. The most important source of contamination is probably the human. Human skin surfaces and nasal passages harbor staphylococci. Also, water supplies contaminated with fecal material may contain pathogens. The contaminants reached the products either during processing or handling[3].

Many yeasts and molds given sufficient time under refrigeration temperatures can spoil dairy products and possibly other foods[4]. Cheeses, although they have been characterized as one of the safest food products by some authors[5], in 2006 the consumption of contaminated cheese accounted for the 0.4% of the total food borne outbreaks in Europe [6].

Microbial contamination of cheese may originate from various sources. Such sources during cheese production might be: starter culture, brine, floor and packaging material, cheese vat, cheese cloth and curd cutting knife, cold room and production room air[5]. Storage coolers have been also demonstrated to be the source of contamination of cheese made from pasteurized milk[7]**.**Several types of control method are effective in preventing or minimizing microbial contamination of product and inhibiting the growth or destroying microbial contaminants.

Considering the above facts the present study was to evaluate the microbiological quality of some processing dairy products sold under market conditions at Cairo area.

Materials and methods

Samples collection

A total of 100 samples of the Egyptian dairy products factories (45 samples of Domiati cheese, 25 samples of Feta cheese and 30 samples of sterilized milk) were collected from Cairo governorate markets and microbiologically examined.

Microbiological examination:

Enumeration of *Staphylococcus aureus*

Staphylococcus aureus in samples was counted on Baird Parker medium (Oxoid).

Determination of coliforms and *Escherichia coli*

Coliform group was determined using solid medium method onto plates of violet red bile agar (VRBA) (Difco) according to the method reported by [8]**.**Plates were incubated 24 hrs. At 32-35^0 C a portion of purple red colonies (5/a plate) per each plate was transferred (loopful) into tube of McConkey broth medium (Oxoid, England) which were incubated at 35^0 C. Positive acid and gas tubes, after 24 and/or 48 h, where further transferred into EC broth which in turn are incubated at 45.5^0 C for 48h. Positive tubes were streaked onto MacConkey agar (Merck, Germany) according to **[9].** Suspected red colonies were tested for IMVIC test for typical *E.coli*.

Molds and yeasts counts

Enumeration and counts of yeasts and molds were carried out in the samples using the media of acidified potato dextrose agar (Oxoid). The method recommended by FDA [8] was followed**.**

Aerobic sporeformers count

The count was carried out as the conventional method, FDA [8] using plate count agar (Oxoid) after heat dilutions to 80 ^{0} C for 10 minutes.

Clostridium perfringens

Enumeration was carried out in the samples using the Clostridium perfringens agar medium (Oxoid).

Results and Discussion

Table (1) Incidence and counts of coliform group and *Escherichia coli* in the examined samples .

Tested microorganism	Positive samples		Counts (c.f.u./gm or ml)	
	No.	%	Min.	Max.
coliform group *E.coli*	1-Domiati cheese (45 samples)			
	8	18	23	24x10
	3	7	4	23
coliform group *E.coli*	2-Feta cheese (25 samples)			
	ND	-	-	-
	ND	-	-	-
coliform group *E.coli*	3- Sterilized milk (30 samples)			
	ND	-	-	--
	ND	-	-	-

Min.- Minimum, Max.- Maximum, ND- Not Detected

As shown in Table (1) coliforms could be detected in 8 (18%) of the Domiati cheese samples examined, counts ranged from 23 to 24x10 c.f.u./gm. The presence of coliforms in cheese particularly *Aerobacter aerogenes* has been reported to be responsible for blown tins of Domiati cheese[10]. This problem is rare in modern dairies, provided that efficient pasteurization and good manufacturing practices are applied [11].However, the post manufacturing contamination may be attributed to the method of handling, transportation and marketing of dairy products. These observations were similar to those reported by [12,13].

In concern to Feta cheese and UHT milk, coliforms could not be detected in all examined samples (Table 1).

Table (1), also proved that *E.coli* was isolated from 3 (7%) of Domiati cheese samples. None of the Feta cheese and sterilized milk samples surveyed contained *E.coli*. *Escherchia coli* are an enteric organism. Most strains of *E.coli* are harmless; however, several are known to be pathogenic. The pathogenic strains may be categorized based on the mechanism underlying the illness. Currently four categories of pathogenic *E.coli* have been associated with food borne illness: Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC) and Enterohaemorrhagic (EHEC) *E.coli*. *E.coli* is often used as an indicator of faecal contamination in food. Its presence in cheese suggests that other food borne pathogens of faecal origin may also be present [2,11].

Table (2) Incidence and counts of *Staph. aureus* in the examined samples .

Positive samples		**Counts (c.f.u./gm or ml)**	
No.	%	Min	Max.
1-Domiati cheese (45 samples)			
2	4.5	30x10	$40x10^2$
2-Feta cheese (25 samples)			
ND	-	-	-
3- Sterilized milk (30 samples)			
ND	-	-	-

Min.- Minimum, Max.- Maximum, ND- Not Detected

The results in Table (2) indicated that *Staph.aureus* was present in 2(4.5%) of the Domiati cheese samples. *Staph.aureus* counts ranged from 30x10 to 40 $x10^2$ cfu/g. None of the Feta cheese and sterilized milk samples contained *Staph.aureus*.

The presence of coagulase- positive *staphylococcus aureus* in a food give an indication about its contamination from skin, mouth or handling the food, but inadequately cleaned utensils or equipment may also a source of contamination [14]. Staphylococcal food poisoning is a major form of food borne illness and appears to continue to be so as time goes on when the environmental conditions are favorable for growth and multiplication of Staphylococcal[15]. The enterotoxins of *Staphylococcus aureus* are antigenically different types include (A, B, Cl, C2, D, E and TST "toxic shock toxin"). All these types of enterotoxins except TST were involved in food borne diseases [16].

Foods that require considerable handling during preparation and that are kept at slightly elevated temperatures after preparation are frequently involved in staphylococcal food poisoning. Staphylococci exist in air, dust, sewage, water, milk, and food or on food equipment, environmental surfaces, human, and animals. Humans and animals are the primary reservoirs. Staphylococci are present in the nasal passages and throats and on the hair and skin of 50 present or more of healthy individuals. This incidence is even higher for those who associate with or who come in contact with sick individuals and hospital environments. Although food handlers are usually the main source of food contamination in food poisoning outbreaks, equipment and environmental surfaces can also be sources of contamination with *Staph. aureus*[17].

Table (3) Incidence and counts of yeasts and molds in the examined samples.

Tested microorganism	**Positive samples**		**Counts (c.f.u./gm)**	
	No.	%	Min.	Max.
	1-Domiati cheese (45 samples)			
Yeasts	10	22.5	5x10	$60x10^2$
Molds	2	4.5	3x10	90x10
	2-Feta cheese (25 samples)			
Yeasts	1	4	<30	-
Molds	ND	-	-	-

Min.- Minimum, Max.- Maximum, ND- Not Detected

The data in Table (3) shows incidence and counts of yeasts and molds in examined samples. Yeasts were implicated in 22.5% and 4% of Domiati and Feta cheese respectively. Molds were found in two samples of Domiati cheese and one sample of Feta cheese.

The high total yeast counts have resulted from inadequate processing[18]. Yeast spoilage constitutes a major economic loss in the cheese industry through developing undesirable changes, such as slimness, red color and yeasty flavor[19]**.**

Yeasts in some cheese types can periodically cause both economic and public health problems. Yeasts themselves are not commonly the cause of defects in cheese unless they ferment lactose in this case; they can grow rapidly and produce a characteristic yeasty or fruity flavor and obvious gas [20,21]. There are numerous references concerning the significance of the presence of yeasts in dairy products, where they may contribute positively to the characteristic taste and flavor development during the stage of maturation or, on the contrary, may lead to product spoilage[22]. The main defects caused by spoilage yeasts are fruity, bitter or yeasty off-flavors, gas production, discoloration changes and texture. In fact, continued lactose fermentation could be lead to increased acidity, gassiness and fruity flavors, while continued hydrolysis of protein and fat could contribute to bitter and rancid flavors as well as a softening of product texture [23]. El-Diasty and Salem[24] reported that yeasts may cause cheese spoilage by breaking down its components and liberating different acids and gas with subsequent change of its odour and flavour. Moreover, mould growth on cheese causes economic losses from

discoloration, poor appearance and off flavour. In addition, some moulds are capable of producing toxic metabolites known as mycotoxins such as aflatoxins which are known carcinogenic.

The presence of moulds and yeasts in butter and cheese are objectionable, as they grow at a wide range of temperature and pH values, resulting in spoilage of the product. Their count is used as an index of storability and sanitary quality of the product. Such moulds and yeasts can cause gas and off flavor in cheese and rancidity or other flavor defects in butter due to their proteolytic activity. Also, Bintsis and Papademas[25] reported that excessive yeast growth will cause softening of cheese, a condition that is usually associated with an unpleasant yeasty or ester-like odor or gas formation, in the case of white-brined cheeses, swelling of the cans can be caused by yeasts that ferment lactose, e.g. *Kluyveromyces* spp. Discoloration of the surface of a Portuguese ewe's milk cheese has been attributed to pigment-producing yeasts. In addition, yeast can increase the pH of the cheese surface, thus spurring the growth of *Staphylococcus aureus* and possibly other pathogenic and /or spoilage bacteria. For Feta stored over a year, a definite deterioration of quality was noticed when the pH of the cheese increased to more than 5.0.

Yeasts and moulds counts in cheese are used as an index of the proper sanitation quality. Defects in this un-ripened soft cheese such as rancidity, softness and colour defects arise mainly from contamination by yeast and mould. Moreover, some species constitutes a public health due to production of mycotoxins[26]. Common contaminating yeasts of cheeses include *Candida* spp., *Kluyveromyces marxianus*, Geotrichum *candidum*, *Debaryomyces hansenii*, and *Pichia* spp.[27]. Molds can grow well on the surfaces of cheeses when oxygen is present, with the low pH being selective for them. In packaged cheeses, mold growth is limited by oxygen availability, but some molds can grow under low oxygen tension. Molds commonly found growing in vacuum-packaged cheeses include *Penicillium* sp. and *Cladosporium* sp.[28]. *Penicillium* is the mold genus most frequently occurring in cheeses[29].

Table (4) Incidence and counts of aerobic sporeformers and *Clostridium perfringens** in the examined samples.

Positive samples		**Counts (c.f.u./gm or ml)**	
No.	%	Min	Max.
1-Domiati cheese (45 samples)			
18	40	3x10	$65x10^2$
2-Feta cheese (25 samples)			
2	2	2x10	30x10
3- Sterilized milk (30 samples)			
2	6.7	4x10	5x10

Min.- Minimum, Max.- Maximum, ND- Not Detected
* All samples were free from *Cl. perfringens*

Data of Table (4) show that aerobic spore formers were present in 18 (40%) of Domiati cheese, 2 (8%) of Feta cheese and 2 (6.7%) of sterilized milk. Sporeformering bacteria that are present in foods are important because the formation of the spore by bacterium allows it to be resistant to heat, freezing, chemicals and other adverse environments that our food undergoes during processing and preparation. Although the vegetative cell is killed by these conditions, the spores can survive and need harsher conditions to be inactivated. Some of the bacteria that are important belong to the genus *Bacillus*, which are aerobic to facultative anaerobic rod-shaped microbes. The other important group of spore forming bacteria is the *Clostridium* species. These are anaerobic bacteria that can grow at temperatures that are both mesophilic and thermophilic, depending on the species involved. They are of interest in foods because they also cause food spoilage and some species cause food-borne disease.

Bacillus species proved to induce certain objectionable changes in milk and some dairy products. Certain Bacillus species can grow at low temperature and produce enzymes which lead to bitterness, sweet

curding, bitty cream and blood red sediment ion the surface of milk. Also, they can cause carbolic fishiness and phenol flavor. While, some strains had been implicated in cases of food poisoning[16].

Bacillus cereus is a food poisoning microorganism, produces one emesis-causing toxin and three enterotoxins that elicit diarrhea. There are two types of food poisoning syndromes caused by *B. cereus*. The first is diarrhea type syndrome, while the second is vomiting type [30]. It has been recognized that high number of *B.cereus* ranged from 106-108/ml is needed to elicit symptoms of food poisoning[31]. However, in compromised consumers a much smaller dose of $1.2x10^3$/ml may cause illness [32]. Neither two forms of illness should be considered life-threating to normal healthy individual. The contamination of milk and milk products by such organisms may be attributed to the fact that *B.cereus* is widely distributed in nature and usually contaminate milk during milking or storage on the farm, then gain entrance to dairy products from which they are prepared. However, the extent of *B.cereus* contamination depends on the effectiveness of hygienic measures applied during processing, handling and distribution of milk products[17].

Conclusion

In conclusion, cheese can be considered a good medium for bacterial growth due to their nutrient content and long storage duration. Several steps in their production can cause bacteriological hazards. The results indicate the unhygienic conditions prevailing during distribution, sale or storage. Our researchers in this work have noted when compiling samples that there are some supermarkets do not take into account rules of public health upon receipt of products in addition to the faults in the refrigerators and unclean places.

It is recommended to use improvement of product quality and safety could be applying Good Manufacturing Practices (GMP), Good Hygienic Practices (GHP) and Hazard Analysis and Critical Control Point (HACCP) system, attempts to provide HACCP guidelines. However, educating food handlers, particularly food vendors, the food hygiene is a strategy that can be used in efforts aimed at to preventing food-borne diseases. Our observations coincide with results by Kousta *et al*. [33] and Adetunjui and Arigbede [34].

Conclusively, there is a need for continuous monitoring products by educating producers, distributors and retailer on good sanitary practices during processing and sale of the product and the possible danger or contaminated dairy products.

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