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Microbiological Quality and Safety of Fluid Milk Marketed in Cairo and Giza Governorates

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ABSTRACT

In this study, the microbial quality and safety of the market and UHT milk were studied. A total of 158 milk samples (125 raw market milk and 33 UHT milk) was collected randomly from different supermarkets and retailer shops in Cairo and Giza governorates. Samples were analyzed for Aerobic Plate Count (APC), total coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and yeast and mold counts, as well as for the pathogens *E. coli* O157:H7 and *Salmonella* spp. All market milk samples were found to be contaminated, having bacterial load exceeding the acceptable limit. The microbiological quality of market milk was judged a poor, whereas the quality of UHT milk samples was excellent. Pathogenic bacteria (*E. coli*, *B. cereus* and *S. aureus*) were detected in some of the market milk samples but none of the UHT milk samples contained any bacteria. None of the market and UHT milk samples taken contained detectable levels of *Salmonella* and *E. coli* O157:H7. A high microbial load of market milk may present a public health hazard to the consumers and emphasizes the need for improved hygienic standards.

Key words: Market milk, UHT, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*

INTRODUCTION

Milk is a highly nutritious food for human beings, but it also serves as an ideal medium for the growth of many organisms because of its near-neutral pH, complex biochemical composition and high water content (Touch and Deeth, 2009). Microbial contamination occurs mainly during and after milking. Microorganisms are introduced to raw milk in a number of ways, such as excretion from the udders of infected animals or contamination from the dairy farm environment and production facilities (Vissers and Driehuis, 2009).

In developed countries, only 2% of the milk produced is consumed in its raw form. Most of the milk in developing countries is consumed raw (Metwally *et al*., 2011). Total milk production within Egypt is estimated to be 5.8 million tons (FAO., 2013), with a total cattle population of around 7.3 million heads (Selim, 2009). The main producers of milk are smallholder farmers, with two to three animals, lacking the hygienic conditions of production and veterinary services. A large percentage of milk is retailed directly to consumers by farmers and small-scale traders, including hawkers with virtually no quality control at all levels (Metwally *et al*., 2011).

From the point of production up to consumption, milk passes through various stages and in the process, the microbial population increases enormously, particularly when it has been handled improperly. Fresh milk drawn from a healthy cow normally contains a low microbial load

($<10^3$ CFU mL^{-1}) but the load may increase up to 100 fold or more once it is stored for some time at ambient temperature. Therefore, keeping milk in clean containers at refrigeration temperatures immediately after the milking process may delay the increase of initial microbial load and prevent the multiplication of microorganisms in milk between milking at the farm and transportation to the dairy shop (Chye *et al*., 2004).

The dairy industry uses a heat treatment process to ensure the safety of fluid milk and to extend its shelf life. In Egypt, the industry processes mostly Ultra-Heat Treatment (UHT) sterilized milk, which is expensive for most consumers, a matter that encourages them to consume retailed raw milk (Metwally *et al*., 2011). Moreover, many people, especially in rural areas, may believe that the raw milk and their products have advantages over the heat treated milk.

However, from a food safety point of view, it is important that the raw milk should be protected from contamination during both the pre-and post-milking stages. Although, this has proven to be a challenge in Egypt, due to low milking hygiene standards and lack of cooling facilities during handling and transportation of milk, it is imperative that the microbiological quality of unprocessed milk be determined in order to monitor food safety. Several pathogens as *Salmonella* spp., verotoxigenic *E. coli*, *S. aureus* and *B. cereus* are recognized as important agents of food-borne illness associated with consumption of raw milk and milk products (Baylis, 2009). Both raw and sterilized milk can be contaminated during bottling, shipment and storage. Sterilization only destroys the pathogens in the milk at the time of processing; if unsanitary conditions allow pathogens to re-enter the milk later, it will be contaminated again. Therefore, the objectives of this study were to assess the microbiological quality of raw (market) milk and UHT milk marketed in Cairo and Giza governorates and to study the prevalence of food-borne pathogens especially *S. aureus*, *E. coli* O157:H7, *Salmonella* spp. and *B. cereus*.

MATERIALS AND METHODS

Collection of samples: A total of 158 milk samples (125 raw market milk and 33 UHT milk) was collected randomly from different supermarkets and retailer shops in Cairo and Giza governorates. Raw market milk samples (75 from Cairo and 50 from Giza) were taken in sterile milk containers. UHT milk samples (18 from Cairo and 15 from Giza) were obtained in their retail packages. Samples were transported to the laboratory under refrigerated conditions (4±2°C) within 1-3 h of collection and analyzed immediately upon arrival.

Microbiological examination: Samples were diluted in 0.1 % peptone water (Oxoid, UK) (11 mL of samples in 99 mL of 0.1% peptone water for initial dilution), subsequent decimal dilutions up to 10^{-7} were prepared with the same diluent and appropriate dilutions were used to enumerate different classes of microorganisms.

Milk samples were analyzed for Aerobic Plate Count (APC), total coliforms, *E. coli*, *S. aureus*, *Bacillus cereus* and yeast and mold counts according to methods described by Roberts and Greenwood (2003). The APC was enumerated in pour-plates of plate count agar (Oxoid, UK) after incubation at 30°C for 72±3 h. Coliforms and *E. coli* counts were estimated by a three tube Most Probable Number (MPN) technique. *Staphylococcus aureus* was enumerated by surface spread technique onto Baird Parker agar containing egg yolk tellurite emulsion (Oxoid, UK), after incubation at 37°C for 48 h. *B. cereus* was enumerated by surface spread technique onto Polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA), after incubation at 37°C for 24 h.

Further confirmation of *E. coli*, *S. aureus* and *Bacillus cereus* was based on biochemical characteristics. Yeasts and molds were enumerated in pour-plates of oxytetracycline glucose yeast extract agar (Oxoid, UK), after incubation at 25°C for 3-5 days.

Milk samples were also examined for *E. coli* O157:H7 and *Salmonella* (Roberts and Greenwood, 2003). For *E. coli* O157:H7, 25 mL of each sample was added to 225 of modified tryptone soya broth (mTSB), which contain 30 g of TSB (Oxoid, UK), 1.5 g of bile salts no.3 (Oxoid, UK), 1.5 g of dipotassium phosphate and 20 mg of novobiocin (Sigma Chemical Co., St. Louis, Mo.) per liter. The inoculated broth was incubated at 41.5±1°C for 18-24 h. After 6 and 18-24 h, a loopful of the incubated broth was plated on CT-SMAC agar (Sorbitol Mac Conkey agar (Oxoid) supplemented with cefixime and potassium tellurite) (Oxoid, UK). After 18-24 h of incubation at 37±1°C, non-sorbitol fermenting colonies were selected and isolated. Presumptive colonies of *E. coli* O157 were biochemically identified using API 20E (Bio Merieux, France). All biochemically identified non sorbitol fermenting colonies were subjected to slide agglutination with *E. coli* O157 latex test kit (Oxoid).

For Salmonella, 25 mL of each sample was added to 225 mL of buffered peptone water (Oxoid). After incubation at 37°C for 18±2 h, 0.1 mL of the incubated broth was transferred into 10 mL volume of Rappaport Vassiliadis (RV) (Oxoid) enrichment broth and incubated at 42°C for 24 h. Loopfuls of RV broth were streaked onto xylose lysine desoxycholate agar and *Salmonella-Shigella* agar (Oxoid). After 24 h of incubation at 37°C, presumptive colonies of salmonella were selected and subjected to the further biochemical and serological identification.

Statistical analysis: The SPSS pocket program for windows was used for the statistical analysis. Values of different parameters were expressed as the Mean±Standard Error (±SE).

RESULTS AND DISCUSSION

Milk samples were analyzed for their microbiological quality and safety. The quality and safety of milk are strictly related to their microbial content. The microbial counts of market milk samples are shown in Table 1. The APC for market milk samples obtained from Cairo and Giza ranged from 3.5×10^6 to 4.8×10^8 with a mean count of $8.8\times10^7\pm1.5\times10^7$ and from 2.3×10^5 to 6×10^9 with a mean value of $4.4\times10^8\pm1.7\times10^8$ CFU mL^{-1}, respectively. These results were higher than that obtained by Al-Tarazi *et al*. (2003), Chye *et al*. (2004), Al-Tahiri (2005), Shojaei and Yadollahi (2008), El-Diasty and El-Kaseh (2009) and Tasci (2011) who found mean APC of 1.1×10^7, 1.2×10^6, 5×10^5, 1.3×10^7, 6.1×10^5 and 3.95×10^6 CFU mL^{-1}, respectively, but lower than those reported by Moustafa *et al*. (1988) and Mohamed and El Zubeir (2007), who found mean values of 1×10^9 and 5.63×10^9 CFU mL^{-1}, respectively. The APC, usually represented by spoilage and lactic acid

Table 1: Bacterial load of market milk samples

Source and variables	Min.	Max.	Mean±SE
Cairo (n = 75 CFU mL^{-1})			
APC*	3.5×10^6	4.8×10^8	$8.8\times10^7\pm1.5\times10^7$
Total coliforms	7.5×10^2	2.1×10^7	$1.8\times10^6\pm4.0\times10^5$
S. aureus	3.5×10^2	6.2×10^6	$5.9\times10^5\pm1.5\times10^5$
Yeast and mold	2.3×10^2	2.0×10^5	$1.4\times10^4\pm3.7\times10^3$
Giza (n = 50 CFU mL^{-1})			
APC*	2.3×10^5	6.0×10^9	$4.4\times10^8\pm1.7\times10^8$
Total coliforms	2.1×10^2	2.3×10^6	$2.0\times10^5\pm7.7\times10^4$
S. aureus	1.5×10^2	1.4×10^6	$1.3\times10^5\pm3.6\times10^4$
Yeast and mold	1.0×10^2	9.0×10^3	$3.1\times10^3\pm3.9\times10^1$

*APC: Aerobic plate count, Min: Minimum, Max: Maximum, SE: Standard error, n: No. of sample

bacteria, is a good indicator for monitoring the sanitary conditions practiced during production, collection and handling of raw milk (Chambers, 2002). Hence training of milk handlers about hygiene can significantly reduce the bacterial load in milk. All the market milk samples had high bacterial load exceeding the acceptable value of 10^5 CFU mL^{-1} of milk in most European countries (EEC., 1992; IFCN., 2006), therefore, it could be said that the raw milk sold in Cairo and Giza cities were unsafe. The most frequent cause of high bacterial load is poor sanitary conditions during milking, collection and transport. Unsanitary conditions associated with the handling of the milk in the supermarket, limited knowledge on the hygienic production of milk and unavailability of cooling facilities during production, handling and transportation of milk might be also some factors contributing to high bacterial load.

The APC of UHT milk must be not more than 10 CFU mL^{-1} (EOSQ., 2005a). The APC of each of the UHT-processed milk samples were <10, indicating their excellent sanitary quality (Table 2). Our study corroborates the results obtained in previous studies, where counts of <10 CFU mL^{-1} were reported by Hassan *et al*. (2009) and El-Shinawy *et al*. (2011). On the contrary, high levels of APC in UHT milk were reported by Abd Elaal (2008), Shojaei and Yadollahi (2008) and Ayad *et al*. (2009) with mean values of 2.9×10^4, 7.1×10^1 and 3.4×10^1 CFU mL^{-1}, respectively.

The existence of coliform bacteria may not necessarily indicate a direct faecal contamination of milk but it is a precise indicator of poor hygiene and sanitary practices during milking and further handling processes. All market milk samples were contaminated by coliform bacteria (Table 1). The total coliforms count (MPN mL^{-1}) of examined market milk samples in Cairo and Giza ranged from 7.5×10^2 to 2.1×10^7 with a mean value of $1.8\times10^6\pm4\times10^5$ and from 2.1×10^2 to 2.3×10^6 with a mean value of $2\times10^5\pm7.7\times10^4$ MPN mL^{-1}, respectively. Moustafa *et al*. (1988), Chye *et al*. (2004), Al-Tahiri (2005), Mohamed and El Zubeir (2007), Shojaei and Yadollahi (2008), Godic-Torkar and Golc-Teger (2008), Abd-Elrahman *et al*. (2009), El-Diasty and El-Kaseh (2009) and Tasci (2011) determined coliform counts in raw cow milk samples as 1×10^6, 1.7×10^5, 6×10^2, 3.32×10^6, 1.3×10^3, 2.1 log, 4.157 log, 7×10^6 and 2×10^4 CFU mL^{-1}, respectively. The occurrence of coliforms in raw milk has received considerable attention, partly due to their association with contamination of fecal origin and the consequent risk of more enteric pathogens being present, partly because of the spoilage that can result from their growth in milk at ambient temperature. Coliform counts regularly in excess of 100 CFU mL^{-1} are considered by some authorities as evidence of unsatisfactory production hygiene (Tasci, 2011). Possible reasons for the high coliform counts could be due to poor herd hygiene, contaminated water, unhygienic milking procedures and improperly washed and maintained equipment (Hossain *et al*., 2010). All UHT milk samples were completely free from any coliform (Table 2). This finding in the present study was consistent with

Table 2: Bacterial load of UHT milk samples

Source and variables	Min.	Max.	Mean±SE
Cairo (n = 18 CFU mL^{-1})			
APC	<10	<10	0
Total coliforms	<3	<3	0
S. aureus	<10	<10	0
Yeast and mold	<10	<10	0
Giza (n = 15 CFU mL^{-1})			
APC	<10	<10	0
Total coliforms	<3	<3	0
S. aureus	<10	<10	0
Yeast and mold	<10	<10	0

APC: Aerobic plate count, Min: Minimum, Max: Maximum, SE: Standard error

Table 3: Incidence of *Escherichia coli*, *Escherichia coli*, O157:H7, *Bacillus cereus* and *Salmonella* organisms in the examined samples

		E. coli		*E. coli* O157:H7		*B. cereus*		*Salmonella*	
Products and source of samples	No. of samples	No*	%	No	%	No	%	No.	%
Market milk									
Cairo	75	25	33	0	0	12	16	0	0
Giza	50	19	38	0	0	14	28	0	0
UHT milk									
Cairo	18	0	0	0	0	0	0	0	0
Giza	15	0	0	0	0	0	0	0	0

No: Number

the results of Al-Tahiri (2005), Ayad *et al*. (2009), Hossain *et al*. (2010) and El-Shinawy *et al*. (2011). On the contrary, high level of coliform counts in UHT milk was reported by Shojaei and Yadollahi (2008) with a mean value of 9 CFU mL^{-1}.

The presence of bacteria in raw milk indicates possible contamination by infected udders, unhygienic milking procedures or equipment and contaminated water. *Escherichia coli* and coliform bacteria are often used as indicator microorganisms and the presence of *E. coli* implies a risk that other enteric pathogens may be present in the milk sample (Chye *et al*., 2004). *Escherichia coli* was isolated from 25 (33%) Cairo milk samples and 19 (38%) Giza milk samples (Table 3). The contamination rate in raw market milk samples was extremely lower than the findings of Moustafa *et al*. (1988), Sobeih *et al*. (2002), Chye *et al*. (2004) and Altalhi and Hassan (2009) as they found 66.6, 88, 65 and 66% of their samples were contaminated by *E. coli*, respectively, but higher than the rate of 32, 27.5, 3.3 and 10% reported by Ahmed and Sallam (1991), Mezyed *et al*. (2008), El-Prince *et al*. (2010) and Tasci (2011), respectively. The presence of *E. coli* in market milk indicates an extensive deficiency of satisfactory sanitary practices during milk production and post-production handling.

In the present study, all market milk samples were contaminated with *S. aureus* with the mean count of $5.9\times10^5\pm1.5\times10^5$ and $1.3\times10^5\pm3.6\times10^4$ CFU mL^{-1} of the examined market milk samples collected from Cairo and Giza (Table 1), respectively. The contamination rate in market milk samples was generally higher than those reported by Al-Tarazi *et al*. (2003), Chye *et al*. (2004), Guven *et al*. (2010) and Tasci (2011) as they found 47, 61, 33.3 and 86% of their samples were contaminated with *S. aureus*, respectively. According to the EOSQ (2005b), the *S. aureus* numbers must not exceed a maximum of 1×10^2 CFU mL^{-1}, all market milk samples failed to meet the national standard. *Staphylococcus aureus* is frequently found in raw milk and milk products. The high levels of *S. aureus* of market milk may be attributed to high prevalence of the organism in the udders of dairy cows and/or cross contamination by hands of the milk handlers. Moreover, transportation of milk under high environmental temperature permitting growth of *S. aureus* can stimulate the production of *S. aureus* enterotoxins. In food, the minimum count of *S. aureus* required to produce intoxication in human beings is estimated to be about 10^5 CFU mL^{-1}. To produce sufficient enterotoxin, the pH should be >4.6 and the temperature should be above 15°C for more than 3-4 h (Gran *et al*., 2003). Therefore, there is a definite risk of toxin production during collection and transportation of milk. Thus, the general hygienic practices aimed at minimizing bacterial contamination of milk should be implemented, as well as the growth of *S. aureus* must be prevented to avoid potential risk.

Yeasts and molds are common contaminants in food. The results given in Table 1 show that the yeasts and molds were present in all market milk samples. The total yeasts and mold count of examined market milk samples in Cairo and Giza ranged from 2.3×10^2 to 2×10^5 with a mean value of $1.4\times10^4\pm3.7\times10^3$ and from 1×10^2 to 9×10^3 with a mean value of $3.1\times10^3\pm3.9\times10^1$ CFU mL^{-1},

respectively, which are higher than those reported at 2×10^3 and 2.3 log CFU mL^{-1} by Moustafa *et al*. (1988) and Godic-Torkar and Golc-Teger (2008), respectively. Whereas the yeast and mold counts were lower than the result obtained at 4.3×10^5 CFU mL^{-1} by El-Diasty and El-Kaseh (2009). The presence of yeast and mold in milk and its products may result in spoilage of the product. An excessive number of these organisms in milk are indicative of unsanitary practices during milking production and unsatisfactory conditions of utensils. The total yeast and mold count of each of the UHT-processed milk samples were <10, indicating their excellent sanitary quality (Table 2). The detection rate of yeast and mold in UHT milk was in agreement with the result of Al-Tahiri (2005), who reported no yeast and mold in their UHT milk samples.

Bacillus cereus was isolated from 12 (16%) and 14 (28%) of the examined market milk samples collected from Cairo and Giza (Table 3), respectively. Ahmed *et al*. (1983), Mosso *et al*. (1989), Ayoub *et al*. (2003) and Hassan *et al*. (2010) reported 9, 0, 26.67 and 30% of their samples were contaminated with *B. cereus*. Because *B. cereus* is widely distributed in the environment, the organism can be introduced into the milk from soil, air, water, bedding, feeds, pasture, udder, excreta from the cows and milking equipment (Te Giffel *et al.,* 1996).

None of the market and UHT milk samples taken contained detectable levels of *Salmonella* and *E. coli* O157:H7. Also, UHT milk samples were free from *S. aureus, E. coli* and *B. cereus* (Table 3). Nevertheless *S. aureus* can still be destroyed through heat treatment when present in milk. Although the heat may kill *S. aureus* cells, the enterotoxin may persist in milk because it is more heat stable than the microorganism (Mhone *et al*., 2011).

The recovery of high levels *S. aureus*, *E. coli* and *B. cereus* from market milk may present a public health hazard because of the potential pathogenicity and/or toxigenicity of some strains of these bacteria, especially considering that in these communities, there are many vulnerable consumers such as children, the elderly and people living with viral diseases. Since a lot of people still drink raw milk, especially in rural areas, this emphasizes the need for educational efforts on health risks associated with consumption of raw milk.

CONCLUSION

The results of the study clearly indicated that microbiological quality and safety of raw milk marketed in Cairo and Giza governorates was poor while that of the UHT milk was excellent. High bacterial counts are likely to affect the keeping quality and safety of raw milk and products derived from it. The presence of pathogenic bacteria such as *S. aureus*, *E. coli* and *B. cereus* may pose a risk to public health. Therefore, there is a necessity for developing the hygienic status of locally produced raw milk, through educating the farmers, milk sellers and collectors on general hygienic practices and on how to handle their foods, including correct storage to protect them from infection and to save a lot of products from being deteriorated. Also information on health hazards associated with consumption of raw milk should be extended to the public, so that consumption of untreated raw milk and its derivatives could be avoided.

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